Songklanakarin J. Sci. Technol.
36 (2), 189-194, Mar. - Apr. 2014

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Anti-cancer activity of compounds from *Cassia garrettiana* heartwood

Supreeya Yuenyongsawad, Kingkan Bunluepuech, Chatchai Wattanapiromsakul, and Supinya Tewtrakul*

Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand.

Received 8 July 2013; Accepted 12 November 2013

Abstract

The ethanol extract of *Cassia garrettiana* heartwood showed marked inhibitory activity against several cancer cell lines including HT-29, HeLa, MCF-7 and KB cells. Therefore, its extract and compounds were investigated for their anti-cancer effect using the Sulforhodamine B (SRB) assay. The ethanol extract of *C. garrettiana* heartwood was separated to give five compounds which are chrysophanol **(1)**, piceatannol **(2)**, aloe-emodin **(3)**, emodin **(4)** and cassigarol E **(5)**. Of the tested samples, chrysophanol **(1)** showed the highest anti-cancer activity against KB cells (IC_{50} = 0.045 μg/mL), aloe emodin **(3)** was the most active against HT-29 (IC_{50} = 0.29 μg/mL), emodin **(4)** was against HeLa cells (IC_{50} = 0.82 μg/mL), and cassigarol E **(5)** was active against MCF-7 (IC_{50} = 0.021 μg/mL), whereas piceatannol **(2)** was inactive in all tested cell lines. This is the first report of anti-cancer effect against HT-29, HeLa, MCF-7 and KB cells of *C. garrettiana* heartwood.

Keywords: anti-cancer activity, *Cassia garrettiana*, Caesalpiniaceae

1. Introduction

Cancer is known medically as a malignant neoplasm characterized by uncontrolled growth of abnormal cells in the body. Cancer can occur as the result of a disruption of this balance, due to an increase in cell proliferation or a reduction in cell death or both (Kerr et a1., 1972). Cancer is caused by endogenous and exogenous factors that lead to the sequential accumulation of genetic alterations, a scenario known as multi-step oncogenesis (Lee & Park, 2010). It may also spread to more distant parts of the body through the lymphatic system or bloodstream. Moreover, cancer is the primary cause of mortality and morbidity in the elderly. Elderly persons are at a ten times greater risk of developing cancer than persons under 65 years of age. *Cassia garrettiana* Craib, locally known in Thai as Samae-sarn, is one of the plants in the Caesalpiniaceae family. In Thai traditional medicine, the heartwood of this plant has been used as emmenagogue and as blood tonic for women (Tewtrakul *et al.*, 2007). Moreover, *C. garrettiana* heartwood has been used to treat Herpes zoster, leukemia, constipation and nematodes (Boonyapraphatsara & Chokchaicharoenporn, 1998). *C. garrettiana* has been reported to show many biological activities such as antifungal (Inamori *et al.*, 1984), antitumor and antimetastatic effects (Yoshiyuki *et al.*, 2008). Since an ethanol extract of *C. garrettiana* heartwood has shown good inhibitory activity against several cancer cell lines including HT-29, HeLa, MCF-7 and KB cells, this study is aimed to isolate pure compounds with anti-cancer activity.

2. Materials and Methods

2.1 Reagents

Phosphate buffer saline (PBS), penicillin-streptomycin and DMEM were from Invitrogen. 25 cm^3 Flasks and 96-well microplates were from Nunc, fetal bovine serum was from Seromed, Tris was from Gibco BRL, Sulforhodamine B (SRB)

* Corresponding author.
Email address: supinyat@yahoo.com; supinya.t@psu.ac.th

was from SIGMA, trichloroacetic acid (TCA) was from CARLO ERBA. The cell lines were from the Cell Lines Service [(HeLa, CLS No.300194), (KB, CLS No.300446), (HT-29, CLS No.300215), (MCF-7, CLS No.300273)].

2.2 Plant material

Cassia garrettiana heartwoods were collected in 2010 at the Suan Ya Thai Thongnoppakhun herbal garden in Chonburi province and were identified by Thai traditional doctor, Mr Sraupsin Thongnoppakhun, and the voucher specimen number is SKP 034030701. The sample was kept at the Herbarium of Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

2.3 Isolation of compounds from *Cassia garrettiana* extract

The dried power of *C. garrettiana* heartwood (2.1 kg) was extracted three times with ethanol (34 L) at room temperature. The EtOH extract (124.4 g) was successively partitioned to obtain hexane (8.5 g), chloroform (0.7 g), ethyl acetate (47.2 g) and water fractions (68.0 g) respectively. The ethyl acetate fraction (10.0 g) was separated by silica gel column chromatography using 10% $CHCl_3$ in methanol (50 ml, each) to afford 15 fractions (F1-F15). Fraction F2 (40.0 mg) was purified by column chromatography on sephadex LH-20 using 100% methanol (25 ml, each) to give chrysophanol (1) (orange solid, 11.3 mg, 0.113% w/w). Fraction F3 (2.0 g) was purified by column chromatography on silica gel using 10% methanol in chloroform (50 ml, each) to give subfraction (F3/1a–F3/7a). Subfraction F3/2a (120.0 mg) was purified by column chromatography on silica gel using 10% methanol in chloroform (25 ml, each) to give piceatannol (2) (white crystal, 50.3 mg, 0.503% w/w). Fraction F3/5a (600.0 mg) was purified by column chromatography on silica gel using 20% methanol in chloroform (20 ml, each) to give cassigarol E (5) (pale yellow solid, 207.5 mg, 2.075% w/w). Fraction F3/7a (60.0 mg) was purified by sephadex LH-20 using 100% methanol (10 ml, each) to give aloe-emodin (3) (yellow solid, 2.0 mg, 0.02% w/w) and emodin (4) (yellow solid, 2.3 mg, 0.023% w/w), respectively.

The structures of compounds 1-5 were elucidated using spectroscopic techniques and compared with reported spectral data (García-Sosa *et al.*, 2006; Li *et al.*, 2005a; Kametani *et al.*, 2007; Yao *et al.*, 2006; Li *et al.*, 2005b).

2.4 Anti-cancer activity assay

The anti-cancer compounds were detected using MCF-7 (human breast adenocarcinoma), HeLa (human cervical adenocarcinoma, HT-29 (human colon adenocarcinoma) and KB (oral cavity cancer) cells. The cancer cell lines were grown in Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10% fetal bovine serum (FBS). Cells were seeded into 96-well microplates (4000 cells/well) and allowed to adhere for 24 h at 37°C in a 5% CO_2 fully humidified incubator. Then 100 µL of a 25 µg/mL crude extract or a five-fold diluted pure compound in medium (final concentration 0.00006, 0.00032, 0.0016, 0.008, 0.04, 0.2, 1, 5 µg/mL) were dispensed into the wells of the cell plates and incubated further for 72 h. After removal of the sample medium, the bound cells were submerged in 200 µL of D-MEM medium and incubated for 72 h. Cells were then fixed with cold 40% trichloroacetic acid and kept at 4°C for 1 h and washed with tap water. The cells were determined by the Sulforhodamine B (SRB) assay. The absorbance was measured at 492 nm using a microplate reader. The results were interpreted based on the ability of extracts to inhibit cell growth compared with the control (cells in media without extract). The IC_{50} values were calculated using probit analysis. Camptothecin, an anti-cancer drug, was used as a positive control.

% Inhibition = [(OD control - OD sample)/ OD control] x 100

3. Results and Discussion

An ethanol extract of *C. garrettiana* heartwood showed marked anti-cancer activity at concentration of 25 µg/mL with % inhibition value ranging from 100 to 85.39 for the HT-29, KB and MCF-7 cells. A water extract of *C. garrettiana* heartwood produced a lower % inhibition than the ethanol extract (Table 1). Therefore five compounds were isolated from the ethanol extract of *C. garrettiana* heartwood (Figure 1) and were tested for their cytotoxic effect against HT-29, HeLa, MCF-7 and KB cell lines. The result indicated that cassigarol E (5) possessed potent activity against MCF-7 (IC_{50} = 0.021 µg/mL) and HeLa cells (IC_{50} = 1.064 µg/mL). Chrysophanol (1) also showed good activity against KB (IC_{50} = 0.045 µg/mL), MCF-7 (IC_{50} = 0.323 µg/mL) and HT-29 (IC_{50} = 1.0 µg/mL). Emodin (4) showed appreciable activity against HT-29 (IC_{50} = 0.336 µg/mL), HeLa (IC_{50} = 0.82 µg/mL), KB (IC_{50} = 1.195 µg/mL), and MCF-7 cells (IC_{50} = 2.188 µg/mL). Aloe

Table 1. % Inhibition values of extracts from *Cassia garrettiana* on anti-cancer activity

Sample	% inhibition at concentration 25 µg/mL			
	HT-29	HeLa	MCF-7	KB
Ethanol extract	100±0.00	54.94±2.39	98.30±0.29	85.39±2.39
Water extract	100±0.00	43.09±1.39	70.27±3.39	44.45±9.72

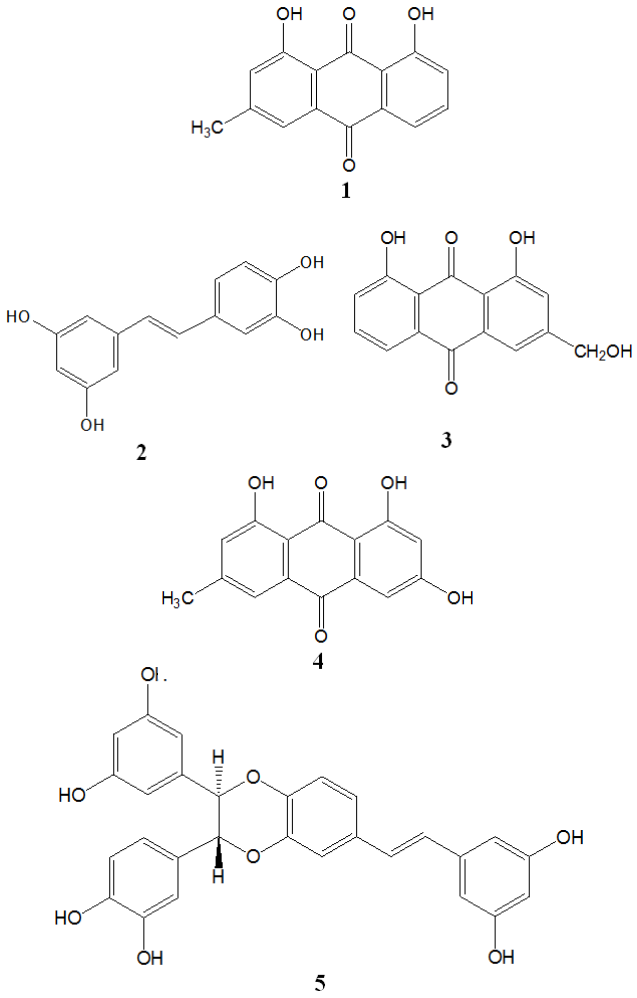


Figure 1. Structures of compounds 1-5 isolated from *Cassia garrettiana* heartwood

emodin (3) showed marked activity against HT-29 (IC_{50} = 0.296 µg/mL) and HeLa (IC_{50} = 1.0 µg/mL), whereas piceatannol (2) was inactive in all tested cell lines (Table 2). Regarding biological activity, there is a report of emodin (4) as anti-breast cancer agent by inhibiting 17β-estradiol binding to human estrogen receptors (ERs) (Matsuda *et al.*, 2001). It has been reported that chrysophanol (1) showed activity against MCF-7 cells (Matsuda *et al.*, 2001) but was inactive on HT-29 (Lee *et al.*, 2011) and HeLa cells (Li *et al.*, 2011) when using a MTT assay. Aloe-emodin (3) was reported to show cytotoxic effects to HT-29 (Kabbash *et al.*, 2008) and HeLa cells (Li *et al.*, 2011). Emodin (4) has been reported to show cytotoxic effects towards HT-29 (Gu *et al.*, 2012), HeLa (Li *et al.*, 2011) and MCF-7 cells (Matsuda *et al.*, 2001). Cassigarol A isolated from *C. garrettiana* heartwood has been reported to inhibit tumor growth and metastasis to the lung (Kimura *et al.*, 2000a) whereas piceatannol did not affect the tumor growth but could inhibit the metastasis to the lung in carcinectomized mice (Kimura *et al.*, 2000b). Cassigarol E (5) has been reported to have anti-allergic activity (Morikawa *et al.*, 2010).

Investigation of the structure-activity relationships (SARs) of hydroxyanthraquinones (Figure 2) for cytotoxicity against HT-29 cells showed that chrysophanol (1), aloe-emodin (3), and emodin (4) bearing the 1- and 8-OH groups were essential for anti-HT-29 activity. CH_2OH and OH groups substituted at position 6 (aloe-emodin, IC_{50} = 0.296 µg/mL; emodin, IC_{50} = 0.336 µg/mL) increased the activity, whereas CH_3 substitution at position 3 (chrysophanol, IC_{50} = 1.00 µg/mL) decreased the activity (Table 3). For cytotoxic effect on HeLa cells, chrysophanol (1), aloe-emodin (3) and emodin (4) having the 1- and 8-OH groups had an effect. CH_2OH and OH groups (aloe-emodin, IC_{50} = 1.00 µg/mL; emodin, IC_{50} = 0.82 µg/mL) substituted at position 6 increased the activity while CH_3 group at position 3 (chrysophanol, inactive) decreased the activity (Table 4). For cytotoxic effect against MCF-7 cells, chrysophanol (1), aloe-emodin (3) and emodin (4) which having the 1- and 8-OH groups showed an effect. A CH_3 at position 3 increased the activity (chrysophanol,

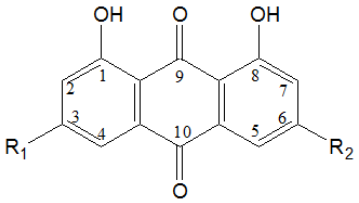


Figure 2. The chemical structure of hydroxyanthraquinone

Table 2. Anti-cancer activity of compounds **1-5** isolated from *Cassia garrettiana* heartwood

Compound	IC_{50} (µg/mL)			
	HT-29	HeLa	MCF-7	KB
Chrysophanol (**1**)	1.00	inactive	0.323±0.030	0.045±0.005
Piceatannol (**2**)	inactive	inactive	inactive	inactive
Aloe-emodin (**3**)	0.296±0.010	1.00	inactive	inactive
Emodin (**4**)	0.336±0.014	0.82±0.07	2.188±0.189	1.195±0.007
Cassigarol E (**5**)	inactive	1.064±0.084	0.021±0.005	inactive
Camptothecin (Positive control)	$0.000263\pm3.75\times10^{-5}$	$0.0449\pm1.0\times10^{-3}$	$0.00116\pm3.72\times10^{-4}$	$0.0057\pm8.7\times10^{-4}$

Table 3. IC_{50} values of hydroxyanthraquinone derivatives against HT-29 cells

Compound	R_1(C3)	R_2(C6)	IC_{50} (μg/mL)
Chrysophanol (**1**)	CH_3	H	1.00
Aloe-emodin (**3**)	H	CH_2OH	0.296±0.010
Emodin (**4**)	CH_3	OH	0.336±0.014
Camptothecin (Positive control)			$0.000263\pm3.75\times10^{-5}$

Table 4. IC_{50} values of hydroxyanthraquinone derivatives against HeLa cells

Compound	R_1(C3)	R_2(C6)	IC_{50} (μg/mL)
Chrysophanol (**1**)	CH_3	H	inactive
Aloe-emodin (**3**)	H	CH_2OH	1.00
Emodin (**4**)	CH_3	OH	0.82±0.07
Camptothecin (Positive control)			$0.0449\pm1.0\times10^{-3}$

Table 5. IC_{50} values of hydroxyanthraquinone derivatives against MCF-7 cells

Compound	R_1(C3)	R_2(C6)	IC_{50} (μg/mL)
Chrysophanol (**1**)	CH_3	H	0.323±0.030
Aloe-emodin (**3**)	H	CH_2OH	inactive
Emodin (**4**)	CH_3	OH	2.188±0.189
Camptothecin (Positive control)			$0.00116\pm3.72\times10^{-4}$

Table 6. IC_{50} values of hydroxyanthraquinone derivatives against KB cells

Compound	R_1(C3)	R_2(C6)	IC_{50} (μg/mL)
Chrysophanol (**1**)	CH_3	H	0.045±0.005
Aloe-emodin (**3**)	H	CH_2OH	inactive
Emodin (**4**)	CH_3	OH	1.195±0.007
Camptothecin (Positive control)			$0.0057\pm8.7\times10^{-4}$

IC_{50} = 0.323 μg/mL; emodin, IC_{50} = 2.188 μg/mL), whereas CH_2OH and OH groups at position 6 decreased the activity (aloe-emodin, inactive) (Table 5). For cytotoxic effect to KB cells, chrysophanol (1), aloe-emodin (3), and emodin (4) having the 1- and 8-OH groups had an effect. A CH_3 group at position 3 increased the activity (chrysophanol, IC_{50} = 0.045 μg/mL; emodin, IC_{50} = 1.195 μg/mL), whereas an additional OH substituted at position 6 decreased the activity as shown in 4 (IC_{50} = 1.195 μg/mL) *vs* 1 (IC_{50} = 0.045 μg/mL) (Table 6). For the stilbene, it was found that piceatannol (2) was inactive against HT-29, HeLa, MCF-7 and KB cells, but the dimer of this compound (5, cassigarol E) increased the effect against HeLa (IC_{50} = 1.064 μg/mL) and MCF-7 cells (IC_{50} = 0.021 μg/mL) (Table 7).

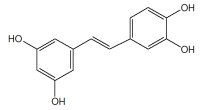
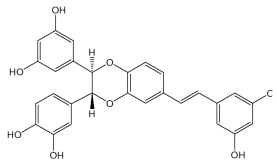
4. Conclusion

This is the first report of anti-cancer effect against HT-29, HeLa, MCF-7 and KB cells of *C. garrettiana* heartwood. The mechanism of action of active compounds will be further investigated.

Acknowledgments

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (PHA540538a). We also thank the Faculty of Pharmaceutical Sciences, Prince of Songkla University for providing labora-

Table 7. IC_{50} values comparison between stilbene (piceatannol) and its derivative (cassigarol E) against cancer cell lines

Compound	IC_{50} (μg/mL)			
	HT-29	HeLa	MCF-7	KB
 Piceatannol (**2**)	inactive	inactive	inactive	inactive
 Cassigarol E (**5**)	inactive	1.064 ±0.084	0.021±0.005	inactive
Camptothecin (Positive control)	$0.000263 \pm 3.75 \times 10^{-5}$	$0.0449 \pm 1.0 \times 10^{-3}$	$0.00116 \pm 3.72 \times 10^{-4}$	$0.0057 \pm 8.7 \times 10^{-4}$

tory facilities and Mr Sraupsin Thongnoppakhun for plant material support.

References

Boonyapraphatsara, N. and Chokchaicharoenporn, A. 1998. Thai Native Herbs No 4. Prachachon Press, Bangkok, Thailand, p. 683.

García-Sosa, K., Villarreal-Alvarez, N., Lübben, P. and Peña-Rodríguez, M.L. 2006. Chrysophanol, an antimicrobial anthraquinone from the root extract of *Colubrina greggii*. Journal of the Mexican Chemical Society. 50(2), 76-78.

Gu, Y., Guo, L. and Cai, J. 2012. Inhibiting effect of emodin on the proliferation of human colon carcinoma cell line RKO, HT-29 and Caco-2 in vitro. Zhongguo Zhongyi Jichu Yixue Zazhi. 18(5), 518-519.

Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M. 1984. The antifungal activity of stilbene derivative. Chemical and Pharmaceutical Bulletin. 32 (2), 801-804.

Kabbash, A., Kamelia, A., Zalat, E., Nagwa, S. and Yagi, A. 2008. Supercritical carbon dioxide extraction of aloe emodin and barbaloin from *Aloe vera* L. leaves and their in-vitro cytotoxic activity. Saudi Pharmaceutical Journal. 16(1), 75-81.

Kametani, S., Kojima-Yuasa, A., Kikusaki, H., Kennedy, D.O., Honzawa, M. and Matsui-Yuasa, I. 2007. Chemical constituents of cape aloe and their synergistic growth-inhibiting effect on ehrlich ascites tumor cells. Bioscience Biotechnology and Biochemistry. 71(5), 1220-1229.

Kerr, J.F., Wyllie, H.A. and Currie, A.R. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer. 26, 239-257.

Kimura, Y., Baba, K. and Okuda, H. 2000a. Inhibitory effects of active substances isolated from *Cassia garrettiana* heartwood on tumor growth and lung metastasis in Lewis lung carcinoma-bearing mice (Part 1). Anticancer Research. 20, 2899-2906.

Kimura, Y., Baba, K. and Okuda, H. 2000b. Inhibitory effects of active substances isolated from *Cassia garrettiana* heartwood on tumor growth and lung metastasis in Lewis lung carcinoma-bearing mice (Part 2). Anticancer Research. 20, 2923-2930.

Lee, M.S., Cha, E.Y., Sul, J.Y., Song, I.S. and Kim, J.Y. 2011. Chrysophanic acid blocks proliferation of colon cancer cells by inhibiting EGFR/mTOR pathway. Phytotherapy Research. 25(6), 833-837.

Lee, S.B. and Park, H. 2010. Anticancer activity of guava (*Psidium guajava* L.) branch extracts against HT-29 human colon cancer cells. Journal of Medicinal Plants Research. 4, 891-896.

Li, R., Yang, F., Lu, L., Zhao, J., Ye, Y. and Xu, W. 2011. Effects of dihydroxy anthraquinones compound on radiosensitivity of cervical carcinoma HeLa cells. Zhongguo Yaofang. 22(15), 1353-1356.

Li, W., He, K., Li, Y. and Hou, Z. 2005b. Total synthesis of (±)-shegansu B, gnetuhainin F, (±)-maackin A and (±)-cassigarol E. Acta Chimica Sinica. 63(17), 1607-1612.

Li, Y., Zhang, D. and Yu, S. 2005a. A new stilbene from *Cercis chinensis* Bunge. Journal of Integrative Plant Biology. 47(8), 1021-1024.

Matsuda, H., Shimoda, H., Morikawa, T. and Yoshikawa, M. 2001. Phytoestrogens from the roots of *Polygonum cuspidatum* (Polygonaceae): Structure-requirement of hydroxyanthraquinones for estrogenic activity. Bio-organic and Medicinal Chemistry Letters. 11, 1839-1842.

Morikawa, T., Xu, F., Matsuda, H. and Yoshikawa, M. 2010. Structures of novel norstilbene dimer, longusone A, and three new stilbene dimers, longusols A, B, and C, with antiallergic and radical scavenging activities from Egyptian natural medicine *Cyperus longus*. Chemical and Pharmaceutical Bulletin. 58(10), 1379-1385.

Tewtrakul, S., Subhadhirasakul, S., Rattanasuwan, P. and Puripattanavong, J. 2007. HIV-1 protease inhibitory substances from *Cassia garrettiana*. Songklanakarin Journal of Science and Technology. 29, 145-149.

Yao, S., Lib, Y. and Kong, L. 2006. Preparative isolation and purification of chemical constituents from the root of *Polygonum multiflorum* by high-speed counter-current chromatography. Journal of Chromatography A. 1115, 64-71.

Yoshiyuki, K., Maho, M., Masahiko, T. and Kimiye, B. 2008. Antitumor and antimetastatic actions of anthrone C-glucoside, cassialoin isolated from *Cassia garrettiana* heartwood in colon 26-bearing mice. Cancer Science. 99(11), 2336-2348.