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Two Flavonoids From Stem Bark of *Casimiroa edulis* and Their Antidiabetic and Antioxidant Activities

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Abstract: *Casimiroa edulis* Llave et Lex (Rutaceae), popularly known as white sapote. The main aim of this study is to isolate and investigate the bioassay of the stem bark of *Casimiroa edulis*. Two flavonoids were isolated from the methanolic fraction of the stem bark of *Casimiroa edulis*. The isolated compounds can be identified as 6,7-dimethoxyflavone (1) and 5,6,2'-trimethoxyflavone (2) by using advance spectroscopic methods, including FT-IR, UV, 1D NMR, 2D NMR. Compounds 1 and 2 were evaluated for their antidiabetic and antioxidant activities. The result revealed that the two compounds did not have antidiabetic activity and antioxidant activity. This is the first phytochemical study of 6,7-dimethoxyflavone from the genus *Casimiroa*.

Key words: *Casimiroa edulis*, white sapote, Rutaceae, flavonoids

1. Introduction

Natural products are used as medicines for treating and preventing various diseases since prehistoric times. According to the record of fossil, human use of plants as medicines for their diseases may be traced back at least 60,000 years. [11; 18]

Casimiroa is a tree belongs to the family of Rutaceae, found in the tropical and subtropical areas of Central America and Mexico, the Caribbean, the Mediterranean region, India, Southeast Asia, South Africa, Australia, and New Zealand. The best-known species is *Casimiroa edulis* [14; 17]. It has been widely used as sedative for the treatment of anxiety and dermatological problem. The early pharmacological studies of an aqueous extract and alcohol extracts of the seeds and leaves of *C. edulis* exhibited the cardiovascular, anticonvulsant, sedative, anti-inflammatory, anti-mutagenic, diuretic, hypnotic, anti-hypertension, anti-inflammatory, muscle relaxant and contractile activities [4; 15]. In Myanmar, local people used this for the treatment of stomach problem.

Many of the phytochemical analysis have been done on the leaves, fruits, seeds and bark of *Casimiroa edulis*. The previous studies indicated that this plant contains flavonoids, coumarin, alkaloids, and limonoids [1-3, 5-9; 12]. In this study, two flavonoids namely, 6,7-dimethoxyflavone (1) and 5,6,2'-trimethoxyflavone (2) have been isolated from the stem bark of *Casimiroa edulis*. Their structures have been elucidated through FT-IR, UV, ¹H-NMR, ¹³C-NMR, and 2D NMR. Furthermore, the antidiabetic and antioxidant activity of isolated compounds were investigated against α -glucosidase inhibition and DPPH assay.

2. Experimental Methods

2.1 General

UV spectra were recorded on UV-Vis Shimadzu spectrometer. IR spectra were recorded on FT IR-8400 spectrophotometer. NMR spectra were recorded in CDCl₃ by using a JEOL ECA-500 (¹H: 500 MHz and ¹³C: 125MHz). Positive mode HRFABMS was obtained by using a JEOL JMS HX-110 mass spectrometer. Column chromatography was carried out on silica gel (BW-820H). Analytical TLC was performed on silica

on pre-coated Kieselgel silica gel 60 F₂₅₄ aluminium sheets. Melting points were measured by melting point apparatus and are uncorrected.

2.2 Plant material

The stem bark of *Casimiroa edulis* Llave et Lex was collected in Namp-see Village, Taunggyi (Shan State), Myanmar during the month of August 2016.

2.3 Extraction and isolation

The air-dried sample of the stem bark of *Casimiroa edulis* (1000 g) was extracted with methanol (3000 mL). Then the methanolic extract was concentrated at room temperature to give MeOH crude extract 250 g. The dried MeOH extracts 250 g were fractionated by partitioning with n-hexane : methanol (v/v) (100 mL × 3). The MeOH extract was evaporated under reduced pressure at 40°C using a rotary evaporator to give the methanolic crude extract 50 g. A methanol extract (50 g) was subjected to VLC separation using 100 g silica gel 60H eluted with a gradient solvent system of n-hexane in Et-OAc (100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 0:100) to afford 28 Fractions (1-28). Based on TLC analysis, the fractions can be grouped to be CF-1, CF-2, CF-3, CF-4, CF-5 and CF-6. Fraction CF-6 (6.19 g) was further fractionated by silica gel column chromatography with a gradient solvent system of n-hexane in Et-OAc (100:0, 95:5, 90:10, 80:20, 70:30, 0:100) to afford 270 fractions. Based on TLC analysis, the fractions can be grouped to be SF-1, SF-2, SF-3, SF-4 and SF-5. Fraction SF-2 (120 mg) was further purified by silica gel column chromatography with a gradient of n-hexane in acetone (100:0, 95:5, 90:10, 80:20, 0:100) to give 70 subfractions. Each fraction was checked by TLC and UV lamp. Then, the sub-fractions of the same R_f value were combined and 5 combined fractions (Fra-1 to Fra-4) were obtained. Among them, Fra-2, and Fra-4 gave only one spot on TLC and UV active. The pure compound white crystalline solid form of compound (1), and compound (2) were obtained.

2.4 α -Glucosidase inhibition assay and DPPH assay

The α -glucosidase inhibition of two compounds was analyzed according to the method reported by Ramadhan & Phuwapraisrisan [13]. Antioxidant activity of two compounds was measured against DPPH radical scavenging activity. The IC₅₀ values of the compound were measured by the linear regression.

2.5 Spectra data

6,7-dimethoxyflavone (1)

White crystalline solids (CHCl₃) (1): UV (MeOH) λ_{max} : 271 nm; IR (ν_{max} , KBr, cm⁻¹): 3070, 2999, 1647, 1571, 1496, 1367, 1288, 1178, 1078, 958, 775; ¹H NMR (CDCl₃, 500 MHz, δ , ppm, J/Hz) : 7.89 (dd, J = 7.7, 1.9 Hz, H-2' and H-6'), 7.51 (m, H-3', H-4' and H-5''), 7.32 (s, H-5 and H-8), 6.69 (s, H-3), 3.98 (s, OCH₃), 3.94 (s, OCH₃); ¹³C NMR (CDCl₃, 125 MHz, δ , ppm): 178.0 (C-4), 161.6 (C-2), 151.6 (C-9), 150.0 (C-7), 148.0 (C-6), 131.7 (C-1'), 131.4 (C-4'), 129.0 (C-3'), 126.1 (C-6'), 119.3 (C-10), 119.1 (C-8), 113.4, 108.0 (C-3), 61.9 (7-OCH₃), 57.2 (6-OCH₃).

5,6,2-trimethoxyflavone(2)

White crystalline solids (CHCl₃) (2) UV (MeOH) λ_{max} : 329, 267, 235 nm; IR (ν_{max} , KBr, cm⁻¹): 3128, 3078, 2972, 2837, 1631, 1612, 1570, 1481, 1357, 1284, 1188, 1083, 964, 744; ¹H NMR (CDCl₃, 500 MHz, δ , ppm, J/Hz) : 7.85 (1H, dd, J = 7.8, 1.7 Hz, H-6'), 7.46 (ddd, J = 8.4, 7.4, 1.8 Hz, H-4'), 7.30 (1H, d, J = 9.2 Hz, H-7), 7.27 (1H, d, J = 9.2 Hz, H-8), 7.09 (1H, td, J = 7.7, 1.0 Hz, H-5'), 7.03 (1H, d, J = 8.0 Hz, H-3'), 6.98 (1H, s, H-3), 3.98 (3H, s, 2'-OCH₃), 3.93 (6H, s, 5-OCH₃ and 6-OCH₃), NMR (CDCl₃, 125 MHz, δ , ppm): 178.4 (C-4), 159.1 (C-2), 158.0 (C-5), 151.9 (C-9), 149.7 (C-6), 147.9 (C-2'), 132.2 (C-4'), 129.1 (C-6'), 120.8 (C-1'), 120.7 (C-5'), 119.2 (C-8), 119.1 (C-10), 113.4 (C-7), 113.1 (C-3), 111.7 (C-3'), 61.9 (2-OCH₃), 57.3 (5-OCH₃), 55.7 (6-OCH₃).

3. Results and discussion

6,7-Dimethoxyflavone (1), and 5,6,2'-trimethoxyflavone (2) were isolated from the methanolic extract of the stem bark of *C. edulis*. 6,7-dimethoxyflavone was the first phytochemical study of this plant. The isolated compounds identified by interpretation of their ¹H NMR and ¹³C NMR spectral data by comparisons to those available in the literature.

Compound (1) was obtained as white crystalline solid with melting point at 236-248°C. IR spectrum of compound (1) displayed the absorption band for methoxy (3431 cm⁻¹), sp² hydrocarbon (3070 cm⁻¹) sp³

hydrocarbon (2999-2839 cm^{-1}), carbonyl (1647 cm^{-1}) and aromatic (1639, 1571 cm^{-1}) groups. The UV spectrum showed an absorption band with λ_{max} 271 nm. According to the ^1H NMR spectrum, compound (1) showed the presence of 14 protons. One singlet sharp peak at δ_{H} 6.69 indicates the presence of H-3. Furthermore, the H-3 proton showed the correlation with the peak at δ_{C} 161.6 (C-2), 178.0 (C-4), 131.7 (C-1') and 119.3 (C-10) in HMBC spectrum. Another two sharp singlets peak at δ_{H} 3.94 and 3.98 (each, 3H, s) indicate the presence of two methoxy groups on the aromatic ring. Moreover, one singlet sharp peak at δ 7.32 (2H, s) indicates the presence of H-5 and H-6 protons. One doublet-doublet at δ 7.89 ppm (2H, $J = 7.7, 1.9$ Hz) indicate the presence of H-2' and H-6' protons. The other remaining one multiplet at δ 7.51 (3H, m) indicates the presence of H-3', H-4' and H-5' protons. The ^{13}C NMR and DEPT spectra of compound (1) showed 17 carbon atoms for the comprising of eight sp^2 methine, two oxygenated sp^3 and seven sp^2 quaternary carbons. Therefore, base above information the compound (1) was identified as 6,7-dimethoxyflavone [16].

Compound (2) was obtained as white crystalline solid with melting point at 144-156°C. IR spectrum of compound (2) displayed the absorption band for methoxy (3128 cm^{-1}), sp^2 hydrocarbon (3078 and 3003 cm^{-1}), sp^3 hydrocarbon (2972-2837 cm^{-1}), carbonyl (1631 cm^{-1}) and aromatic (1612, 1600 and 1570 cm^{-1}) groups. The UV spectrum showed absorption band with λ_{max} 329, 267 and 235 nm. According to the ^1H NMR spectrum, compound (2) showed the presence of 16 protons. One singlet sharp peak at δ_{H} 6.98 (1H, s) indicates the presence of H-3 proton. Furthermore, the H-3 proton showed the correlation with the peak at δ_{C} 159.1 (C-2), 178.4 (C-4), 119.1 (C-1') and 120.8 (C-10) in HMBC spectrum. Two doublets at δ_{H} 7.27 and 7.30 ppm (each, 1H, $J = 9.2$ Hz) indicates the presence of H-7 and H-8. Two singlet sharp peaks at δ_{H} 3.93 (3H, s) and 3.98 ppm (6H, s) indicate the presence of three methoxy groups on the aromatic ring. One doublet-doublet at δ_{H} 7.85 (1H, $J = 7.8, 1.7$ Hz) indicates the presence of H-6' proton. One doublet-doublet-doublet at δ_{H} 7.46 (1H, $J = 8.4, 7.4, 1.8$ Hz) indicates the presence of H-4' proton. One triplet-doublet at δ_{H} 7.09 (1H, 7.7, 1.0 Hz) indicates the presence of H-5' proton. One doublet at δ_{H} 7.03 (1H, $J = 8$ Hz) indicates the presence of H-3' proton. The ^{13}C NMR and DEPT spectra of compound (2) showed 18 carbon atoms for the consisting of seven sp^2 methine, three oxygenated sp^3 and eight sp^2 quaternary carbons, respectively. Therefore, base above information the compound (2) was identified as 5,6,2'-trimethoxyflavone [10].

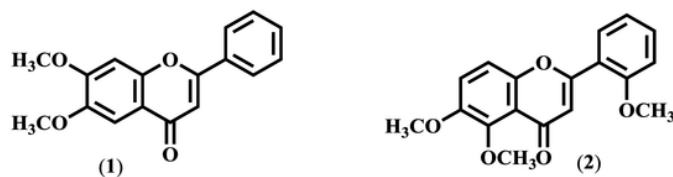


Figure 1. Chemical structure of compound (1) and (2)

3.1 Anidiabetic and Antioxidant activity

Two compounds were isolated from MeOH fraction of the stem bark of *Casimiroa edulis* were screened for antidiabetic and antioxidant activity against α -glucosidase inhibition and DPPH assay. According to the Table (1), these two compounds did not showed antidiabetic and antioxidant activity.

Table 1. Antioxidant and α -glucosidase inhibition activities of isolated compounds

Compound	IC ₅₀ mM	
	Yeast	DPPH
6,7-dimethoxyflavone (1)	NI	NI
5,6,2'-trimethoxyflavone (2)	NI	NI
Acarbose	0.1030	-

NI = No Inhibition

4. Conclusion

Two compounds were isolated from the stem bark of *Casimiroa edulis*. From their spectroscopic data, these two compounds can be identified as 6,7-dimethoxyflavone (**1**), and 5,6,2'-trimethoxyflavone (**2**). The isolated compounds were evaluated for antidiabetic and antioxidant activities. The result revealed that these two compounds did not have antidiabetic activity and antioxidant activity. Base on our knowledge, 6,7-dimethoxyflavone is isolated for the first time from the genus *Casimiroa*.

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