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Anti-proliferative and apoptosis induction activities of extracts from Thai medicinal plant recipes selected from MANOSROI II database

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ABSTRACT

Ethonopharmacological relevances: Traditional medicines have long been used by the Thai people. Several medicinal recipes prepared from a mixture of plants are often used by traditional medicinal practitioners for the treatment of many diseases including cancer. The recipes collected from the Thai medicinal text books were recorded in MANOSROI II database. Anticancer recipes were searched and selected by a computer program using the recipe indication keywords including Ma-reng and San which means cancer in Thai, from the database for anticancer activity investigation.
Aim of study: To investigate anti-cancer activities of the Thai medicinal plant recipes selected from the "MANOSROI II" database.
Materials and methods: Anti-proliferative and apoptotic activities of extracts from 121 recipes selected from 56,137 recipes in the Thai medicinal plant recipe "MANOSROI II" database were investigated in two cancer cell lines including human mouth epidermal carcinoma (KB) and human colon adenocarcinoma (HT-29) cell lines using sulforhodamine B (SRB) assay and acridine orange (AO) and ethidium bromide (EB) staining technique, respectively.
Results and conclusions: In the SRB assay, recipes NE028 and, S003 gave the highest anti-proliferation activity on KB and HT29 with the IC_{50} values of 2.48 ± 0.24 and 6.92 ± 0.49 μg/ml, respectively. In the AO/EB staining assay, recipes S016 and NE028 exhibited the highest apoptotic induction in KB and HT-29 cell lines, respectively. This study has demonstrated that the three Thai medicinal plant recipes selected from "MANOSROI II" database (NE028, S003 and S016) gave active anti-cancer activities according to the NCI classification which can be further developed for anti-cancer treatment.

1. Introduction

Cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis. There are more than 100 distinct types of cancer with its subtypes can be found within the specific organs. Plants have played an important role as the source of effective anti-cancer agents. It is significant that over 60% of the currently used anti-cancer agents are derived from natural sources including plants, marine organisms and microorganisms (Cragg et al., 2005). The search for anti-cancer agents from plant sources started with the discovery and development of the vinca alkaloids, vinblastine and vincristine and the isolation of the cytotoxic podophyllotoxins. This has led to the discovery of many novel chemotypes showing the range of cytotoxic activities. The first group of the agents to advance into clinical use was the so-called vinca alkaloids, vinblastine (VLB) and vincristine (VCR), isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae). These agents are primarily used in the combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers as well as Kaposi's sarcoma (Gueritte and Fahy, 2005). Paclitaxel (Taxol®) was initially isolated from the bark of the Pacific Yew, *Taxus brevifolia* Nutt. (Taxaceae). Paclitaxel has been used effectively in the treatment of breast, ovarian and non-small cell lung cancer (NSCLC), as well as against Kaposi sarcoma (Kingston, 2005). However, the currently used Taxol is produced by biotransformation using plant cells and microorganisms (Frense, 2007).

Thai traditional medicines have long been used for many disease treatments including cancer. The Natural Products Research and

Abbreviations: SRB, sulforhodamine B; AO, acridine orange; EB, ethidium bromide; IC_{50}, half maximal inhibitory concentration; KB, human mouth epidermal carcinoma cell line; HT-29, human colon adenocarcinoma cell line.

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Development Center (NPRDC) at Chiang Mai University in Thailand has developed the database containing the Thai medicinal plant recipes collected from many institutes, temples and folklore doctors (Manosroi et al., 2006). Approximately 56,137 recipes have been collected from the Thai medicinal text books. The total of 633 recipes anti-cancer recipes have been recorded.

Apoptosis was the type of cell death with unique morphological features and a wide significance in tissue homeostasis. Apoptosis is an active process regulated by molecular mechanisms that activate or modify a stereotyped "program" of the effector events. The morphological changes of apoptotic cells are cell shrinkage and rounding-up with the loss of the contact with adjacent cells. Then, the chromatin condenses with a uniform and smooth appearance against the nuclear membrane (crescent shape), followed by nuclear fragmentation and formation of apoptotic bodies (Stewart, 1994). In cancer therapy, apoptosis is the type of programmed cell death which plays an important role in cancer. The deficiency of apoptosis is one of the causes of cancers. In the apoptotic study, staining of apoptotic cells with fluorescent dyes such as acridine orange (AO) and ethidium bromide (EB) is considered to be one of the reliable methods for evaluating the changed nuclear morphology. AO permeates all cells and makes the nuclei appear green. EB is only taken up by cells which their cytoplasmic membrane integrity is lost, and their nuclei are stained red. EB also dominates over AO. Thus, live cells will show a normal green nucleus. Early apoptotic cells should give bright green nucleus with condensed or fragmented chromatin. Late apoptotic cells display condensed and fragmented orange chromatin. Cells that have died from direct necrosis have a structurally normal orange nucleus (Renvoize et al., 1998).

This study has aimed to investigate anti-cancer activities including anti-proliferation and apoptosis activity of the Thai medicinal plant recipe extracts selected from the "MANOSROI II" database by SRB assay and AO/EB staining technique, respectively. KB and HT-29 cancer cell lines were used because these two cancers have high incidences in Thai population. The results from this study will be beneficial for the further development of the recipes to the novel anti-cancer drugs.

2. Materials and methods

2.1. Materials

Acridine orange (AO), ethidium bromide (EB), sulforhodamine B (SRB), quinine sulfate, anthraquinone, fructose, glucose, sucrose and luteolin were purchased from Sigma (St. Louis, MO, USA). β-Carotene, tannin, xanthone and trypsin were from Fluka (Buchs, Switzerland). Trypsin was prepared at 0.25% solution in phosphate buffered saline. Completed DMEM medium was prepared from Dulbecco's Modified Eagle Medium (GIBCO, Invitrogen Corporation, NY, USA) supplemented with 10% fetal bovine serum (PAA, Pasching, Austria), penicillin (100 U/ml) and streptomycin (100 mg/ml). All other chemicals and reagents were analytical grade.

2.2. Recipe selection and plant material preparation

Thai medicinal plant recipes were collected from 4 regions of Thailand. The 121 anti-cancer recipes were selected from 56,137 recipes of the Thai medicinal plant recipe database "MANOSROI II". The keywords of Ma-reng and San which in Thai meant cancer were used to select the recipes. The selected plants were collected from Chiang Mai Province in Thailand during June to August in 2009. The voucher specimens of the plant samples were deposited at NPRDC, Faculty of Pharmacy, Chiang Mai University in Chiang Mai, Thailand.

2.3. Preparation of the extracts

The plants were washed, cut into small pieces, dried at 50 °C and then powdered. The plant powder was mixed according to the compositions in the recipes. For the extraction process, the medicinal plants recipe powder (40 g) was boiled for 2 h in 800 ml of distilled water and then cooled to room temperature (27 ± 2 °C) and centrifuged (IEC clinical centrifuge, International equipment, USA). The supernatant was filtered through the Whatman No. 1 filter paper. The filtrates were concentrated by a rotary evaporator (Büchi, BÜCHI Labortechnik AG, Flawil, Switzerland) and lyophilized (CHRIST: ALPHA 1-2 LD, Martine Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The dried extracts dissolved in serum-free medium at the concentration of 0.01 g/ml were used as the stock solution. The stock solution was diluted to obtain the final concentration of 0.1–1000 μg/ml. All samples were prepared aseptically by filtering through the cellulose acetate filter (0.2 μm) and kept at 4 °C until use.

2.4. Cell cultures

The human mouth epidermal carcinoma (KB) obtained from American Type Culture (ATCC) and human colon adenocarcinoma (HT-29) cell lines provided from Medicinal Microbiology Department, Faculty of Biology, University of Tuebingen, Tuebingen, Germany were grown in complete DMEM medium and maintained in a humidified atmosphere of 5% CO_2 incubator (Contherm mitre 4000, Contherm Scientific, Hutt city, New Zealand) at 37 °C. The cells were trypsinized and counted with a haemacytometer. The cells were plated in 96-well flat-bottomed tissue culture plates for anti-proliferative and apoptotic activity assays.

2.5. Anti-proliferative assay

The anti-proliferative activity of the 121 selected recipes was determined by SRB assay (Monks et al., 1991). Cells (10^4 cells/well) were plated in 96-well plates and incubated overnight in a humidified atmosphere of 5% CO_2 incubator at 37 °C. An amount of 20 μl of the extracts and anti-cancer drugs (cisplatin, doxorubicin, fluorouracil and vincristine) at various final concentrations (0.1–1000 μg/ml) was added to the cells. After 24 h of incubation, the cells were fixed with 50% trichloroacetic acid solution, incubated at 4 °C for 1 h and washed five times with distilled water. Excess water was drained off and the plates were air-dried for 24 h. The cells were stained with 50 μl of 0.4% SRB solution in 1% acetic acid for 30 min at room temperature (25 °C). After incubation, the SRB solution was poured off and the plates were washed with 1% acetic acid. The plates were air-dried and 100 μl of 10 mM Tris-base solution were added to each well to solubilize the dye and shaked for 30 min at room temperature (25 °C). The absorbance at 540 nm was determined by the microplate reader (Biorad model 680, Biorad, Japan). All experiments were performed in triplicate. The % cell growth (% G) was determined using the following equation:

$$\%\,\text{Cell growth}\,(\%G) = \frac{T_{\text{treat}} - T_0}{T_{\text{control}} - T_0} \times 100$$

where T_{treat} was the optical density of the test extracts, T_{control} was the optical density of the control and T_0 was the optical density at time zero. The IC_{50} values were determined by plotting the percentages of cell growth (% G) versus the concentrations of the samples. The anti-proliferative activity in the term of IC_{50} values was classified into four categories as: active ($IC_{50} \leq 20$ μg/ml), moderately active ($20 < IC_{50} < 100$ μg/ml), inactive ($IC_{50} \geq 100$ μg/ml) and not detected (IC_{50} at 1 mg/ml of the extracts) (Cordell et al., 1993). Extracts which were in the categories of active, moderate and