Title: Antiproliferative activity of *Tiliacora triandra* ethanolic extract leaf powder against Lung cancer cell

Field: Biology and Biodiversity

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Abstract:

Lung cancer is one of the most common cancers in many countries and the most common cause of cancer deaths in the past few decades. Lung cancer treatments with current anticancer drugs are associated with drug resistance and many unpleasant side effects. Herbal medicine is an inevitable alternative to reduce toxicity. Yanang (*Tiliacora triandra* (Colebr.) Diels.), a flowering plant native to Southeast Asia and widespread in the northeast of Thailand, has a long history of consumption and use as with IC₅₀ values of 110.03 ± 0.81 and 88.98 ± 9.93 µg/ml at exposure times of 24 and 72 h, respectively. The ethanolic extract was relatively less toxic to the non-cancerous Vero cells at 24 h-exposure with the IC₅₀ value of 466.75 ± 32.73 µg/ml, but relatively toxic to Vero cells at 72 h-exposure with the IC₅₀ value of 86.68 ± 5.29 µg/ml. Our finding suggests the possibility to develop *T.triandra* leaf extract as chemotherapeutic drug for lung cancer treatment.

Keywords: *Tiliacora triandra* (Colebr.) Diels, Lungcancer, MTT assay

Introduction:

Lung cancer is the leading cause of cancer death world-wide (Bray et al., 2018). The statistics of lung cancer has been increased and tends to continuously grow in the future. Cigarette smoking remains the principal cause of lung cancer (Cheng et al., 2016). Tobacco smoke is a toxic mix of more than 7,000 chemicals. At least 70 are known to cause cancer. At present, adolescents' smoking is increasing and causes various health and social problems. Chemotherapy is routinely used for cancer treatment, however, there are some intrinsic problems of chemotherapeutic drugs. Various kinds of toxicities may occur as a result of chemotherapeutic treatments. The toxicity of chemotherapeutic drugs sometimes creates a significant problem in the treatment of cancer established medicine. Moreover, many patients are poor and unable to gain access to high-priced drugs. Therefore, various therapies have been propounded for the treatment of cancer, many of which use plant-derived products. Plants have a long history of use in the treatment of cancer and continue to be a major source of new drugs. Herbal medicines have been recognized as one of the attractive approaches for lung cancer therapy because they have proven to be useful and effective in preventing side effects of chemotherapy and improving quality of life in lung cancer patients (Wu et al., 2016).

Yanang (*Tiliacora triandra*) is a flowering native plant in Southeast Asia and widespread in the northeast of Thailand (Smitinand, 1991). It is traditionally used as anti-pyrogenic agent. It has been reported
to have antioxidant activity and used to treat various chronic diseases. The leaves are used in bamboo shoot soup or raw materials for cooking in Thai and Laos foods. At present, *T. triandra* leaf powder products are available in Thailand and sold to be OTOP product. However, there are no reports about the anticancer activity of these products. Therefore, Yanang leaf powder from one source in Bangkok was randomly selected to study the anti-cancer activity in lung cancer cells. So, this research aimed at studying antiproliferative activity against human lung cancer cell line (A549 cells) of the ethanolic extract of *T. triandra* leaf powder. Antiproliferative activity of the ethanolic extract against A549 cells for 24 and 72 h was determined by MTT assay.

**Methodology:**

**Cells and Cell culture**

The lung cancer cells (A549 cells) were obtained from Dr. A. Ittharat (Thammasat University, Thailand). The non-cancerous cell line (African green monkey; Vero cells) was obtained from Dr. S. Barusrux (Khon Kaen University, Khon Kaen, Thailand). Both cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) (Gibco-BRL) at 37°C in a humidified atmosphere with 5% CO₂.

**Preparation of *Tiliacora triandra* ethanolic leaf powder extract**

*Tiliacora triandra* leaf powder (Figure 1) was purchased from the Rak Samunpai group, Bangkok, Thailand. *T. triandra* leaf powder (4 g) was added in 40 ml of absolute ethanol and stirred with magnetic stirrer for 48 h. The suspension was centrifuged at 10,000 rpm (6,150 x g, Centrifuge, Hitachi, R20A2) for 10 minutes. The supernatant was collected and filtered with the Whatman NO. 4 filter paper. The supernatant was then evaporated to 1-2 mL by using rotary evaporator and to dryness utmostly under a gentle stream of nitrogen. The ethanolic extract was kept at -20°C until used.

**Figure 1** *Tiliacora triandra* leaf powder from the Rak Samunpai group, Bangkok, Thailand.

**MTT assay**

Cells were cultured in a 96 wells-plate at the initial cell number of 8,000 cells/well for 24 hours. The varied concentrations of ethanolic extract were prepared as follows: 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 milligrams per milliliter (mg/ml). The cultured plate was incubated at 37°C for 2-4 hours. DMSO was added to dissolve the formazan and the absorbance of formazan was measured with microplate reader at a wavelength of 550 nm and the reference wavelength of 655 nm. Cell survival rate or cell viability is derived from the equation.

\[
\text{% Cell Viability} = \left( \frac{(A_{550\text{ sample}}-A_{655\text{ sample}})}{(A_{550\text{ control}}-A_{655\text{ control}})} \right) \times 100
\]

**Statistical analysis**

The results were demonstrated as mean ± standard deviation (S.D.) from two independent experiments. The program Statistical Package for the Social Science version 19.0 for windows (SPSS Corporation, Chicago, IL) was used for analysis. The significant difference was analysed using one-way ANOVA with Duncan’s post hoc test and a statistical significance was set at *p* < 0.05.
Results and Discussion:

Anti-proliferative activity of *T. triandra* leaf powder ethanolic extract against lung cancer cell line (A549 cells) and non-cancer cell line (Vero cells) was investigated using MTT assay. The results were shown in the percentage of cell viability and IC$_{50}$ value. The ethanolic extract had a dose- and time-dependent anti-proliferative effect against A549 and Vero cells. As shown in Figure 2, *T. triandra* leaf powder ethanolic extract significantly inhibited proliferation of A549 cells with IC$_{50}$ values of 110.03 ± 0.81 and 88.98 ± 9.93 µg/ml at exposure times of 24 and 72 h, respectively.

![Figure 2](image_url)  
**Figure 2** The effect of *T. triandra* leaf powder ethanolic extract on A549 cell proliferation. The A549 cells were treated with *T. triandra* leaf powder ethanolic extract (2.5-640 µg/ml) for 24 and 72 h. Antiproliferative activity was determined using MTT assay according to manufacturer protocol. Data are expressed as percentage of solvent control, which was defined as 100%.

In addition, cytotoxicity of *T. triandra* leaf powder ethanolic extract in non-tumorigenic Vero cells was investigated in comparison with the cancer cell line (A549 cells). As shown in Figure 3, the *T. triandra* leaf powder ethanolic extract inhibited proliferation of Vero cells with IC$_{50}$ values of 466.75 ± 32.73 and 86.68 ± 5.29 µg/ml at exposure times of 24 and 72 h, respectively.

![Figure 3](image_url)  
**Figure 3** The effect of *T. triandra* leaf powder ethanolic extract on Vero cell proliferation. The Vero cells were treated with *T. triandra* leaf powder ethanolic extract (2.5-640 µg/ml) for 24 and 72 h. Antiproliferative activity was determined using MTT assay according to manufacturer protocol. Data are expressed as percentage of solvent control, which was defined as 100%.

According to the above results, the ethanolic extract was relatively more toxic to A549 lung cancer cells but less toxic to the non-cancerous Vero cells at 24 h-exposure, indicating somewhat greater specificity to cancer cell line of the extract. However, the ethanolic extract was relatively toxic to Vero cells at longer exposure time (72 h).
counterpart of human lung adenocarcinoma A549 cells. Therefore, further study on toxicity of the extract in normal human bronchial epithelial (NHBE) and BEAS-2B non-cancer cell lines should be performed. In addition, toxicity to human peripheral blood mononuclear cells (PBMCs) should also be studied in order to provide more information on the extract’s toxicity.

**Conclusion:**

*T. triandra* leaf powder ethanolic extract inhibited the growth of lung cancer A549 cells. In addition, *T. triandra* leaf powder ethanolic extract was less toxic to non-tumorigenic Vero cells at 24 h exposure. *T. triandra* leaf powder ethanolic extract has potential for development as a chemotherapeutic drug for human lung cancer treatment. However, further studies in more cell lines and in animal models are needed prior to clinical applications.

**References :**


**Acknowledgement :**

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