Songklanakarin J. Sci. Technol.
36 (4), 459-463, Jul. - Aug. 2014

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

Original Article

Antibacterial and antioxidative compounds from *Cassia alata* Linn.

Trinop Promgool[1], Orasa Pancharoen[2], and Suwanna Deachathai[1]*

[1] *School of Science,*
Mae Fah Luang University, Mueang, Chiang Rai, 57100 Thailand.

[2] *Department of Chemistry, Faculty of Science,*
Silpakorn University, Mueang, Nakhon Pathom, 73000 Thailand.

Received 15 August 2013; Accepted 9 April 2014

Abstract

Phytochemical investigation of *Cassia alata* Linn. led to the isolation of six known anthraquinones: aloe-emodin (**1**), emodin (**2**), ω-hydroxyemodin (**3**), lunatin (**4**), physcion (**5**), and ziganein (**6**), six flavonoids: apigenin (**7**), 7,4′-dihydroxy-5-methoxyflavone (**8**), diosmetin (**9**), kaempferol (**10**), luteolin (**11**), and *trans*-dihydrokaempferol (**12**) as well as one stilbene, *trans*-resveratrol (**13**). Their structures were elucidated on the basis of spectroscopic methods, including UV, IR, NMR and MS. Nine compounds (**3-9** and **12-13**) were reported for the first time as metabolites of *C. alata.* Compound **2** exhibited strong antibacterial activity against methicillin resistant *S. aureus* (MRSA)-SK1 and *B. cereus* TISTR 687 with MIC values of 4 and 8 μg/mL, respectively. Compound **10** was found to exhibit antioxidative activity with IC_{50} value of 9.67 μM that was three times stronger than that of ascorbic acid (IC_{50} 25.41 μM).

Keywords: *Cassia alata* Linn., anthraquinones, flavonoids, antibacterial, antioxidation

1. Introduction

Cassia genus, belong to the Leguminosae family. The plants in this genus generally produce a variety of secondary metabolites including anthraquinones, alkaloids, flavonoids, pyrrolizine and pyrrolizidine alkaloids, triterpenes, steroids and tannins (Hemlata and Kalidhar, 1993; Miralles and Gaydou, 1986; Idu *et al*., 2007) some of which possess interesting biological and pharmacological activities, such as antimicrobial, antioxidative, anti-inflammatory, antitumor as well as cytotoxic activities (Ibrahim and Osman, 1995; Panichayupakaranant and Kaewsuwan, 2004; Fernand *et al*., 2008). Due to these properties, *Cassia* species have attracted attention as important sources for medicinal treatment. They have been used to treat eczema, itching and skin infection in human (Palanichamy and Nagarajan, 1990). *Cassia alata* Linn., locally known in Thai as Chum-Hed-Thed. It was observed that methanol extracts of leaves, flowers, stem and root barks of *C. alata* shown to have a broad spectrum of antibacterial activity (Khan *et al*., 2001). On the basis of DPPH radical scavenging assay-guided isolation, kaempferol from *C. alata* leaves exhibited antioxidant activity with ED_{50} 9.99 μM that was six times stronger than that of BHT with ED_{50} 57.41 μM and fifty-eight times stronger than that of emodin with ED_{50} 578.87 μM (Panichayupakaranant and Kaewsuwan, 2004). Previous research studies have led to the isolation of a number of constituents with many biological activities, making these potential agents for the treatment of diseases (Gurib-Fakim, 2006). In a continuing search for bioactive metabolites from *C. alata*, we now report the result of a phytochemical investigation of *C. alata* (leaves, roots and twigs) yielding thirteen compounds. Moreover, this work demonstrates that *C. alata* is among the potential sources of antibacterial and antioxidative compounds.

* Corresponding author.
Email address: suwanna.dea@mfu.ac.th

2. Experimental

2.1 General

Melting points were measured on a BÜCHI B-540 melting point apparatus and uncorrected. Ultraviolet spectra (UV) were measured with a Perkin Elmer Lambda 35 UV/VIS spectrophotometer. Principal bands (λ_{max}) were recorded as wavelengths (nm) and log ε on methanol solutions. Infrared spectra (IR) were recorded with a Perkin Elmer Spectrum GX spectrophotometer. Major bands (ν_{max}) were recorded in wavenumber (cm^{-1}). 1D and 2D NMR spectra were performed on a Bruker AVANCE 300 or a Bruker FTNMR Ultra Shield 400 MHz. Spectra were recorded on $CDCl_3$ or acetone-d_6 solutions. Quick column chromatography (QCC) was performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was carried out using silica gel 100 (0.063-0.200 mm, Merck).

2.2 Plant materials

The leaves, roots and twigs of *C. alata* Linn. were collected from Nong Khai Province, in the northeast of Thailand, in December 2009. The plant was identified by Mr. James Maxwell, Chiang Mai University Herbarium, and a specimen (957) was deposited at Chiang Mai University Herbarium, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

2.3 Extraction and isolation

The chopped dried leaves (0.267 kg), roots (6.735 kg) and twigs (2.030 kg) of *C. alata* were successively extracted with acetone 6, 31, and 11 liters, respectively, over a period of 7 days at room temperature. The extracts were evaporated to dryness on a rotary evaporator to give crude acetone extracts of the leaves (LA), the roots (RA) and the twigs (TA).

Crude LA (15.88 g) was subjected to QCC over silica gel eluted with CH_2Cl_2/Me_2CO (100:0 to Me_2CO 100%) to give nine fractions (LA1-LA9). Fractions LA3 and LA4 were combined (0.10 g) and purified by CC eluted with hexane/acetone (80:20 to acetone 100%) to give compounds **1** (17.8 mg) and **2** (20.7 mg). Fraction LA8 (3.86 g) was isolated by CC using hexane/EtOAc (50:50 to EtOAc 90%) to give compounds **9** (3.2 mg) and **10** (54.1 mg). Crude RA (40.42 g) was separated by QCC over silica gel using CH_2Cl_2/Me_2CO (100:0 to Me_2CO 100%) in a polarity gradient manner to give twenty fractions (RA1-RA20). Fraction RA2 (26.4 mg) was purified by CC eluted with hexane/CH_2Cl_2 (100:0 to CH_2Cl_2 15%) to give compound **6** (10.5 mg). Fraction RA4 (0.17 g) was purified by CC eluted with hexane/CH_2Cl_2 (100:0 to CH_2Cl_2 15%) to yield compound **5** (35.0 mg). Fraction RA14 (3.92 g) was subjected to QCC and CC over silica gel eluted with CH_2Cl_2/ Me_2CO (100:0 to Me_2CO 100%) to give compounds **3** (17.0 mg), **7** (3.0 mg), and **13** (110.9 mg). Crude TA (10.30 g) was subjected to QCC over silica gel using a gradient of CH_2Cl_2/ Me_2CO (100:0 to Me_2CO 100%) as a solvent system to give ten fractions (TA1-TA10). Fraction TA7 (1.31 g) was separated by CC and gradiently eluted with CH_2Cl_2/Me_2CO (95:5 to Me_2CO 40%) to give compound **12** (1.3 mg). Fraction TA8 (0.92 g) was subjected to CC eluted with hexane-EtOAc (100:0 to EtOAc 40%) to give compounds **4** (2.7 mg) and **11** (17.0 mg). Fractions TA9 and TA10 were combined (3.34 g) and subjected to CC eluted with hexane-Me_2CO (70:30 to Me_2CO 75%) to afford compound **8** (7.8 mg). Their structures were elucidated on the basis of UV, IR, NMR, and MS data as well as by comparison of their NMR spectral data with reported values (Lim, 1999; Jadulco *et al*., 2002; Miyazawa and Hisama, 2003; Fujimoto *et al*., 2004; Chu *et al*., 2005; Kametani *et al*., 2007; Lee *et al*., 2007; Mboungouere *et al*., 2007; Jeong *et al*., 2009; Ahn *et al*., 2011).

2.4 Antibacterial assay

Five microorganism cultures (*Bacillus cereus* TISTR 687, *Escherichia coli* TISTR 780, *Pseudomonas aeruginosa* TISTR 781, *Salmonella typhimurium* TISTR 292 and *Staphylococcus aureus* TISTR 1466) were derived from the Microbiological Resources Centre of the Thailand Institute of Scientific and Technological Research whereas Methicillin resistant *Staphylococcus aureus* (MRSA)-SK1 was donated by the Department of Microbiology, Faculty of Science, Prince of Songkla University. The minimum inhibitory concentrations (MICs) were determined by a two-fold serial dilution method (CLSI, 2002). The test samples were dissolved in DMSO. Serial 2-fold dilutions of the test samples were mixed with melted Mueller Hinton broth (MHB) in microtiter plates. The final concentrations of the test crude sample and pure compounds in broth ranged from 2.5-1,280 μg/mL and 0.25-128 μg/mL, respectively. 50 μL of inoculum suspension was added to each well (final concentration of $1x10^4$ CFU/well). The inoculated plates were incubated at 35-37ºC for 16-18 h. 10 μL of 0.18% resazurin was added to the microtiter plate and incubated at 35-37ºC for 2-3 h. A blue color indicated that the sample was capable of inhibiting bacterial growth, while a pink color indicated that the sample was incapable of inhibiting bacterial growth. MICs were recorded by reading the lowest concentration capable of inhibiting visible growth. The tests were performed at least in triplicate. Vancomycin and gentamicin were used as positive control drugs.

2.5 Antioxidation assay

The DPPH assay is one of the methods used for the evaluation of antioxidative activity. The following assay procedure was modified from that described in a previous report (Deachathai *et al*., 2006). The pure compound was dissolved in absolute ethanol to give a 3.05 mM solution. The solution of each sample (50 μL) was mixed with 0.05 mM DPPH ethanolic solution (3 mL) in a cuvette to give a 50 μM final solution. The trapping effect was assessed by measuring the absorbance change of the solution at 517 nm against

0.05 mM DPPH ethanolic solution after 15, 30, 45 and 60 min. Ascorbic acid and BHT were used as positive controls. The degree of loss in color is a measure of the activity. The absorbances were measured at 517 nm for 30 min. The concentration needed to decrease % inhibition of the DPPH solution to 50% inhibition (IC_{50}) was obtained by linear regression analysis of the dose-response curve.

3. Results and Discussion

Phytochemical investigation of the leaves, roots, and twigs of *C. alata* led to the isolation and identification of thirteen known compounds (Figure 1). Four compounds [aloe emodin (**1**), emodin (**2**), diosmetin (**9**) and kaempferol (**10**)] were isolated from the leaves. Five compounds [ω-hydroxyemodin (**3**), physcion (**5**), ziganein (**6**), apigenin (**7**) and *trans*-resveratrol (**13**)] were isolated from the roots and four compounds [lunatin (**4**), 7,4′-dihydroxy-5-methoxyflavone (**8**), luteolin (**11**), and *trans*-dihydrokaempferol (**12**)] were isolated from the twigs. Nine of the compounds (**3-9** and **12-13**) were isolated and reported for the first time as metabolites of *C. alata*. The crude extracts of *C. alata* (LA, RA and TA) were tested for antibacterial activity against Gram-positive (*B. cereus* TISTR 687, methicillin resistant *S. aureus* (MRSA)-SK1, *S. aureus* TISTR 1466) and Gram-negative (*E. coli* TISTR 780, *Ps. aeruginosa* TISTR 781, *S. typhimurium* TISTR 292) by Broth Microdilution Method (CLSI, 2002). Crude RA exhibited strong antibacterial activity against *B. cereus* with a MIC value of 40 µg/mL. The crude LA and TA showed moderate antibacterial activity against Gram-positive in the range of MICs 160-320 µg/mL. All crude extracts (LA, RA, and TA) showed weak antibacterial activity against Gram-negative bacteria with MIC values of 640-1280 µg/mL (Table 1). Some of the pure compounds were evaluated for their antibacterial activity. Emodin (**2**) showed strong antibacterial activity against MRSA with MIC value of 4 µg/mL and moderate antibacterial activity against *B. cereus* and *S. aureus* MIC value of 16 µg/mL, whereas compounds **1**, **3**, **5**, **6**, **7**, **10**, **11**, and **13** showed weak antibacterial activity against Gram-positive bacteria with a MIC range of 64-128 µg/mL. This strong antibacterial activity of emodin (**2**) against MRSA was supported by antimicrobial activity of emodin isolated from *Rheum palmatum* L., and the compound showed antimicrobial activity against 17 different strains of MRSA with

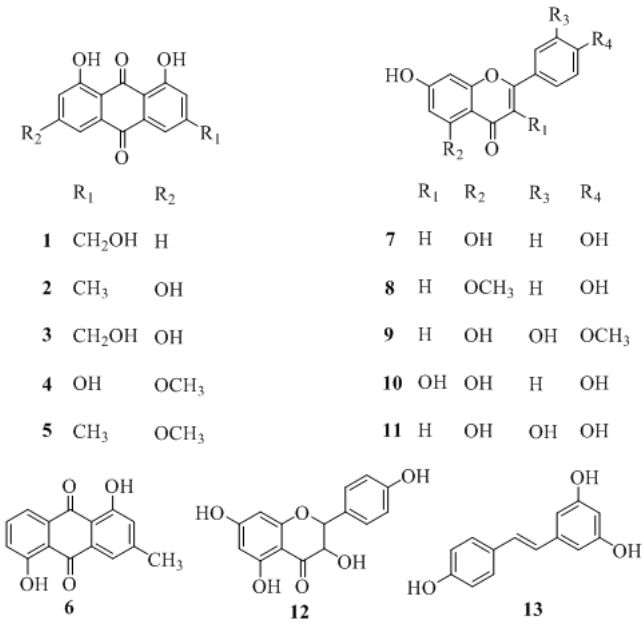

Figure 1. Structure of compounds (**1-13**)

Table 1. Antibacterial activity of crude extracts and isolated compounds from *C. alata*

Sample	MICs (µg/mL)					
	Gram-positive			Gram-negative		
	B.C	MRSA	S.A	E.C	Ps.A	S.T
LA	160	320	160	640	1280	1280
RA	40	160	160	640	1280	1280
TA	160	320	320	640	1280	1280
2	16	4	16	-	128	128
3	128	128	128	128	128	128
5	128	-	-	-	128	128
6	128	-	-	-	64	128
7	64	64	128	64	128	128
10	128	128	128	128	128	128
11	128	64	128	128	128	128
13	64	64	-	128	64	128
Gentamicin	-	-	-	0.5	1	0.5
Vancomycin	0.5	0.5	0.5	-	-	-

the minimum inhibitory concentrations (MICs) in the range of 1.5-25 μg/mL (Lee *et al*., 2010). All isolated compounds showed weak antibacterial activity against Gram-negative bacteria (MICs 64-128 μg/mL) as shown in Table 1.

Antioxidation activity of crude LA, RA, and TA extracts was tested at a final concentration of 100 μg/mL (Table 2). Crudes TA and RA showed moderate antioxidative activity with IC_{50} values of 26.23 and 29.51 μg/mL, respectively, whereas crude LA showed antioxidation activity with an IC_{50} value of 41.80 μg/mL. Compounds **1**, **2**, **3**, **5**, **6**, **7**, **8**, **10**, and **13** were tested for screening scavenging assay whereas the other compounds **4**, **9**, **11**, and **12** were not tested. The activity was monitored by following the decrease of absorbance of the solution at 517 nm for 30 min. Compounds **10** and 13 showed % inhibition more than 50%; both compounds were further tested for 50% inhibition concentration (IC_{50}). Kaempferol (**10**) showed antioxidative activity with IC_{50} 9.67 μM that was three times stronger than that of ascorbic acid with IC_{50} 25.41 μM and five times stronger than that of BHT with IC_{50} 46.56 μM (Table 3). Kaempferol (**10**) has been reported antioxidant activity with ED_{50} 9.99 μM that was six times stronger than that of BHT with ED_{50} 57.41 μM (Panichayupakaranant and Kaewsuwan, 2004). *trans*-Resveratrol (**13**) showed moderate antioxidative activity (IC_{50} 45.90 μM) which was almost effective than BHT (IC_{50} 46.56 μM) as shown in Table 3.

Table 2. Antioxidation activity of crude extracts of *C. alata* at final concentration of 100 μg/mL

Sample	IC_{50} (μg/mL)
Ascorbic acid	2.79
BHT	12.30
TA	26.23
RA	29.51
LA	41.80

Table 3. Antioxidation activity of isolated compounds from *C. alata* at final concentration of 50 μM

Sample	IC_{50} (μM)
10	9.67
Ascorbic acid	25.41
13	45.90
BHT	46.56

4. Conclusion

Phytochemical investigation of the *C. alata* has led to the isolation and identification of thirteen compounds: six anthraquinones (**1-6**), six flavonoids (**7-12**) and one stilbene (**13**). Moreover, nine compounds (**3-9** and **12-13**) were reported for the first instance as constituents of *C. alata*. Emodin (**2**) exhibited strong antibacterial activity against methicillin resistant *S. aureus* with MIC value of 4 μg/mL. In addition, kaempferol (**10**) showed antioxidative activity (IC_{50} 9.67 μM) that was three times stronger than that of ascorbic acid (IC_{50} 25.41 μM). *trans*-Resveratrol (**13**) showed moderate activity (IC_{50} 45.90 μM), which was almost better than BHT (IC_{50} 46.56 μM).

Acknowledgements

The authors are grateful to the Mae Fah Luang University for financial support. We also thank the Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand, for providing microorganism cultures and Mr. James Maxwell for plant identification.

References

Ahn, D., Lee, S. I., Yang, J. H., Cho, C. H., Hwang, Y.-H., Park, J.-H. and Kim, D. K. 2011. Superoxide radical scavengers from the whole plant of *Veronica peregrina*. Natural Product Sciences. 17, 142-146.

Chu, X., Sun, A. and Liu, R. 2005. Preparative isolation and purication of five compounds from the Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc by high-speed counter-current chromatography. Journal of Chromatography A. 1097, 33-39.

Clinical and Laboratory Standards Institute. 2002. Reference method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. approved standard M7-A7. Wayne, PA, USA.

Corner, E. J. H. 1952. Wayside Trees of Malaya, Vol. 1, Second Edition. Singapore: Government Printing Office.

Deachathai, S., Mahabusarakam, W., Phongpaichit, S., Taylor, W. C., Zhang, Y.-J. and Yang, C.-R. 2006. Phenolic compounds from the owers of *Garcinia dulcis*. Phytochemistry. 67, 464-469.

Fernand, V. E., Dinh, D. T., Washington, S. J., Fakayode, S. O., Losso, J. N., Ravenswaay, R. O. V. and Warner, I. M. 2008. Determination of pharmacologically active compounds in root extracts of *Cassia alata* L. by use of high performance liquid chromatography. Talanta. 74, 896-902.

Fujimoto, H., Nakamura, E., Okuyama, E. and Ishibashi, M. 2004. Six immunosuppressive features from an ascomycete, *Zopfiella longicaudata*, found in a screening study monitored by immunomodulatory activity. Chemical Pharmaceutical Bulletin. 52, 1005-1008.

Gurib-Fakim, A. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine. 27, 1-93.

Hemlata and Kalidhar, S. B. 1993. Alatinone, an anthraquinone from *Cassia alata*. Phytochemistry. 32, 1616-1617.

Ibrahim, D. and Osman, H. 1995. Antimicrobial activity of *Cassia alata* from Malaysia. Journal of Ethnopharmacology. 45, 151-156.

Idu, M., Omonigho, S. E. and Igeleke, C. L. 2007. Preliminary investigation on the phytochemistry and antimicrobial activity of *Senna alata* L. flower. Pakistan Journal of Biological Sciences. 10, 806-809.

Irwin, H. S. and Barneby, R. C. 1982. The American Cassiinae: a synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the new world. York Botanical Garden. 35, 918.

Jadulco, R., Brauers, G., Edrada, R. A., Ebel, R., and Wray, V., Sudarsono and Proksch, P. 2002. New metabolites from sponge-derived fungi *Curvularia lunata* and *Cladosporium herbarum*. Journal of Natural Products. 65, 730-733.

Jeong, G.-S., Lee, S.-H., Jeong, S.-N., Kim, Y.-C. and Kim, E.-C. 2009. Anti-inammatory effects of apigenin on nicotine- and lipopolysaccharide-stimulated human periodontal ligament cells via heme oxygenase-1. International Immunopharmacology. 9, 1374-1380.

Kametani, S., Yuasa-Kojima, A., Kikuzaki, 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 & Biochemistry. 71, 1220-1229.

Khan, M. R., Kihara, M. and Omoloso. A. D. 2001, Antimicrobial activity of *Cassia alata*. Fitoterapia. 72, 561-564.

Lee, D.-Y., Lyu, H.-N., Kwak, H. Y., Jung, L., Lee, Y.-H., Kim, D.-K., Chung, I.-S., Kim, S.-H. and Baek, N.-I. 2007. Isolation of flavonoids from the fruits of *Cornus kousa* Burg. Journal of Applied Biological Chemistry. 50, 144-147.

Lee, Y.-S., Kang, O.-H., Choi, J.-G., Oh, Y.-C., Keum, J.-H., Kim, S.-B., Jeong, G.-Sa., Kim, Y.-C., Shin, D.-W. and Kwon, D.-Y. 2010. Synergistic effect of emodin in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. Pharmaceutical Biology. 48, 1285-1290.

Lim, C.-H. 1999. Monocerin and ziganein: phytotoxins from pathogenic fungus *Exserohilum monoceras* Inu-1. Agricultural Chemistry and Biotechnology. 42, 45-47.

Mbouangouere, R. N., Tane, P., Ngamga, D., Djemgou, P., Choudhary, M. I. and Ngadjui B. T. 2007. Piptaderol from *Piptadenia africana*. African Journal of Traditional, Complementary and Alternative Medicine. 4, 294-298.

Miralles, J. & Gaydou, E. M. 1986. Fatty acid and sterol composition of oils extracted from seed of three *Cassia* (Caesalpiniaceae) species from Senegal. Revue Francaise des Corps Gras. 33, 381-384.

Miyazawa, M. and Hisama, M. 2003. Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. Bioscience, Biotechnology & Biochemistry. 67, 2091-2099.

Palanichamy, S. and Nagarajan, S. 1990. Antifungal activity of *Cassia alata* leaf extract. Journal of Ethnopharmacology. 29, 337-340.

Panichayupakaranant, P and Kaewsuwan, S. 2004. Bioassay-guided isolation of the antioxidant constituent from *Cassia alata* L. leaves. Songklanakarin Journal of Science and Technology. 26, 103-107.