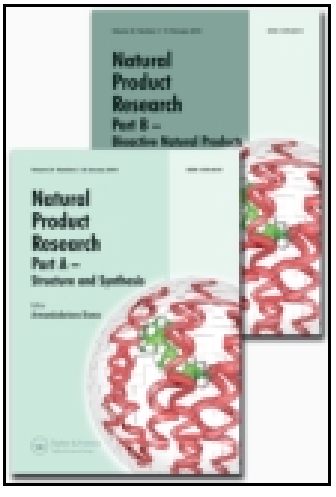

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Lalith Jayasinghe [a], Nilupa R. Amarasinghe [a], B.G. Suranga Arundathie [a], G. Kalinga Rupasinghe [a], N.H. Ayona N. Jayatilake [a] & Yoshinori Fujimoto [b]

[a] Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

[b] Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan

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Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*

Lalith Jayasinghe[a]*, Nilupa R. Amarasinghe[a], B.G. Suranga Arundathie[a], G. Kalinga Rupasinghe[a], N.H. Ayona N. Jayatilake[a] and Yoshinori Fujimoto[b]

[a]*Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka;*
[b]*Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo 152-8551, Japan*

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Chemical investigation of the leaves of *Elaeocarpus serratus* yielded myricitrin (**1**), mearnsetin 3-*O*-β-D-glucopyranoside (**2**), mearnsitrin (**3**), tamarixetin 3-*O*-α-L-rhamnopyranoside (**4**) and the fruits of *Filicium decipiens* yielded three flavonol glycosides, kaempferol 3-*O*-rutinoside (**5**), kaempferol 3-*O*-robinobioside (**6**) and trifolin (**7**). Compound **1** showed strong antioxidant activity against DPPH.

Keywords: *Elaeocarpus serratus*; *Filicium decipiens*; Elaeocarpaceae; Sapindaceae; flavonol glycosides; antioxidant activity; DPPH

1. Introduction

Oxidation is one of the major causes of chemical spoilage resulting in rancidity, and/or deterioration of the nutritional quality, colour, flavour, texture and safety of food (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). Antioxidants are widely used as additives in fats and oils and in food processing to prevent or delay spoilage of food (Suhaj, 2006). Recently interest has increased considerably in finding naturally occurring antioxidants for use in food or medicinal materials to replace synthetic antioxidants which are being restricted due to their carcinogenicity (Velioglu, Mazza, Gao, & Oomah, 1998). Butylated hydroxyltoluene (BHT), butylated hydroxylanisole (BHA) and tertiary butylhydroquinone (TBH) are extensively used as antioxidants at present, in order to reduce the harm caused by free radicals (Tepe, Eminagaoglu, Akpulat, & Aydin, 2007). Synthetic antioxidants such as BHA and BHT have restricted uses in food as they are reported to be carcinogenic (Ito, Fukushima, & Tsuda, 1985). Antioxidants are also important in the prevention of human diseases such as alzheimer's disease, aging, diabetic rheumatoid arthritis, cataracts, etc. The harmful actions of free radicals can be reduced by antioxidants. Earlier, scientists have paid attention to natural compounds such as vitamin C, E and carotenoids, and during the recent years the powerful antioxidant capacity of phenolics aroused more interest (Shahidi, Janitha, &

*Corresponding author. Email: ulbj2003@yahoo.com

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Wanasundara, 1992). Hence it is very important to pay attention to search for human friendly natural antioxidants. For this purpose, compounds of plant origin can play a very prominent role.

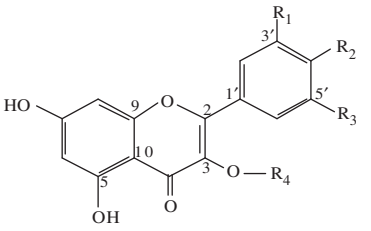
In a continuation of our studies towards the antioxidant active compounds from Sri Lankan plants with special reference to high polar secondary metabolites, the present investigation is carried out on the leaves of *Elaeocarpus serratus* of the family Elaeocarpaceae and the fruits of *Filicium decipiens* of the family Sapindaceae. Both are moderate size trees growing in Sri Lanka. Fruits of the *E. serratus* is a popular edible fruit in Sri Lanka. Water extracts of the leaves of *E. serratus* are used in anti-head lice and anti-dandruff treatments in rural areas of Sri Lanka. No previous chemical work on *E. serratus* has been reported. *Filicium decipiens* is very popular in Sri Lanka as a shade and ornamental plant. Several triterpenoidal saponins (Lavaud et al, 1998), 24-norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7 caffeate (Jayasinghe, Bandara, Hara, & Fujimoto, 2001) and some flavonol glycosides (Jayasinghe, Balasooriya, Hara, & Fujimoto, 2004) have been reported from the stem and leaves of the plant.

2. Results and discussion

The dried leaves of *E. serratus* were defatted with *n*-hexane and extracted with dichloromethane and methanol. Chromatographic separation of the *n*-butanol extract of the methanol extract of *E. serratus* over silica, sephadex LH-20 and reversed phase HPLC afforded 3-*O*-α-L-rhamnopyranosyl-5,7-dihydroxy-2-(3,4,5-trihydroxy-phenyl)-chromen-4-one (myricitrin) (**1**) (Nicollier & Thompson, 1983), 3-*O*-β-D-glucopyranosyl-5,7-dihydroxy-2-(3,5-dihydroxy-4-methoxy-phenyl)-chromen-4-one (mearnsetin 3-*O*-β-D-glucopyranoside) (**2**) (Braca, Tommasi, Mendez, & Morelli, 1999), 3-*O*-α-L-rhamnopyranosyl-5,7-dihydroxy-2-(3,5-dihydroxy-4-methoxy-phenyl)-chromen-4-one (mearnsitrin) (**3**) (Noreen, Serrano, Perera, & Bohlin, 1998) and 3-*O*-α-L-rhamnopyranosyl-5,7-dihydroxy-2-(3-hydroxy-4-methoxy-phenyl)-chromen-4-one (tamarixetin 3-*O*-α-L-rhamnopyranoside) (**4**) (Rao, Hanumaiah, Vemuri, & Rao 1983).

The fruits of *F. decipiens* were defatted with *n*-hexane and extracted with methanol. Chromatographic separation of the *n*-butanol extract from the methanol extract over silica, sephadex LH-20 and reversed phase HPLC afforded kaempferol 3-*O*-α-L-rhamnopyranosyl-(1$\rightarrow$6)-β-D-glucopyranoside (kaempferol 3-*O*-rutinoside) (**5**) (Sang et al., 2002), kaempferol 3-O-α-L-rhamnopyranosyl-(1$\rightarrow$6)-β-D-galactopyranoside (kaempferol 3-*O*-robinobioside) (**6**) (Rastrelli, Saturnino, Schettino, & Dini, 1995) and kaempferol 3-*O*-β-D-galactopyranoside (trifolin) (**7**) (Scharbert, Holzmann, & Hofmann, 2004), respectively. Structure elucidation of **1**–**7** was based on the detailed analysis of the spectral data and comparison with the reported data (Figure 1).

This is the first report of the isolation of compounds **1**–**4** from *E. serratus* and **5**–**7** from *F. decipiens*. Antioxidant properties of compounds **1**–**7** were evaluated against the DPPH radical by TLC bio-autography method (Takao, Kitatani, Watanabe, Yagi, & Sakata, 1994). All these compounds **(1–7)** exhibited off-white spots in purple background at the level of 1 µg/spot indicating the antioxidant activity of compounds. Only the compound **1** showed strong off-white spot on TLC, even at



1	R_1=OH	R_2=OH	R_3=OH	R_4=-rha
2	R_1=OH	R_2=OMe	R_3=OH	R_4=-glu
3	R_1=OH	R_2=OMe	R_3=OH	R_4=-rha
4	R_1=OH	R_2=OMe	R_3=H	R_4=-rha
5	R_1=H	R_2=OH	R_3=H	R_4=-glu^6-rha
6	R_1=H	R_2=OH	R_3=H	R_4=-gal^6-rha
7	R_1=H	R_2=OH	R_3=H	R_4=-gal

glc-=β-D-glucopyranosyl
gal-=β-D-galactopyranosyl
rha- =α-L-rhamnopyranosyl

Figure 1. Structures of compounds **1**–**7**.

the concentration of **1**–**7** reduced to 0.1 μg/spot. Hence the antioxidant property of **1** was compared with ascorbic acid and butylated hydroxylanisole (BHA) using spectrophotometric method and IC_{50} values were determined as **1** (3.2 μg mL^{-1}), ascorbic acid (3.9 μg mL^{-1}) and BHA (3.6 μg mL^{-1}) (Burtis & Bucar, 2000; Cuendet, Hostettmann, Dyatmiko, & Potterat, 1997). The antioxidant activity was expressed in terms of IC_{50} (μg mL^{-1}, concentration required to inhibit the DPPH radical formation by 50%). Antioxidant activity of **1** measured with different methods have been reported (Keim et al., 2010; Moharram, Marzouk, Ibrahim, & Mabry, 2006).

3. Experimental

3.1. *General*

Mps (uncorr.) were determined on a Gallenkamp apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL 400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer. FABMS were obtained on a JEOL JMSAx505 HA spectrometer with glycerol as matrix.

3.2. *Plant material*

Plants: Leaves of *E. serratus* were collected from the North Western Province of Sri Lanka in April 2001 and the fruits of *F. decipiens* were collected from the Central Province of Sri Lanka in June, 2003. Voucher specimens (IFS/10/ES1 and IFS/10/FD1) are deposited at the National Herbarium, Royal Botanic Gardens, Peradeniya, Sri Lanka.

3.3. *Extraction and isolation*

Dried ground leaves of *E. serratus* (550 g) were defatted with *n*-hexane and sequentially extracted with dichloromethane and methanol. Evaporation of CH_2Cl_2 gave a dark green coloured solid (20 g) and evaporation of methanol gave a dark brown solid (36 g). TLC analysis of CH_2Cl_2 extract indicated the presence of chlorophyll as the major constituent. Methanol extract (30 g) was partitioned with *n*-butanol and water. Evaporation of *n*-butanol gave a dark brown solid (7.8 g). Chromatographic separation of the solid (7 g) over silica gel with EtOAc-MeOH

followed by sephadex LH-20 with MeOH and reverse phase HPLC (STR Prep-ODS 20x250 mm column; 60% H_2O-MeOH, 5 ml min^{-1}; UV detection 254 nm) furnished **1** (11 mg), **2** (8 mg), **3** (87 mg) and **4** (17 mg).

Dried, ground whole fruit of *F. decipiens* (225 g) were defatted with *n*-hexane and extracted with methanol. Evaporation of methanol gave a dark brown solid (46 g). A portion of the methanol extract (25 g) was partitioned with *n*-butanol and water. Chromatographic separation of *n*-butanol extract (7 g) over silica gel with EtOAc-MeOH followed by Sephadex LH-20 with MeOH and reverse phase HPLC (STR Prep-ODS 20x250 mm column; 65% H_2O-MeOH, 5 ml min^{-1}; UV detection 254 nm) furnished **5** (120 mg), **6** (80 mg) and **7** (110 mg).

3.4. *Antioxidant activity*

TLC bioautography method: Compounds **1**–**7** were spotted on silica gel (1 µg/spot) coated aluminum plates and eluted with $CHCl_3$-MeOH-H_2O (7 : 3 : 1) solvent system. Plates were air dried and sprayed with 0.004% DPPH solution in ethanol.

Spectrophotometry method: The antioxidant activity of compound **1** was evaluated by the DPPH radical scavenging effect (Burtis & Bucar, 2000; Cuendet et al, 1997). 5 µg, 10 µg, 15 µg, 25 µg, 50 µg and 100 µg of compounds **1** in 100 µl of ethanol was added to 5 mL of 0.004% ethanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was measured against a blank (only DPPH in methanol) at 517 nm. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Inhibition of free radical DPPH in percent (I%) was calculated as follows.

$$I\% = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100$$

$$A_{blank} = \text{absorbance of the control reaction (only DPPH in ethanol)}$$

A graph was plotted with I% versus the weight of the compound in 5 mL of DPPH in ethanol and IC_{50} values determined. The antioxidant activity is expressed in terms of IC_{50} (µg mL^{-1}, concentration required to inhibit the DPPH radical formation by 50%). Ascorbic acid and BHA were used as positive control.

References

Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst, 127*, 183–198.

Braca, A., Tommasi, N.D., Mendez, J., & Morelli, I. (1999). Flavonoids and triterpenoids from *Licania heteromorpha* (Chrysobalanaceae). *Biochemical Systematics and Ecology, 27*, 527.

Burtis, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research, 14*, 323–328.

Cuendet, M., Hostettmann, K., Dyatmiko, W., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Ito, N., Fukushima, S., & Tsuda, H. (1985). Carcinogenicity and modification of the carcinogenic response by BHA, BHT and other antioxidants. *CRC Critical Reviews in Toxicology, 15*, 109–150.

Jayasinghe, U.L.B., Bandara, A.G.D., Hara, N., & Fujimoto, Y. (2001). A new neohopane caffeate from *Filicium decipiens*. *Fitoterapia, 72*, 737–742.

Jayasinghe, U.L.B., Balasooriya, B.A.I.S., Bandara, A.G.D., & Fujimoto, Y. (2004). Glycosides from *Grewia damine* and *Filicium decipiens*. *Natural Product Research, 18*, 499–502.

Keim, P.V., Mai, N.T., Minh, C.V., Khoi, N.H., Dang, N.H., Thao, N.P., . . . , Kim, Y.H. (2010). Two new C-glucosyl benzoic acids and flavonoids from *Mallotus nanus* and their antioxidant activity. *Archives of Pharmaceutical Research, 33*, 203–208.

Lavaud, C., Voutquenne, L., Massiot, G., Le Men-Oliver, L., Das, B.C., Laprevote, O., . . . , Becchi, M. (1998). Saponins from the stem bark of *Filicium decipiens*. *Phytochemistry, 47*, 441–449.

Moharram, F.A., Marzouk, M.S.A., Ibrahim, M.T., & Mabry, T.J. (2006). Antioxidant galloylated flavonol glycosides from *Calliandra haematocephala*. *Natural Product Research, 20*, 927–934.

Nicollier, G., & Thompson, A.C. (1983). Flavonoids of *Desmanthus illinoensis*. *Journal Natural Products, 46*, 112–117.

Noreen, Y., Serrano, G., Perera, P., & Bohlin, L. (1988). Flavan-3-ols isolated from some medicinal plants inhibiting COX-1 and COX-2 catalysed prostaglandin biosynthesis. *Planta Medica, 64*, 520–524.

Rao, C.P., Hanumaiah, T., Vemuri, V.S.S., & Rao, K.V.J. (1983). Flavonol 3-glycosides from the leaves of *Flemingia stricta*. *Phytochemistry, 22*, 621–622.

Rastrelli, L., Saturnino, P., Schettino, O., & Dini, A. (1995). Studies on the constituents of *Chenopodium pallidicaule* (Canihua) seeds. Isolation and characterization of two new flavonol glycosides. *Journal of Agricultural and Food Chemistry, 43*, 2020–2024.

Sang, S., Lapsley, K., Jeong, W-S., Lachance, P.A., Ho, C-T, & Rosen, R.T. (2002). Antioxidative phenolic compounds isolated from almond skins (*Prunus amygdalus* Batsch). *Journal of Agricultural and Food Chemistry, 50*, 2459–2463.

Scharbert, S., Holzmann, N., & Hofmann, T. (2004). Identification of the astringent compounds in black tea infusions by combining instrumental analysis with human bioresponse. *Journal of Agricultural and Food Chemistry, 52*, 3498–3508.

Shahidi, F., Janitha, P.K., & Wanasundara, P.D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Suhaj, M. (2006). Spice antioxidants isolation and their antiradical activity: a review. *Journal of Food Composition and Analysis, 19*, 531–537.

Takao, T., Kitatani, F., Watanabe, N., Yagi, A., & Sakata, K. (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscience, Biotechnology and Biochemistry, 58*, 1780–1783.

Tepe, B., Eminagaoglu, O., Akpulat, H.A., & Aydin, E. (2007). Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. *verticillata* and S. *verticillata* (L.) subsp. *amasiaca* (Freyn & Bornm.) Bornm. *Food Chemistry, 100*, 985–989.

Velioglu, Y.S., Mazza, G., Gao, L., & Oomah, B.D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural Food and Chemistry, 46*, 4113–4117.