Original Articles

In Vitro Antitumor Activity of Extracts from Thai Plants in Guttiferae and Schisandraceae Families on Human Cancer Cell Lines

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Purpose: *To screen the in vitro antitumor activity of extracts from the selected Guttiferae and Schisandraceae plants.* ***Materials and Methods:*** *Twenty-two methanol and chloroform extracts from Guttiferae and Schisandraceae families collected from the northern region of Thailand were tested for antitumor activity on HeLa (cervical carcinoma), KB (epidermoid carcinoma) and B16F10 (melanoma) human tumor cell lines using the sulforhodamine B (SRB) binding assay.* ***Results:*** *All extracts showed an antitumor activity with a dose response relationship. The chloroform extract of G. speciosa showed the potent inhibitory effect with the 50% growth inhibition (GI_{50}) value of 4.0, 6.6 and 3.7 μg/mL from the leaves and 9.9, 15.7 and 8.1 μg/mL from the wood against HeLa, KB and B16F10 tumor cell lines, respectively. Chloroform extracts of H. hookerianum and G. xanthochymus showed the inhibitory effect on cell growth with GI_{50} value less than 20 μg/mL.* ***Conclusion:*** *The information from this study can support the use of these plants in Thai traditional medicine and the further development of these extracts to new pharmaceuticals.*

Key Words: ● *Guttiferae* ● *Schisandraceae* ● *SRB assay* ● *Antitumor activity* ● *Thai plants* ● *Extracts*

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Introduction

The Guttiferae, mainly found in tropical and northern temperate regions and well known to be rich in secondary metabolites such as xanthonoid, biflavonoid and triterpenoid[1] are widely used in traditional medicine. These plants have been screened and found to exhibit significant pharmaceutical activity. *Hypericum hookerianum*, a traditional tribal wound healing agent, have been evaluated for antibacterial activity[2] and cinnamate esters isolated by our group exhibited significant inhibitory effect against MCF-7, NCI-H460 and SF-268 tumor cell lines, and were moderately effective in influencing the mitogenic response of human lymphocytes to phytohemagglutinin[3]. *Garcinia speciosa* have been studied for antiviral activity and effects on

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apoptosis[4-5]. Compounds isolated from wood of *G. xanthochymus* which had NGF-potentiating activity also have been reported[6]. Wood of *C. formosum* ssp. *Pruniflorum* was found to contain quercetin, hyperoside, xanthones, mangiferin and isomangifin[7] but the biological activities of these compounds were not known.

For plants in the Schisandraceae family, the winding stem twist around the trunks of trees and climb to their top, over 19 species have been widely used in Chinese traditional medicine. These plants have been proved to be rich in lignans and triterpenoids with various biological activities[8]. Isolated compounds from *Schisandra propinqua* fruit, seed and stem, exhibited the antihepatotoxic, antioxidant and antitumor activities[9].

In the framework of our chemical and biological investigations on plant species, the methanol and chloroform extracts from wood and leaves of the six Thai plant species in the Guttiferae (*Hypericum hookerianum* Wight & Arn, *Garcinia speciosa* Wall, *G. xanthochymus* Hook.f ex T.Anderson, *Cratoxylum formosum* ssp. *pruniflorum* (Kurz) Gogel and *Calophyllum polyanthum* Wall ex Choisy) and Schisandraceae (*Schisandra verruculosa* Gagnap) were studied for free radical scavenging activity[10]. In the present work, these extracts were found to possess *in vitro* antitumor properties against three human tumor cell lines, HeLa (cervical carcinoma), KB (epidermoid carcinoma) and B16F10 (melanoma).

Materials and Methods

Plant material.

The plants materials in the present investigation were collected from Chiang Mai Province, Thailand in November-December 2002. Voucher specimens were authenticated and deposited at the herbarium of Biology Department, Faculty of Science and Faculty of Pharmacy, Chiang Mai University, Thailand.

Preparation of the extracts and stock solution.

The extracts and stock solutions were prepared by a modified method of Wilairat et al.[3]. Wood and leaves from the plants were separately chopped to small pieces, dried at 40°C in a hot air oven and ground to powder. The dried powder samples (100-300 g) were macerated in methanol for 48 h. The solution was evaporated under reduced pressure by a rotary evaporator. The residues were re-extracted with chloroform and concentrated by partial evaporation under reduced pressure.

Stock solutions of methanol and chloroform extracts were prepared in DMSO (Sigma Chemical Co., MO, USA) and stored at -20°C. The frozen samples were diluted with cell culture medium prior to the assay. The concentration ranges of the extracts were 3 to 250 μg/ml.

Cell lines and culture medium.

HeLa, KB and B16F10 cell lines used in the experiments were kindly provided by National Cancer Institute, Bangkok, Thailand. Cells were routinely maintained as adherent cell cultures in DMEM medium (Sigma Chemical Co., MO, USA) supplemented with 10% heat-inactivated FCS (Gibco BRL, Canada) and 50 μg/mL of gentamicin (Sigma Chemical Co., MO, USA) at 37°C in a humidified air incubator containing 5% CO_2.

Treatment of cells with extracts.

When the cultures reached approximately 80% to 90% confluency they were sub-cultured by treating with 0.25% trypsin, and cell viability was tested by the tryphan blue dye exclusion method. Cell counts were performed in quadruplicated on a hemocytometer. The cell viability was always found to be greater than 98%. Each cell line was plated at a density of 2.0×10^5

cells/ml for HeLa and $1.0x10^5$ cells/ml for KB and B16F10 in 96-well plates and allowed to attach overnight. The following day, cells were exposed to five serial concentrations of extracts. Doxorubicin hydrochloride (Dabur Pharma Ltd, UK) was used as positive control. The plates were incubated at 37°C for 48 h.

SRB assay.

The effect of extracts on the growth of human tumor cell lines were evaluated according to the procedure of the National Cancer Institute (NCI, USA) for the *in vitro* anticancer drug screening using the protein-binding dye, SRB to assess cell growth[11]. After incubation period, the adherant cells were fixed in situ, washed and dyed with SRB (Sigma Chemical Co., MO, USA). The bound dye was solubilized and the absorbance was measured at 492 nm in a micro-plate reader. The dose-response curves were generated for each extract tested and for each cell line, and the GI_{50}, corresponding to the concentration of compounds that inhibit 50% of the cell growth was determined as described by Monks et al.[12].

Results

The effect of the methanol and chloroform wood and leaves extracts on HeLa, KB and B16F10 cell lines were shown in Table 1 and 2, respectively. Final concentration of DMSO ($\leq$ 0.25%) did not interfere with the biological activities tested. All extracts exhibited a dose dependent growth inhibitory effect with no significant difference in GI_{50} in the three cell lines understudied.

The chloroform leaves extract of *G. speciosa* showed the most potent inhibitory effect with GI_{50} values of 4.0, 6.6 and 3.7 μg/mL on HeLa, KB and B16F10 cell lines, respectively. These values were 13, 20 and 142 folds less potent than doxorubicin, the positive control, which gave the GI50 values of 300 nM, 330 nM and 26 nM on HeLa, KB and B16F10 cell lines, respectively. The strong growth inhibitory effects were also detected in the chloroform leaves extract of *C. polyan-thum* with GI_{50} value of 13.3, 19.0 and 11.0 μg/mL.

From the wood, the chloroform extract of *G. speciosa* showed the strong cell growth inhibition with the GI_{50} values of 9.9, 15.7 and 8.1 μg/mL on HeLa, KB and B16F10 cell lines, respectively. Chloroform extracts of *H. hookerianum*, and *G. xanthochymus* also exhibitedthe inhibitory effect on cell growth with GI_{50} value less than 20 μg/mL.

Moderate inhibitory effect were found in the methanol leaves extract of *G. speciosa*, the chloroform leaves extract of *G. xanthochymus*, the chloroform wood extract of *C. formosum* ssp. Pruniflorum and the methanol wood extract of *H. hookerianum*. Both of the methanol and chloroform leaves extracts of *C. formosum* ssp. *Pruniflorum*, and *S. verruculosa* showed no inhibitory activity at any concentration (GI_{50} > 100 μg/mL).

Discussion

For the *in vitro* primary screening of anticancer drug, an assay using the protein-binding dye sulforhodamine B (SRB) is used by the NCI. The SRB binds to the basic amino acids of cellular macromolecules and the solubilized stain is measured spectrometrically to determine relative cell growth in treated and untreated cells[11].

In Guttifeare family, the aerial part of *H. empetrifolium* that was collected from different locations in Greece were tested on brine shrimps, human colon carcinoma (Caco-2) and human hepatoma cell lines (HepG2) for cytotoxic activities. The results showed that methanol extract of *H. empetrifolium* exhibited high activities on cell lines with the 50% cell killed or 50% lethal concentration (LC_{50}) values ranging from 25

Table 1. Effect of methanol and chloroform extracts from wood of the selected Thai plants in family Guttiferrae and Schisandraceae on the growth of cell lines.

Plant species		GI_{50} (μg/mL) HeLa	KB	B16F10
Guttiferae				
H. hookerianum	M	42.3±1.5	46.3±1.5	51.0±8.5
	C	19.7±1.2	19.3±1.5	14.5±0.7
G. speciosa	M	67.3±2.5	75.0±0.6	82.0±4.2
	C	9.9±1.2	15.7±0.6	8.1±0.1
G. xanthochymus	M	> 100	> 100	> 100
	C	13.3±1.5	19.0±1.0	11.5±0.7
C. formosum ssp. pruniflorum	M	> 100	> 100	> 100
	C	41.3±1.5	37.3±0.6	44.5±2.1
C. polyanthum	M	> 100	> 100	> 100
	C	90.3±3.1	74.7±3.2	52.5±3.5
Schisandraceae				
S. verruculosa	M	> 100	70.7±6.4	> 100
	C	> 100	> 100	70.0±1.4

Note: M = methanol extract; C = chloroform extract

Results are expressed as GI_{50} that are arithmetical means±SD of 3 independent experiments performed in duplicate. Doxorubicin was used as positive control (GI_{50} Hela = 300±0.9 nM ; GI_{50} KB = 330±0.9 nM; GI_{50} B16F10 = 26±0.2 nM)

to 46 mg/ml and moderate activities on brine shrimps, ranging from 22 to 150 mg/ml[13]. Griffipavixanthone, a novel bixanthone with cyclized prenyl groups providing the xanthone-xanthone linkage isolated from bark of Malaysian plants, *G. griffithii* and *G. pavifolia* showed high *in vitro* cytotoxicity against mouse leukemia (P388), mouse Lewis lung carcinoma (LL/2) and mouse fibrosarcoma (Wehil 64) cell lines with the 50% effective dose (ED_{50}) of 3.40, 6.80 and 4.60 μg/ml, respectively[14]. Coumarins isolated from bark of Myanmar plant, *Kayea assamica* (Clusiaceae), have been evaluated for their cytotoxicity on human cancer cell lines using SRB assay. They exhibited strong cytotoxicity activity againts Col2 (colon), KB (epidermoid) and LNCaP (lung) human cancer cell lines with the 50% inhibition concentration (IC_{50}) values in the range 3.5-13.1 μM[15]. Ethanol extract of the stems of Taiwanese plants, *Schisandra arisanensis* which was useful as an antirheumatic, exhibited cytotoxicity against KB *in vitro*. Bioassay-directed fraction of this extract led to the isolation and characterization of four unique C19 homo lignans with a 5, 4'-butano-2, 4-cyclohexadienone-6-spiro-3'(2;-3'-dihydrobenzo [b]furan) skeleton : schiarisanrin A-C, and the biological evaluation of them demonstrated cytotoxicity against KB, colon carcinoma (COLO-205), hepatoma (HEPA) and cervix

Table 2. Effect of methanol and chloroform extracts from leaves of the selected Thai plants in family Guttiferrae and Schisandraceae on the growth of cell lines.

Plant species		GI_{50} (μg/mL) HeLa	KB	B16F10
Guttiferae				
H. hookerianum	M	ND	ND	ND
	C	ND	ND	ND
G. speciosa	M	34.7±2.3	23.7±0.6	25.7±0.6
	C	4.0±0.3	6.6±0.2	3.7±0.4
G. xanthochymus	M	> 100	> 100	> 100
	C	17.0±1.0	29.3±0.6	37.7±4.0
C. formosum ssp. pruniflorum	M	> 100	> 100	> 100
	C	> 100	> 100	> 100
C. polyanthum	M	> 100	> 100	> 100
	C	19.0±2.7	13.3±0.6	11.0±1.1
Schisandraceae				
S. verruculosa	M	> 100	> 100	> 100
	C	> 100	> 100	> 100

Note: M = methanol extract; C = chloroform extract ; ND = not determined

Results are expressed as GI_{50} that are arithmetical means±SD of 3 independent experiments performed in duplicate. Doxorubicin was used as positive control (GI_{50} Hela = 300±0.9 nM ; GI_{50} KB = 330±0.9 nM; GI_{50} B16F10 = 26±0.2 nM)

(HELA) cancer cells[15].

In our study, all of plant extracts which were in the genus related to the aforementioned studies also showed antitumor activity on HeLa, KB and B16F10 cell lines. The chloroform extracts from wood and leaves of all plants showed stronger inhibition than the methanol extracts. This might be due to the presence of more active non polar compounds which are more soluble in chloroform.

The results from this study supported that the selected plants from Guttiferae and Schisandraceae family had significant growth inhibitory activity. Further study on the mechanism of action and isolation of pure compounds in the extracts for potential uses as new pharmaceuticals should be encouraged.

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ฤทธิ์ต้านเซลล์มะเร็งของสารสกัดจากพืชวงศ์ Guttiferae และ Schisandraceae

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ศูนย์วิจัยและพัฒนาวัตถุดิบยา เครื่องสำอาง และผลิตภัณฑ์ธรรมชาติ สถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยเชียงใหม่

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บทคัดย่อ

วัตถุประสงค์ของการวิจัย: *เพื่อศึกษาฤทธิ์ต้านเซลล์มะเร็งของสารสกัดหยาบจากพืชวงศ์ Guttiferae และ Schisandraceae* ***วัสดุและวิธีการวิจัย:*** *ในการทดลองได้สกัดสารสกัดหยาบจากใบและเนื้อไม้โดยใช้เมธานอลและคลอโรฟอร์มของบัวทอง (Hypericum hookerianum) พะว้าหรือสารภีป่า (Garcinia speciosa) มะดะหลวง (Garcinia xanthochymus) ติ้วขนหรือติ้วเหลือง (Cratoxylum formosum ssp. Pruniflorum) พะองหรือมะแหนดอย (Calophyllum polyanthum) และ Schisandra verruculosa ซึ่งเก็บในพื้นที่จังหวัดเชียงใหม่และนำสารสกัดหยาบที่ได้มาทดสอบฤทธิ์ในการยับยั้งการเจริญเติบโตของเซลล์มะเร็งโดยใช้สาร sulforhodamine B* ***ผลการศึกษาและสรุป:*** *สารสกัดโดยคลอโรฟอร์มของ G. speciosa ออกฤทธิ์สูงสุดในการยับยั้งการเจริญเติบโตของเซลล์มะเร็งปากมดลูก (HeLa) มะเร็งในช่องปาก (KB) และมะเร็งผิวหนัง (B16F10) โดยจากส่วนของใบมีค่าความเข้มข้นที่สามารถยับยั้งการเจริญเติบโตของเซลล์มะเร็งได้ 50 เปอร์เซ็นต์ (GI_{50}) เท่ากับ 4.0, 6.6 และ 3.7 ไมโครกรัมต่อมิลลิลิตร และจากส่วนของเนื้อไม้มีค่าเท่ากับ 9.9, 15.7 และ 8.1 ไมโครกรัมต่อมิลลิลิตรตามลำดับ สารสกัดคลอโรฟอร์มจาก H. hookerianum และ G. xanthochymus แสดงฤทธิ์ในการยับยั้งการเจริญของเซลล์มะเร็งทั้งสามชนิดโดยมีค่า GI_{50} น้อยกว่า 20 ไมโครกรัมต่อมิลลิลิตร จากผลการศึกษาครั้งนี้ชี้ให้เห็นว่าพืชที่ทำการศึกษานี้มีศักยภาพที่จะสามารถพัฒนาเพื่อเป็นยาใหม่ได้ต่อไป*

Key Words: ● *Guttiferae* ● *Schisandraceae* ● *SRB assay* ● *Antitumor activity* ● *Thai plants* ● *Extracts*

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