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Original Research Article

Cytotoxic activity of the chemical constituents of *Clerodendrum indicum* and *Clerodendrum villosum* roots

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ABSTRACT

Objective: The roots of two Thai medicinal plants, *Clerodendrum indicum* and *Clerodendrum villosum* are found in traditional medicine practices. The aim of this research was to preliminarily study the cytotoxicity of extracts of their roots, and the parts that possessed cytotoxic activity were separated on a chromatograph to identify their active compounds.

Methods: The extracts of both plants were screened for cytotoxicity on the SW620 cell line and the compounds isolated from the active extracts were further evaluated for their cytotoxic activity against five human cancer cell lines, including SW620, ChaGo-K-1, HepG2, KATO-III and BT-474 using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay.

Results: Dichloromethane extracts of *C. indicum* and *C. villosum* were active against the SW620 cell line. Triterpenoids were mostly obtained from the extracts of these plants (0.28% and 1.02%, respectively) and exhibited varying degrees of cytotoxicity and specificity against the tested cell lines. Two triterpenoids, oleanolic acid 3-acetate and betulinic acid, displayed moderate to strong cytotoxicity toward all cancer cell lines, with 50% inhibitory concentration (IC_{50}) values of 1.66–20.49 μmol/L, whereas 3β-hydroxy-D:B-friedo-olean-5-ene and taraxerol were cytotoxic to only the SW620 cell line (IC_{50} = 23.39 and 2.09 μmol/L, respectively). Triterpenoid, lupeol, showed potent cytotoxicity on both SW620 (IC_{50} = 1.99 μmol/L) and KATO-III cell lines (IC_{50} = 1.95 μmol/L), while a flavonoid, pectolinarigenin, displayed moderate cytotoxicity against these cells (IC_{50} = 13.05 and 24.31 μmol/L, respectively). Although the widely distributed steroid, stigmasterol, was effective against the SW620 cell line (IC_{50} = 2.79 μmol/L) and β-sitosterol was also active against SW620 (IC_{50} = 11.26 μmol/L), BT-474 (IC_{50} = 14.11 μmol/L) and HepG2 cancer cells (IC_{50} = 20.47 μmol/L), none of the characteristic 24β-ethylsteroids of either *Clerodendrum* species were shown to be cytotoxic.

Conclusion: This study is the first report on the presence of cytotoxic triterpenoids from the roots of these medicinal plants, which have been used in herbal formulas as an antipyretic. Our findings support further in-depth study of this pharmacological activity as an anticancer agent.

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1. Introduction

The genus *Clerodendrum* belongs to the family Lamiaceae, and includes more than 500 plant species found in the tropics and subtropics of the world, among which are several important medicinal plants. These plants are attractive targets for phytomedicinal research to support their traditional uses and discover new pharmacological activities. Some biological activities of *Clerodendrum* species that have already been described include: anti-asthma [1], anti-inflammatory and antipyretic [2], antifungal [3], antioxidant and wound healing [4], anti-obesity [5], antinociception [6], antimicrobial [7], inhibition of angiotensin converting enzyme and α-glucosidase [8] and antimutagenicity [9]. *Clerodendrum indicum* (L.) Kuntze and *Clerodendrum villosum* Blume are two species of this genus which are endemic to Thailand [10]. *C. indicum* is a shrub locally known as "*Thao-Yaai-Mom*" and can be found throughout the country. Decoction and alcoholic extract of its leaves and roots have been used in Thai traditional medicine to treat fever, inflammation and asthma [11]. Its roots are also used

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as an important ingredient in the antipyretic herbal formula called "*Ya Ha Rak*" [12] or "*Ben-Cha-Lo-Ka-Wi-Chian*" remedy [13]. Another species, *C. villosum*, is a shrub which grows wild in several Asian countries. In India, the plant is applied to the scalp to kill head lice and its decoction has been used to treat liver diseases [14]. While phytochemical and bioactivity studies of *C. indicum* have previously been reported [1,2], phytomedicinal research on *C. villosum* has rarely been done. We recently reported on the chemical constituents of the roots of both plants and their chemotaxonomic significance [15]. In our continuing study of these medicinal plants, we evaluated the cytotoxic activity of extracts from both *C. indicum* and *C. villosum* roots against human colon adenocarcinoma (SW620) cells. Further, the chemical constituents that were obtained in our preceding study (i.e., triterpenoids, steroids and flavonoids) were tested for their cytotoxicity toward five human cancer cell lines including SW620, lung bronchus carcinoma (ChaGo-K-1), hepatocellular carcinoma (HepG2), gastric carcinoma (KATO-III) and breast carcinoma (BT-474). The data from this study will be useful in the development of anticancer treatments of Thai medicinal plants.

2. Materials and methods

2.1. Plant materials

The roots of *C. indicum* and *C. villosum* were collected from Khao Kho, Phetchabun Province, Thailand in October, 2011. The plants were authenticated by one of the authors (R. Suttisri). Voucher specimens (No. RS1101 and RS1102, respectively) were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

2.2. Plant extraction and isolation of compounds

The dried, powdered roots of *C. indicum* (1.0 kg) were sequentially macerated with *n*-hexane (3 × 3 L), dichloromethane (3 × 3 L) and methanol (3 × 3 L). Extracts from each solvent were combined and concentrated under reduced pressure at 45 °C to yield *n*-hexane (5.0 g), dichloromethane (8.0 g) and methanol extracts (20.5 g). The dried, powdered roots of *C. villosum* (1.0 kg) were similarly extracted to yield *n*-hexane (4.5 g), dichloromethane (3.4 g) and methanol extracts (30.2 g), respectively. Chemical constituents of the dichloromethane extracts from each plant were isolated and identified [15]. Chromatographic separation of *C. indicum* root extract identified 4 triterpenoids, 3β-hydroxy-D: B-friedo-olean-5-ene (1), oleanolic acid 3-acetate (2), taraxerol (3) and lupeol (4), 6 steroids, (22*E*)-stigmasta-4,22,25-trien-3-one (6), stigmasta-4,25-dien-3-one (7), stigmasta-4,22-dien-3-one (8), 22-dehydroclerosterol (9), clerosterol (10), stigmasterol (11), a mixture of three steroid glycosides, 22-dehydroclerosterol-3-*O*-β-D-glucopyranoside, clerosterol-3-*O*-β-D-glucopyranoside and stigmasterol-3-*O*-β-D-glucopyranoside (13) and two flavonoids, pectolinarigenin (15) and hispidulin (16). *C. villosum* root extract yielded three triterpenoids 1, 4 and betulinic acid (5), three steroids 9, 10, β-sitosterol (12) and a mixture of three steroid glycosides, 22-dehydroclerosterol-3-*O*-β-D-glucopyranoside, clerosterol-3-*O*-β-D-glucopyranoside and β-sitosterol-3-*O*-β-D-glucopyranoside (14).

2.3. Cytotoxic assay

An *in vitro* cytotoxic assay against human cancer cell lines was performed using the 3-(4,5)-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) colorimetric method [16,17]. All extracts from the roots of *C. indicum* and *C. villosum* were dissolved in dimethylsulfoxide (DMSO) and diluted with culture medium to make a stock solution. A series of 10-fold dilutions of each stock solution was prepared for the assay and the final concentration of DMSO did not exceed 0.5% in each experiment. The extracts were tested against the SW620 cell line in the preliminary investigation of the cytotoxicity; the compounds isolated from the active extracts were further subjected to the MTT assay in order to evaluate their cytotoxicity toward five human cancer cell lines including SW620, ChaGo-K-1, HepG2, KATO-III and BT-474.

All cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 5% fetal calf serum, and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Exponentially growing cells were seeded in 96-well plates (200 μL/well at a density of 2.5×10^4 cells/mL). The cells were incubated in growth medium for 24 h. Various concentrations of the test compounds were added (2 μL/well) and the cells were incubated for up to 72 h. At the end of this incubation period, 10 mL of MTT solution (5 μg/mL) was added and the plate was further incubated for 4 h at 37 °C. After removal of the culture supernatants, 150 μL of DMSO and 25 μL of glycine buffer (pH 10.4) were sequentially added to each well in order to facilitate solubilization of the formazan product. The plate was shaken, and the absorbance was measured by the optical density (OD) at 540 nm in a Synergy™ absorbance microplate reader (Bio-Tek). The percentage of cell survival was calculated as follows: Cell survival % = $[OD_{test}/OD_{control}] \times 100$. OD_{test} and $OD_{control}$ are the absorbance from treated condition and untreated condition, respectively.

Dose response curves were plotted from 5 concentrations of a 10-fold serial solution of the extracts (0.1–1000 μg/mL) and isolated compounds (0.001–10 μg/mL) against their percentage of cell survival in triplicate. The concentration of each compound that reduced the growth of a cancer cell line by 50% was also calculated from these curves and reported as the 50% inhibitory concentration (IC_{50} value). Results of the MTT assay for each extract were expressed in μg/mL, whereas the IC_{50} values of active chemical constituents were calculated and reported as μmol/L. The cytotoxicity of each compound was compared with that of doxorubicin. Doxorubicin was also used as a positive control since it is an antibiotic drug which has been potentially cytotoxic to various cancer cell lines and widely used as a control for the cytotoxic screening of natural products.

3. Results

3.1. Cytotoxic activity of C. indicum and C. villosum root extracts

Among the extracts of *C. indicum* and *C. villosum* roots, the dichloromethane portions of both plants exhibited cytotoxicity to SW620 cell line in the MTT assay. Dichloromethane extracts of *C. indicum* and *C. villosum* inhibited SW620 cell growth with the average IC_{50} values of (212.24 ± 2.28) and (247.12 ± 1.83) μg/mL, respectively. None of the *n*-hexane and methanol extracts from these plants were cytotoxic in the range of concentrations tested (0.1–1 000 μg/mL). Thus, the dichloromethane extracts from each plant were subsequently separated chromatographically to yield the expected cytotoxic constituents.

3.2. Chemical constituents of C. indicum and C. villosum roots

The chemical constituents of these root extracts were isolated by chromatographic methods and their structures were identified as described in our previous work [15]. Fifteen compounds were obtained from the dichloromethane extract of *C. indicum* and nine compounds from similar extract of *C. villosum*. As shown in Tables 1 and 2, four groups of phytochemicals were found in *C. indicum* roots (i.e., triterpenoids, steroids, steroid glycosides

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