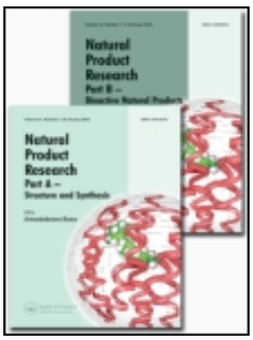


Natural Product Research

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Dihydrochalcones with radical scavenging properties from the leaves of Syzygium jambos

U. L. B. Jayasinghe , R. M. S. Ratnayake , M. M. W. S. Medawala & Y. Fujimoto

To cite this article: U. L. B. Jayasinghe , R. M. S. Ratnayake , M. M. W. S. Medawala & Y. Fujimoto (2007) Dihydrochalcones with radical scavenging properties from the leaves of Syzygium jambos , Natural Product Research, 21:6, 551-554, DOI: 10.1080/14786410601132238

To link to this article: http://dx.doi.org/10.1080/14786410601132238

Published online: 11 May 2007.

Natural Product Research, Vol. 21, No. 6, 20 May 2007, 551–554

Dihydrochalcones with radical scavenging properties from the leaves of *Syzygium jambos*

U. L. B. JAYASINGHE*†, R. M. S. RATNAYAKE†,
M. M. W. S. MEDAWALA† and Y. FUJIMOTO‡

†Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka
‡Department of Chemistry and Materials Science,
Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan

(*Received 2 November 2005; in final form 10 August 2006*)

Chemical investigation of the dichloromethane extract of the leaves of *Syzygium jambos* furnished three dihydrochalcones, phloretin 4′-O-methyl ether (2′,6′-dihydroxy-4′-methoxydihydrochalcone) (**1**), myrigalone G (2′,6′-dihydroxy-4′-methoxy-3′-methyldihydrochalcone) (**2**), and myrigalone B (2′,6′-dihydroxy-4′-methoxy-3,5′-dimethyldihydrochalcone) (**3**) with radical scavenging properties towards the DPPH radical by spectrophotometric method.

Keywords: *Syzygium jambos*; Myrtaceae; Dihydrochalcone; Antioxidant activity; DPPH

1. Introduction

During past two decades scientists have paid much attention to the research on free radical science. Alzheimer's disease, rheumatoid arthritis, cardiovascular disease, cataracts, diabetes, hypertension, and aging itself, may be caused by a phenomenon known as oxidative or free radical damage. Antioxidants are substances, which can prevent, stop, or reduce oxidative damage. It is well known that a diet rich in fruits and vegetables significantly reduces the incidence and mortality rates of cardiovascular diseases and certain cancers in humans [1]. Hence it is very important to search for human friendly antioxidants. For this purpose compounds of plant origin can play a very prominent role.

In a continuation of our studies towards the search for biologically active compounds from Sri Lankan plants the present study was carried out on the leaves of *Syzygium jambos* (L.) Alston of the family Myrtaceae. *Syzygium jambos* is a tree of moderate size, cultivated in the home gardens of Sri Lanka for its delicious fruit, as well as an ornamental plant. Several phenolic compounds have been reported from the various parts of the plant [2–4]. We have noted the presence of antioxidant active compounds in the dichloromethane extract of this plant. In this article we report the isolation and

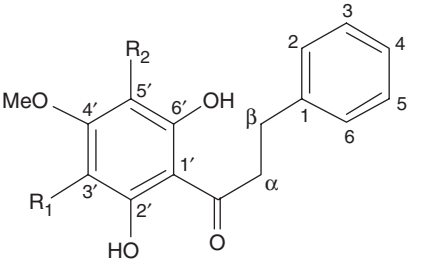
*Corresponding author. Email: ulbj2003@yahoo.com

Natural Product Research
ISSN 1478-6419 print/ISSN 1029-2349 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/14786410601132238

structure elucidation of three dihydrochalcone from the leaves of *S. jambos* and their antioxidant activity against 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical.

2. Results and discussion

The leaves of *S. jambos* were extracted with dichloromethane. Preliminary investigation indicated the presence of compounds with DPPH radical scavenging properties in the dichloromethane extract by thin layer chromatography (TLC) bioautography method [5]. Further the dichloromethane and methanol extracts did not show antifungal activity against *Cladosporium cladosporioides* by TLC bioautography method [6].



1: R_1, R_2=H
2: R_1=Me, R_2=H
3: R_1, R_2=Me

Chromatographic separation of the dichloromethane extract by a combination of chromatographies afforded three compounds. Detailed analysis of ^{1}H NMR, ^{13}C NMR, HMQC, HMBC, FABMS(−) spectral data unambiguously established the structures of **1**–**3** as phloretin 4′-O-methyl ether (2′,6′-dihydroxy-4′-methoxydihydrochalcone) (**1**) [7], myrigalone G (2′,6′-dihydroxy-4′-methoxy-3′-methyldihydrochalcone) (**2**) [8], and myrigalone B (2′,6′-dihydroxy-4′-methoxy-3′,5′-dimethyldihydrochalcone) (**3**) [9]. None of these compounds have been reported from *Syzygium* species, although isolation of a related chalcone, 4′,6′-dihydroxy-2′-methoxy-3′,5′-dimethylchalcone, has been reported from *S. samarangense* [10]. Antioxidant activity of compounds **1**, **2**, and **3** were determined by a TLC bioautography method as well as spectrophotometry method [11]. In this assay antioxidant reacts with the free radical 2,2′-phenyl-1-picrylhydrazyl. α-Tocopherol was used as the positive control. Compound **3** showed antioxidant activity higher than that of α-tocopherol by spectrophotometry method (table 1). Antioxidant activity of dihydrochalcones including compound **1** [11] and compound **3** [12] has been reported [13]. Antifungal and antibacterial activities of compounds **2** and **3** have been reported previously [14].

3. Experimental

Melting points were determined by Gallenkamp apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-160 A spectrometer. ^{1}H NMR and ^{13}C NMR

spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ or CD_3OD solution. Tetramethylsilane or d_4-methanol ($\delta = 3.30$) signals was used as an internal standard for ^{1}H shifts, and $CDCl_3$ ($\delta = 77.0$) or CD_3OD ($\delta = 49.0$) signal was used as a reference for ^{13}C shifts. EIMS (70 eV) and FABMS were obtained on a JEOL JMS-AX700 spectrometer. HPLC analyses were carried out on Shimadzu LC-6A apparatus equipped with UV detector using a reverse phase C_{18} column under isocratic solvent condition.

3.1. *Plant material*

Leaves of the *S. jambos* were collected from the North Central Province of Sri Lanka in January, 2004. A voucher specimen is deposited at the Institute of Fundamental Studies, Kandy, Sri Lanka.

3.2. *Extraction and isolation*

The dried leaves of *S. jambos* (65 g) were extracted with dichloromethane. Evaporation of dichloromethane gave a dark green colored solid (2.1 g). A portion of the dichloromethane extract (2 g) was fractionated over a column of silica gel with *n*-hexane-ethylacetate-methanol gave nine major fractions (F_1–F_9). TLC indicated the presence of UV active ($\lambda = 254$ nm) and antioxidant active compounds only in fraction no. F_2, F_3, and F_5. These fractions were combined (800 mg) and further purified by a combination of chromatographies over column of silica gel, Sephadex LH-20, reverse phase silica gel, and reverse phase HPLC (STR Prep ODS 20×250 mm column, 5 mL min^{-1}, 20% H_2O–MeOH, UV detection 254 nm) to give compounds **1** (30 mg), **2** (48 mg), **3** (75 mg).

Phloretin 4′-O-methyl ether (1). Amorphous; ^{1}H NMR (400 MHz, CD_3OD) δ: 2.84 (2H, t, $J = 9.9$ Hz, H-β), 3.23 (2H, t, $J = 9.9$ Hz, H-α), 3.65 (3H, s, –OCH_3 at C-4′), 5.82 (2H, s, H-3′, H-5′), 7.04–7.16 (5H, m, H-2 to H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.0 (C-β), 47.0 (C-α), 55.8 (–OCH_3 at C-4′), 94.3 (C-3′, C-5′), 106.0 (C-1′), 126.9 (C-4), 129.3 (C-2, C-6), 129.4 (C-3, C-5), 143.1 (C-1), 165.6 (C-2′, C-6′), 167.5 (C-4′), 206.4 (C=O); FABMS(−) m/z:271$[M-H]^-$.

Myrigalone G (2). Amorphous; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.91 (3H, s, –CH_3 at C-3′), 2.94 (2H, t, $J = 7.8$ Hz, H-β), 3.34 (2H, t, $J = 7.8$Hz, H-α), 3.80 (3H, s, –OCH_3 at C-4′), 6.0 (1H, s, H-5′), 7.12–7.25 (5H, m, H-2 to H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 7.25 (–CH_3 at C-3′), 32.2 (C-β), 47.1 (C-α), 55.9 (–OCH_3 at C-4′), 91.0 (C-5′), 104.7 (C-3′), 105.7 (C-1′), 126.8 (C-4), 129.3 (C-2, C-6), 129.4 (C-3, C-5), 143.2 (C-1), 161.9 (C-2′), 163.7 (C-6′), 165.1 (C-4′), 206.6 (C=O); FABMS(−) m/z: 285 $[M-H]^-$.

Myrigalone B (3). Amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.08 (6H, s, –CH_3 at C-3′ & C-5′), 3.01 (2H, t, $J = 7.8$ Hz, H-β), 3.43 (2H, t, $J = 7.5$ Hz, H-α), 3.69 (3H, s, –OCH_3 at C-4′), 7.16–7.28 (5H, m, H-2 to H-6), 9.49 (2H, bs, 2-OH, 6-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 8.1 (–CH_3 at C-3′ & C-5′), 30.5 (C-β), 46.1 (C-α), 60.2 (–OCH_3 at

C-4′), 107.0 (C-1′), 108.6 (C-3′, C-5′), 125.9 (C-4), 128.3 (C-2, C-6), 128.4 (C-3, C-5), 141.5 (C-1), 158.2 (C-2′, C-6′), 162.7 (C-4′), 205.8 (C=O); FABMS(−) m/z: 299 $[M-H]^-$, EIMS m/z: 300$[M]^+$, 283, 281, 195, 168, 152, 149, 123, 104, 91.

3.2.1. Antioxidant activity. The antioxidant activity was evaluated by the DPPH radical scavenging effect. The 0.1 M acetate buffer was prepared by dissolving 1.64 g of CH_3COONa in 16 mL of H_2O and 150 µL of CH_3COOH. The final volume was adjusted to 20 mL by adding H_2O. The 0.2 mM DPPH solution was prepared by dissolving 3.9 mg of DPPH in 50 mL of ethanol. α-Tocopherol (1 mg) in 10 mL of ethanol solution was prepared. A series of test tubes with 1.0 mL of buffer solution was mixed well with 0.5 mL of DPPH solution. A series of various concentrations of compounds **1**–**3** (1–20 µg in 1 mL of ethanol) were added to each tube and mixed well. After 30 min in room temperature, the absorptions of each solution was measured by UV spectrophotometer at 517 nm. A mixture of buffer solution and ethanol was used as the reference for the spectrophotometer. A graph was plotted with the weight of the compound *versus* absorptions and IC_{50} values determined. Results are given in the following table. The antioxidant activity was expressed in terms of IC_{50} ($\mu g\,mL^{-1}$, concentration required to inhibit the DPPH radical formation by 50%). α-Tocopherol was used as a positive control.

Compound No.	IC_{50} ($\mu g\,mL^{-1}$)
1	30
2	10.6
3	3.8
α-Tocopherol	7.3

References

[1] U.M. Acuna, D.E. Atha, J. Ma, M.H. Nee, E.J. Kennelly. *Phytother. Res.*, **16**, 63 (2002).
[2] A.K. Chakravarty, B. Das, T. Sarkar, K. Masuda, K. Shiojima. *Indian J. Chem. B*, **37B**, 1316 (1998).
[3] K. Slowing, E. Carretero, A. Villar. *Phytother. Res.*, **10**(Suppl 1), S126 (1996).
[4] K. Slowing, M. Shollhuber, E. Carretero, A. Villar. *Phytochemistry*, **37**, 255 (1994).
[5] T. Takao, F. Kitatani, N. Watanabe, A. Yagi, K. Sakata. *Biosci. Biotech. Biochem.*, **58**, 1780 (1994).
[6] A.L. Homans, A. Fuchs. *J. Chromatogra.*, **51**, 327 (1970).
[7] J.F. Eykman. *Ber.*, **16**, 2769 (1883).
[8] K.E. Malterud. *Acta. Pharm. Nordica.*, **4**, 65 (1992).
[9] T. Anthonsen, I. Falkenberg, M. Laake, A. Midelfart, T. Mortensen. *Acta Chem. Scand.*, **25**, 1929 (1971).
[10] R. Srivastava, A.K. Shaw, D.K. Kulshreshtha. *Phytochemistry*, **38**, 687 (1995).
[11] C. Masuoka, M. Ono, Y. Ito, T. Nohara. *F. Sci. Tec. Int.*, **3**, 285 (1997).
[12] L. Mathiesen, K.E. Malterud, R.B. Sund. *Free Radical Biol. Med.*, **22**, 307 (1996).
[13] Y. Nakamura, S. Watanabe, N. Miyake, H. Kohno, T. Osawa. *J. Agric. F. Chem.*, **51**, 3309 (2003).
[14] S. Gafner, J.-L. Wolfender, S. Mavi, K. Hostettmann. *Planta Med.*, **62**, 67 (1996).