A Flavone from *Vitex parviflora*

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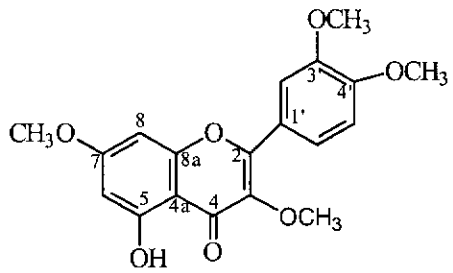
The chloroform extract of the air-dried leaves of Vitex parviflora afforded retusin by silica gel chromatography. Its structure was elucidated by extensive 1D and 2D NMR spectroscopy. Antimicrobial tests indicated that retusin has moderate activity against the fungi, A. niger and low activity against the bacteria, P. aeruginosa. It was found inactive against the fungi, C. albicans and T. mentagrophytes and the bacteria: E. coli, S. aureus and B. subtilis.

INTRODUCTION

Vitex parviflora Juss. commonly known as molave is found in open primary forests at low altitudes throughout the Philippines. It is well known for its extremely hard wood. Medicinally, its bark is used as decoction for diarrhea, and an infusion of the wood is considered a good remedy for poison because it induces vomiting. There are no reported studies on the plant. However, a number of studies on *Vitex* reported the isolation of flavones[1-7] which are of relevance to our present report. Casticin, a flavone isolated from *V. negundo* was reported to inhibit the growth of the fungi, *C. albicans* and *A. niger* and the bacteria, *S. aureus* and *P. aeruginosa*[7]. Another flavone, 5,7,3'-trihydroxy-6,8,4'-trimethoxyflavone from *V. negundo* has estrogenic and pregnancy interceptory effects[1].

We now report the isolation, structure elucidation and antimicrobial assay of retusin (**1**) from the leaves of molave. This is the first report on the isolation of **1** from *Vitex parviflora*.



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RESULTS AND DISCUSSION

The chloroform extract of the air-dried leaves of *Vitex parviflora* afforded retusin (**1**). The structure of the compound was elucidated by extensive 1D and 2D NMR analysis as follows:

Table 1. 300 MHz ^{1}H and ^{13}C Spectral Data of 1 in $CDCl_3$

Carbon No.	^{13}C, δ	^{1}H, δ
C-2	155.6	—
C-3	139.1	—
C-4	177.6	—
C-4a	106.1	—
C-5	162.1	—
C-6	97.8	6.36 (1H, d, *J*=1.6 Hz)
C-7	165.4	—
C-8	92.3	6.45 (1H, d, *J*=1.6 Hz)
C-8a	156.6	—
C-1'	122.9	—
C-2'	111.5	7.69 (1H, d, *J*=2.1 Hz)
C-3'	148.6	—
C-4'	151.9	—
C-5'	111.0	6.99 (1H, d, *J*=8. 6 Hz)
C-6'	122.2	7.73 (1H, dd, *J*=2.1, 8.5)
C-5(OH)	—	12.63 (s, br)
OCH3	55.8, 56.1(2C), 60.2	3.973 (3H, s), 3.967 (3H, s) 3.88 (3H, s), 3.87 (3H, s)

The ^{1}H NMR spectrum of **1** (Table 1) indicated resonances for four methoxy groups at δ 3.973 (3H, s), 3.967 (3H, s), 3.88 (3H, s), 3.87 (3H, s) and a chelated hydroxyl at δ 12.63 (s br). Two ortho aromatic protons were attributed to the resonances at δ 6.99 (1H, d, J = 8. 6 Hz) and 7.73 (1H, dd, J=2.1, 8.5 Hz) which is meta coupled to the resonance at δ 7.69 (1H, d, J=2.1 Hz). Furthermore, two meta aromatic protons were assigned to the resonances at δ 6.36 (1H, d, J = 1.6 Hz) and 6.45 (1H, d, J= 1.6 Hz). The J-modulated ^{13}C NMR spectral data (Table 1) indicated resonances for nineteen carbon atoms with the following functionalities: four methoxy carbons at δ 55.8, 56.1 (2C), and 60.2; seven oxygenated aromatic carbons at δ 139.1, 148.6, 151.9, 155.6, 156.6, 162.1, 165.4; five protonated aromatic carbons at δ 92.3, 97.8, 111.0, 111.5, 122.9; and a conjugated carbonyl carbon at δ 177.6. These resonances are characteristics of flavones.

The ^{1}H and ^{13}C connectivities were verified by the inverse long-range heteronuclear experiment HMBC optimized for J = 10 Hz. The following long-range correlations were observed. The carbons at δ 139.1 (C-3), 148.6 (C-3'), 151.9 (C-4') and 165.4 (C-7) are long-range correlated to the methoxy groups at δ 3.87 (3-OCH_3), 3.967 (3'-OCH_3), 3.973 (4'-OCH_3) and 3.88 (7-OCH_3), respectively. The carbons at δ 106.2 (C-4a), 162.1 (C-5) and 165.4 (C-7) are long-range correlated to the aromatic proton at δ 6.36 (H-6). The carbons at δ 156.6 (C-8a) and 151.9 (C-4') are long-range correlated to the protons at δ 6.45 (H-8) and 7.69 (H-2'), respectively. The carbons at δ 122.9 (C-1'), 148.6 (-3') and 122.2 (C-6') are long-range correlated to the proton at δ 6.99 (H-5'). All long-range correlations observed were consistent with the structure of **1**.

The NOESY spectral data of **1** indicated that the aromatic protons at δ 6.36 (H-6) and 6.45 (H-8) are close in space to the methoxy group at δ 3.88 (7-OCH_3). The aromatic proton at δ 6.99 (H-5') is close to the methoxy group at δ 3.973 (4'-OCH_3) and the aromatic proton at δ 7.73 (H-6'). The aromatic proton at δ 7.69 (H-2') is close in space to the methoxy group at δ 3.967 (3'-OCH_3). The methoxy group at δ 3.87 (3-OCH_3) is close to the aromatic proton at δ 7.73 (H-6'). This confirms the structure of **1.** Literature search revealed that **1** is retusin.[8] Confirmatory evidence is the ^{1}H NMR spectra of **1** and retusin.[8] The spectra matched in all essential respects, thus

1 is retusin.

Since plants of the genus *Vitex* and many flavones have antimicrobial properties, compound **1** was tested for possible activity against seven microorganisms using the agar cup method. Results of the study (Table 2) indicated that **1** is active against the fungus, *A. niger* with antimicrobial index (AI) of 0.3 at a concentration of 30 μg, and has slight activity against the bacterium *P. aeruginosa* with AI of 0.2 at the same concentration. It was inactive against *E. coli, S. aureaus, B. subtilis, C. albicans* and *T. mentagrophytes.*

Table 2. Antimicrobial Test Results on Compound 1

Organism	Sample (30 μg)	Clearing Zone, mm			AI
		Replicate 1	Replicate 2	Replicate 3	
E. coli	**1**	—	—	—	0
	Tetracycline	30			2.0
P. aeruginosa	**1**	12	12	12	0.2
	Tetracycline	20	—	—	1.0
S. aureus	**1**	—	—	—	0
	Chloramphenicol	30	—	—	0.2
B. subtilis	**1**	—	—	—	0
	Chloramphenicol	40	—	—	3.0
C. albicans	**1**	—	—	—	0
	Clotrimazole	25	—	—	1.5
T. mentagrophytes	**1**	—	—	—	0
	Clotrimazole	38	—	—	2.8
A. niger	**1**	13	13	14	0.3
	Cycloheximide	16	—	—	0.6

EXPERIMENTAL

General Experimental Procedures

NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C. 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-H_2SO_4 and warming.

Sample Collection

The plant material was collected from the University of the Philippines, Diliman in October 1998. It was identified as *Vitex parviflora* at the Philippine National Museum.

Isolation

The air-dried leaves (500 g) was extracted with $CHCl_3$. The crude extract (20 g) was treated with 4% aqueous $Pb(OAc)_2$ to precipitate the more polar components[9]. The treated extract was chromatographed using increasing proportions of Me_2CO in $CHCl_3$ (10% increment) as eluents. The fraction eluted with 10% Me_2CO in $CHCl_3$ was rechromatographed (5x) in 10% EtOAc in petroleum ether to afford **1** (yellow crystals, m.p. 158-160°C, 10 mg) after recrystallization from diethyl ether.

Antimicrobial Tests

The microorganisms used in these tests 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.

Microbial suspension containing approximately 6×10^8 cells/ml was prepared from each test organism for 24-hour culture of *S. aureus, E. coli, P. aeruginosa, B. subtilis* and *C. albicans* and from a 5-day-old *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water. One-tenth (0.1) ml of the bacteria, yeast and molds were transferred into pre-poured nutrient agar (NA, DISCO Laboratories, Detroit, Michigan), glucose yeast peptone agar (GYP)[10] and potato dextrose agar (PDA, DISCO Laboratories, Detroit, Michigan), respectively. About 5 ml of corresponding medium, autoclaved and cooled to about 45°C was poured into the 90 mm petri dish. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. Three 10 mm wells were cut from equidistant points of the seeded agar plates using sterile cork borer. Thirty (30) μg of samples dissolved in 95 % EtOH were transferred in each well. For the standard agent, 30 mg were used.

The NA, GYP and PDA-based cultures were incubated at 30 ± 1°C for 24, 48 and 72 hours, respectively. Antimicrobial effects were determined by measuring the zone of the growth inhibition represented by a clear zone in mm. The average diameter of the clear zones was used to calculate an antimicrobial index.

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