Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(3):256-260
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Chemical constituents of *Syzygium samarangense*

Consolacion Y. Ragasa[1,2*], Francisco C. Franco Jr.[2], Dennis D. Raga[3] and Chien-Chang Shen[4]

[1]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines*
[2]*Chemistry Department and Center for Natural Sciences and Ecological Research, De La Salle University, 2401 Taft Avenue, Manila, Philippines*
[3]*Biology Department and Center for Natural Sciences and Ecological Research, De La Salle University-Manila*
[4]*National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec 2, Taipei, Taiwan*

ABSTRACT

*The dichloromethane extract of the leaves of Syzygium samarangense (Blume) Merr. & Perry afforded 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (**1**), 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**), 2′-hydroxy-4′,6′-dimethoxy-3′-methyl chalcone (**3**), squalene (**4**), betulin (**5**), lupeol (**6**), sitosterol (**7**), and a mixture of cycloartenyl stearate (**8a**), lupenyl stearate (**8b**), β-sitosteryl stearate (**8c**), and 24-methylenecycloartenyl stearate (**8d**). The structures of **1**-**3**, **5**, **7**, and **8a**-**8d** were elucidated by 1D and 2D NMR spectroscopy. Sample **8** was tested for hypoglycemic and antimicrobial potentials. It showed negative hypoglycemic potential and exhibited moderate antifungal activity against C. albicans, low activity against T. mentagrophytes and low antibacterial activity against E. coli, S. aureus and P. aeruginosa. It was inactive against B. subtilis and A. niger.*

Keywords: *Syzygium samarangense*, Myrtaceae, cycloartenyl stearate, lupenyl stearate, β -sitosteryl stearate, 24-methylenecycloartenyl stearate

---

INTRODUCTION

Syzygium samarangense (syn. *Eugenia javanica* Linn.) commonly known as 'makopa' is grown throughout the Philippines for its fruits. The tree is used as an antipyretic and a diuretic [1]. Four flavonoids isolated from the hexane extract of *S. samarangense* showed dose-dependent spasmolytic activity [2]. Another study reported that 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone from *S. samarangense* exhibited significant differential cytotoxicity against the MCF-7 cell line and significant selective cytotoxicity against RAD 52 yeast mutant strain [3]. Compounds isolated from the hexane extract of the leaves of *S. samarangense*: 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone, 2′,4′-dihydroxy-6′-methoxy-3′-methyl dihydrochalcone, 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone, α- and β-carotene, lupeol, betulin, epi-betulinic acid, and β-D-sitosterylglucoside exhibited significant and selective inhibition against prolyl endopeptidase [4]. An earlier study reported that the methanol extract of makopa leaves exhibited high antidiabetic activity [5], while a recent study reported that 2′,4′-di hydroxy-6′-methoxy-3′,5′-dimethylchalcone and 5-*O*-methyl-4′-desmethoxy matteucinol from *S. samarangense* significantly lowered the blood glucose levels in hyperglycaemic mice when administered 15 minutes after glucose load, while 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone significantly lowered the blood glucose levels of alloxan diabetic mice [6]. Recently, we reported the potent analgesic and anti-inflammatory activities and the negligible toxicity on zebrafish embryonic tissues of a mixture of cycloartenyl stearate (**8a**), lupenyl stearate (**8b**), sitosteryl stearate (**8c**), and 24-methylenecycloartenyl stearate (**8d**) from the dichloromethane extract of the air-dried leaves of *S. samarangense* [7].

We report herein the isolation and identification of 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (**1**), 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**), 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (**3**), squalene (**4**), betulin (**5**), lupeol (**6**), sitosterol (**7**), and a mixture of cycloartenyl stearate (**8a**), lupenyl stearate (**8b**), β-sitosteryl stearate (**8c**), and 24-methylenecycloartenyl stearate (**8d**) (Fig.1) from the air-dried leaves of *S. samarangense*. To the best of our knowledge this is the first report on the isolation of **8a**-**8d** from the tree. Results of the hypoglycemic and antimicrobial tests on a mixture of **8a**-**8d** are likewise reported.

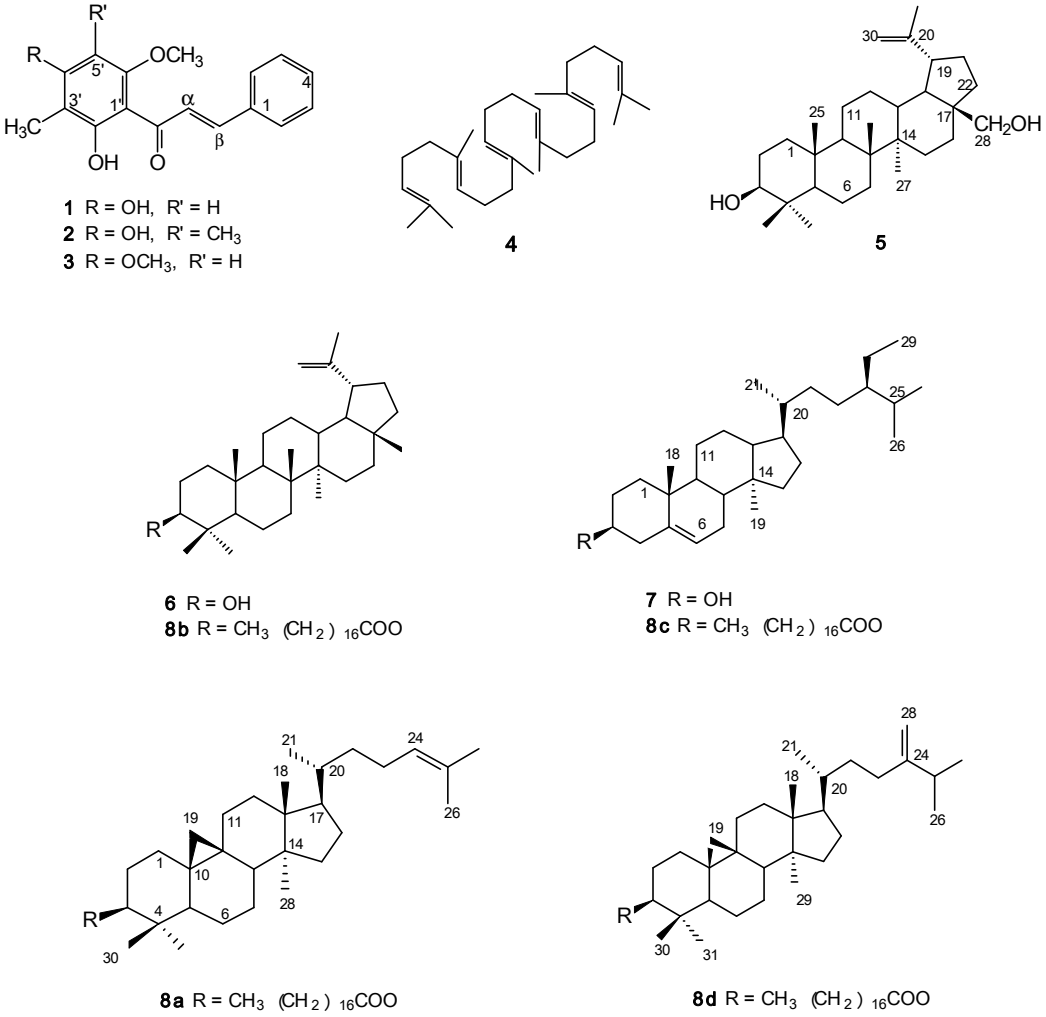

Figure 1: The compounds from *S. samarangense*: 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (1), 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (2), 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (3), squalene (4), betulin (5), lupeol (6), sitosterol (7), cycloartenyl stearate (8a), lupenyl stearate (8b), β-sitosteryl stearate (8c), and 24-methylenecycloartenyl stearate (8d)

MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. 2D NMR (COSY, HSQC, HMBC) spectra were recorded on a Varian VNMRS spectrometer. MS was obtained on a Finnigan MAT LCQ mass spectrometer. Column chromatography was performed with silica gel 60 (70-230 mesh); TLC was performed with plastic backed plates coated with silica gel F_{254} ; plates were visualized by spraying with vanillin sulfuric acid, followed by warming.

Sample Collect

Fresh leaves of *Syzygium samarangense* (5 kg) were collected from Antipolo City in December 2008. Specimens of the sample were authenticated at the Institute of Biology, University of the Philippines, Diliman, Quezon City. A voucher specimen # 140 was deposited at the Chemistry Department, De La Salle University-Manila.

Isolation

The air-dried leaves of *Syzygium samarangense* (1 kg) were ground in an osterizer, soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (45.86 g) which was chromatographed in increasing volumes of acetone in CH_2Cl_2 at 10 % increment. The CH_2Cl_2 and 10 % acetone in CH_2Cl_2 fractions were combined and rechromatographed in petroleum ether. The less polar fractions afforded **4** (55 mg). The more polar fractions were rechromatographed in increasing percentage of EtOAc in petroleum ether (0.5%, 1%, 2.5% and 5% by volume) to afford sample **8** which is a mixture of **8a**-**8d** (1g). The 20% and 30% acetone in CH_2Cl_2 fractions were combined and rechromatographed (5 ×) in CH_2Cl_2 to afford **6** (25 mg) and **7** (35 mg). The 40% and 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8) by volume ratio. The less polar fractions were rechromatographed (4 ×) in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9) by volume ratio to afford **3** (18 mg) and **5** (8 mg). The more polar fractions were rechromatographed (6 ×) in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8) by volume ratio to afford **1** (24 mg) and **2** (32 mg).

Antimicrobial Test

The microorganisms used were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* (UPCC 1244), *Bacillus subtilis* (UPCC 1149), *Escherichia coli* (UPCC 1195), *Staphylococcus aureus* (UPCC 1143), *Candida albicans* (UPCC 2168), *Trichophyton mentagrophytes* (UPCC 4193) and *Aspergillus niger* (UPCC 3701). Sample **8** was tested for antimicrobial activity against these microorganisms using the procedure reported in the literature [8].

Experimental Animals

A total of 50 male albino mice (*Mus musculus* L.) of an inbred ICR strain (7 weeks old) weighing 19.0 ±2.0 g were acclimatized for 7 days prior to conducting the bioassay. The animals (n = 9) were procured from the Food and Drugs Authority, Muntinlupa City, Philippines and housed at the animal containment unit of DLSU-Manila with 12h daylight and 12h darkness with free access to food pellets and water. A 16h fasting period was carried out prior to each treatment procedure [9]. All animal handling procedures were in accordance with the existing policies and guidelines of the Philippine Association of Laboratory Animal Science (PALAS) for care and use of laboratory animals and with Administrative Order 40 of the Bureau of Animal Industry relative to the Rep. Act. No.8485.

Hypoglycemic Test

The anti-diabetes assay was performed modified from the procedure [9]. Oral Glucose Tolerance Test (5 g/kg BW) was performed on normoglycemic mice, followed by measurement of blood glucose level (mg/dL) using OneTouch Horizon Glucometer (Lifescan, Johnson & Johnson, USA). Polysorbate 80 (25 mg/kg BW, Tween-80, AJAX, Finechem Pty. Ltd., Australia) as the negative control for sample **8** (**8a**-**8d**). Solosa (16.7 µg/kg BW, Glimepiride solosa, Aventis, Italy) dissolved in distilled H_2O was orally administered as the positive control, while sample 8 (100 mg/kg BW, 50 mg/kg BW, and 25 mg/kg BW) dissolved in Polysorbate 80 were given as the test compounds. Blood glucose was measured within a 3h period at 30 minutes intervals. Blood glucose reduction was computed as percent reduction ([initial blood glucose – final blood glucose] / initial x 100) and was used in the statistical analysis.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried leaves of *S. samarangense* afforded 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone (**1**), 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**2**), 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (**3**), squalene (**4**), betulin (**5**), lupeol (**6**), sitosterol (**7**), and a mixture of cycloartenyl stearate (**8a**), lupenyl stearate (**8b**), β-sitosteryl stearate (**8c**), and 24-methylenecycloartenyl stearate (**8d**).

The structures of **1**, **2**, **3**, **5** and **7** were elucidated by extensive 1D and 2D NMR analyses and confirmed by comparison of their ^{1}H and ^{13}C NMR data with those of 2′,4′-dihydroxy-6′-methoxy-3′-methylchalcone [10], 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone [11], 2′-hydroxy-4′,6′-dimethoxy-3′-methylchalcone (aurentiacin) [12], betulin [13] and sitosterol [14], respectively. Compound **4** was identified by comparison of its ^{1}H NMR data with those of squalene [15]. The structure of **6** was deduced by comparison of its ^{13}C NMR data with those of lupeol [13].

The structures of **8a**-**8d** were elucidated by extensive 1D and 2D NMR spectroscopy. The resonances attributed to the major compound, **8a** suggested a cycloartenol esterified to a fatty acid. Confirmatory evidences are the ^{13}C NMR data of **8a** and cycloartenyl acetate [16] for the triterpene part and the fatty acid ester of 16-hydroxycycloartenyl palmitate [17] for the fatty acid part, which match in all essential respects. The fatty acid chain length was determined by the mass spectrum of sample **8** which gave a molecular ion at *m/z* = 694.2 corresponding to the molecular formula of $C_{48}H_{86}O_2$ and an [M^+-$C_{18}H_{35}O_2$] of *m/z* 409 which resulted from the loss of stearic acid

from the molecular ion peak. The resonances assigned to **8b** indicated that it is lupenyl stearate [18], while **8c** is β-sitosteryl stearate [19]. On the other hand, **8d** is 24-methylenecycloratenyl stearate as confirmed by similar ^{13}C NMR data with 24-methylenecycloartenyl acetate [20] for the triterpene part and the fatty acid ester of 16-hydroxycycloartenyl palmitate [17] for the fatty acid part.

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, sample **8** was tested for possible antimicrobial activities by the agar well method. Results of the study (Table 1) indicated that sample **8** is moderately active against the fungus, *C. albicans* with an activity index (AI = 0.3), slightly active against the fungus, *T. mentagrophytes* (AI = 0.3), slightly active against the bacteria: *E. coli* (AI = 0.1), *P. aeruginosa* (0.3) and *S. aureus* (AI = 0.1). It was inactive against *B. subtilis* and *A. niger.*

Table 1. Antimicrobial Test Results on Sample 8

Organism	Sample (30µg)	Clearing Zone (mm)[a]	Activity Index (AI)
E. coli	Sample **8**	11	0.1
	Chloramphenicol[b]	23	2.8
P. aeruginosa	Sample **8**	12	0.2
	Chloramphenicol[b]	14	1.3
S. aureus	Sample **8**	12	0.2
	Chloramphenicol[b]	25	3.2
B. subtilis	Sample **8**	-[d]	0
	Chloramphenicol[b]	20	2.3
C. albicans	Sample **8**	13	0.3
	Canesten, 0.2g[c]	18	0.8
T. mentagrophytes	Sample **8**	12	0.2
	Canesten, 0.2g[c]	55	4.5
A. niger	Sample **8**	-	0
	Canesten, 0.2g[c]	23	1.3

[a] Average of three trials; [b]Chloramphenicol disc - 6 mm diameter;
[c]Contains 1% chlotrimazile; [d]No clearing zone

An earlier study reported that the methanol extract of the leaves of makopa exhibited high antidiabetes activity [5]. Another study reported that the chalcones (**1-3**) isolated from *S. samarangense* have been tested for hypoglycemic activity where **2** significantly lowered the blood glucose levels of alloxan diabetic mice [6]. Since **8a-8d** were obtained for the first time from *S. samarangense* and the leaves are known to have antidiabetes property, sample **8** was tested for hypoglycemic potential.

Glucose challenged mice were given three doses of sample **8** (25 mg/kg BW, 50 mg/kg BW, 100 mg/kg BW), Solosa® or P80 as experimental, positive and negative controls, respectively. Blood glucose was measured 30 minutes after oral gavage and after every 30 minutes for 3h. Percent blood glucose reduction was found highest in mice administered with 50 mg/kg BW sample **8** at 0.5h (Table 2). This observed reduction however is statistically similar with the negative control and 100 mg/kg BW. This implies that there was minimal blood glucose reduction as affected by the treatment. The effect however was very minimal that it is not possible to statistically identify it from the effects of the negative control. Glimipiride Solosa on the other hand was found to have its effects at 1.0h similar to our previous reports [21]. Although the observed blood glucose reduction at 0.5h revealed significant differences ($P<0.05$) between means, such percent reduction cannot be accounted to the effect of sample **8** in all dosages of the treatment groups but rather to the net effects of insulin. The results however revealed no hypoglycemic potential of sample **8**.

Table 2. Percent blood glucose reduction in mice administered with sample 8 across a 3h observation period

Group	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h
Control (P80)	62.07±2.91 [ab]	27.78±5.94 [b]	17.40±5.36	-4.91±4.99	4.22±2.37
Glymipiride Solosa	32.96±3.72 [c]	59.24±3.40 [a]	19.81±3.44	-11.28±4.7	0.15±4.57
25 mg/Kg BW sample **8**	53.60±4.54 [b]	26.01±4.10 [b]	15.30±2.54	0.31±4.83	-0.15±4.39
50 mg/Kg BW sample **8**	64.67±2.76[a]	33.63±4.39 [b]	10.13±2.86	-16.89±3.91	10.16±3.24
100 mg/Kg BW sample **8**	63.05±2.81 [ab]	21.80±6.09 [b]	6.87±4.38	-7.93±2.47	6.57±2.62

**means followed by the same letter are not significantly different at α=0.05 DMRT.*

Statistical Analysis

The results were analyzed using SPSS ver. 10.5 for windows. One way Analysis of Variance was performed to determine the significant effects on anti-diabetic potentials of sample 8. Significant differences within group

variables were determined by post hoc analysis at 95% DMRT. Results were considered significant at $\alpha = 0.05$. The data was presented as Mean±SD.

Acknowledgment
The antimicrobial tests were conducted at the University of the Philippines-Natural Sciences Research Institute (UP-NSRI). A research grant from the De La Salle University Science Foundation through the University Research Coordination Office is gratefully acknowledged.

REFERENCES

[1] Y. Kuo, L. Yang, L. Lin, *Planta Med.*, **2004**, 70, 1237-1239.
[2] M. N. Ghayur, A. H. Gilani, A. Khan, I. M. Villasenor, M. I. Choudhary, *Phytother. Res.*, **2006**, 20(1). 49-52.
[3] E. Amor, I. M. Villasenor, R. Antemano, Z. Perveen; G. P. Concepcion, M. I. Choudhary, *Pharm. Biol.*, **2007**, 45(10), 777-783.
[4] E. Amor, I. M. Villasenor, A. Yasin, M. Choudhary, *Z. Naturforsch.* **2004**, 59c, 86-92.
[5] I. M. Villasenor, M. A. Cabrera, K. B. Meneses, V. R. R. Rivera, R. M. Villasenor, *Philipp. J. Sci.*, **1998**, 127(4), 261-266.
[6] M. H. C. Resurreccion-Magno, I. M. Villasenor, N. Harada, K. Monde, *Phytother. Res.*, **2005**, 19(3), 246-251.
[7] D. D. Raga, C. L. C. Cheng, K. C. I. C. Lee, W. Z. P. Olaziman, V. J. A. De Guzman, C.-C. Shen, F. C. Franco, C. Y. Ragasa, *Z. Naturforsch. C.* **2011**, 66c, 235-244.
[8] B. Q. Guevara, B. V. Recio, *Acta Manilana Suppl.*, **1985**, 45-50.
[9] B. Meddah, R. Ducroc, M. E. A. Faouzi, B. Eto, L. Mahraoui, A. Benhaddou-Andaloussi, L. C. Martineau, Y. Cherrah, P. S. Haddad, *J. Ethnopharmacol.*, **2009**, 121, 419-424.
[10] Y. Kuo, L. Yang, L. Lin, *Planta Med.*, **2004**, 70, 1237-1239.
[11] M. M. Salem, K. A. Werbovertz, *J. Nat. Prod.*, **2005**, 68, 108-111.
[12] G. Solladie, N. Gehrold, J. Maignan, *Eur. J. Org. Chem.*, **1999**, 9, 2309-2314.
[13] M. Sholichin, K. Yamasaki, R. Kasai, O. Tanaka, *Chem. Pharm. Bull.*, **2004**, 28(3), 1006-1008.
[14] J. M. Cayme, C. Y. Ragasa, *Kimika*, **2004**, 20(1-2), 5-12.
[15] V. M. L. Inte, C. Y. Ragasa, J. A. Rideout, *Asia Life Sci.*, **1998**, 7(1), 11-21.
[16] J. De Pascual Teresa, J. G. Urones, I. S. Marcos, P. Basabe, M. J. Sexmero Cuadrado, R. Fernandez Moro, *Phytochem.*, **1987**, 26(6), 1767-1776.
[17] C. Y. Ragasa, F. Tiu, J. Rideout, *Nat. Prod. Res.*, **2004**, 18(4), 319-323.
[18] X. K. Liu, Z. R. Li, M. H. Qui, R. L. Nie, *Acta Botanica Yunnanica*, **1998**, 20(3), 369-373.
[19] V. Parmar, S. C. Jain, S. Gupta, S. Talwar, V. K. Rajwanshi, R. Kumar, A. Azim, S. Malhotra, N. Kumar, R. Jain, N. K. Sharma, O. M. Tyagi, S. J. Lawrie, W. Errington, O. W. Howarth, C. E. Olsen, S. K. Singh, J. Wengel, *Phytochem.*, **1998**, 49(4), 1069-1078.
[20] C. Y. Ragasa, H. Ngo, J. A. Rideout, *J. Asian Nat. Prod. Res.*, **1998**, 7(1), 7-12.
[21] C. Y. Ragasa, A. B. Alimboyoguen, S. Urban, D. D. Raga, *Nat. Prod. Commun.*, **2008**, 3(3), 1663-1666.