New phenolic Constituents from bark of *Walsura trifoliata*

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Abstract

Phytochemical investigation of methanolic extract from *Walsura trifoliata* bark led to the isolation of two new compounds viz phenylpropanoid substituted flavan-3-ol (2) and anthraquinone glucoside (3), along with known compound, chrysophanol (1). The structures of all these compounds were established by the interpretation of NMR and Mass spectral data.

Keywords: *Walsura trifoliata* (synonym: *Walsura piscidia* Roxb.), phytochemical investigation; phenylpropanoid, anthraquinone

1. Introduction

*Walsura trifoliata* (synonym: *Walsura piscidia* Roxb.) belongs to meliaeace family and is an evergreen tree distributed widely in the tropical areas of Asia, such as India, Southern China, Malaysia, and Indonesia \[1,2\]. The plant is well known in traditional system of medicine to treat various ailments such as skin allergies, astringent and diarrhea \[3\]. The triterpenoids are by far the most abundant metabolites of this plant and have been shown to possess various biological activities including antifeedant activity against important insect pests \[4-6\].

As part of our programme on isolation and identification of the structurally interesting and biologically significant secondary metabolites from Indian Meliaceae plants \[7,8\], we were fascinated about *walsura trifoliata* and these structurally diverse and biologically interesting metabolites have encouraged us to continue our phytochemical study of this plant. This paper deals the extraction, isolation, and structural characterization of the bark. Which results leads the two new compound along with know compounds.

2. Results and discussion

The MeOH extract from the bark of *Walsura trifoliata* was partitioned between ethylacetate and water to give EtoAc-soluble fraction. The EtoAc-soluble extract of *Walsura trifoliata* was chromatographed on silica gel, and the resultant fractions were subjected to repeated column chromatography resulted in the isolation of three compounds (1-3) (fig-1), out of which compounds 2 and 3 were new compounds and structures were established using IR, MS, 1D and 2D NMR (HSQC, HMBC, COSY, ROESY and NOESY) spectroscopic techniques.

Compound 2 was isolated as a pale yellow amorphous powder. The molecular formula was determined as C24H21O9 by HRESIMS which provided a pseudo molecular ion peak at m/z 453.2564 [M + H]+ (calcd. 453.2572). The IR absorption bands at 3444, 2988 and 1633 cm-1 suggested the presence of hydroxyl and aromatic functional groups. 1H NMR spectra (300MHz, CD3OD) displayed the signals due to 1,3,5-trisubstituted phenyl group [δH 6.80 (2H, s), 6.98 (1H, s)] and 1,3,4-trisubstituted phenyl group [δH 6.61 (1H, s), 6.67 (1H, d, J = 8.1 Hz), 6.43 (1H, dd, J = 8.1,1.8 Hz)]. Further it also showed AMX2-type signals at δH 4.87 (1H, brs, H-2), 4.42-4.48 (1H, m) and 2.94 (2H, dd, J = 16.9, 5.0 Hz) as drawn with bold lines in
The connectivity of these fragments were confirmed by the analysis of its HMBC spectrum (Figure-2). The key HMBC correlations were observed from the H-3″ (δH 4.44 (t, J = 4.5 Hz)); to C-4″ (δC 114.7) and C-9″ (δC 118.9) which suggested the bonding position of the catechol group to be at C-3″. Further, correlations of H-3″ to C-4″, C-9″, C-7, C-8 and H-6 to C-7, C-8, C-4, and C-5 in HMBC spectra established the connections and indicated the flavan-3-ol skeleton.

The relative configuration of 2 corresponded to that of cinchonain Ia on the basis of similar NMR and NOE data (Figure 2). Which clearly indicated configuration of C-2/C-3 cis relationship and further supported by negligible coupling between H-2 and H-3. On the basis of the above evidence, the structure of 3 was assigned as shown in figure 1.

Compound 3 was isolated as pale yellow solid and the molecular formula was determined as C21H20O12 by m/z 465.1013 [M + H]+ (calcld. 465.1028). The IR spectrum of 3 found to exhibit the absorptions of OH (3428 cm⁻¹), and ester (1764 cm⁻¹) functionalities. The 1H NMR spectrum showed signals due to an anomic proton [δH 5.45 (1H, d, J = 7.3 Hz, Glcα-H1)], a characteristic peak for glucoside attached anomic protons, five aromatic protons [δH 7.58 (1H, d, J = 8.2 Hz, H-4), 7.58 (1H, t, J = 4.5 Hz, H-3), 6.83 (1H, d, J = 4.0 Hz, H-2), 6.40 (1H, d, J = 4.5 Hz, H-2)], and a characteristic of one chelated carboxyhydroxyl group of anthraquinones [δH 12.64 (1H, br s, 1-OH)]. In addition, 13C NMR displaying 21 resonances which were classified by A DEPT 135° NMR (Fig. 17) experiment into one methylene, ten methines, and ten quaternary carbons including one carbonyl carbons. Based on above data compound 3 were similar to those of revandchinone-2 [10], which is isolated from Rheum emodi. The major differences between the two compounds were that the methyl group in revandchinone-2 was replaced by the hydroxytolyl resonances in compound 3, and also replacement of -OCO-group by glucopyranosyl unit at C-9. this difference was concluded by 2D NMR spectra of Compound 3, especially 1H-1H COSY (Fig.-3), HSQC and HMBC allowed the establishment of the planar structure. Key HMBC correlation between H-1′ and C-9 indicate glucopyranosyl unit at C-9 position and correlation between H-2, H-4 and C-11 indicates COOH at 5″ position. Therefore, structure of 3 was assigned as shown in Fig. -1.

Optical rotations were measured using a JASCO DIP 300 digital polarimeter and at 1 ml cell at 25°C. IR spectra were recorded on a Nicolet-740 spectrometer with KBr pellets. The NMR spectra were recorded on a Bruker FT-600 MHz spectrometer at 600 MHz for 1H NMR and 150 MHz for 13C NMR respectively, using TMS as internal standard. The chemical shifts are expressed as δ values in parts per million (ppm) and the coupling constants (J) are given in hertz (Hz). Mass spectra were performed on a LC-MS/MS (Agilent Technologies 6510) Q-TOF Mass spectrometer. The 2D experiments (1H-1H COSY, HSQC, HMBC, NOESY) were performed using standard Bruker microprograms. Column chromatography was performed with silica gel (100-200 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Analytical TLC was performed on precoated Merck plates (60 F₂₅₄, 0.2mm) and compounds were viewed under a UV lamp (254 and 365 nm) and sprayed with 10% H₂SO₄, followed by heating.

The bark of Walsura trifoliata were collected from the tirumula forest Area, chittoor district and were identified by taxonomist, Dr. K. Madva Chetty, Sri Venkateswara University, Tirupati. The Voucher specimen was deposited in the laboratory.

The areal parts of Walsura trifoliata (2 kg) were powdered, and extracted with methanol at room temperature for 48 h. The resulting methanol extract was evaporated to dryness under reduced pressure to give syrupy residue (5 g). This residue was then suspended in H₂O (500ml) and extracted with ethyl acetate to give 2 g of ethyl acetate soluble portion, which was further subjected to column chromatography (silica gel, 100-200 mesh, eluting with CHCl₃/MeOH mixture of increasing polarity) to give 30 fractions. All the column fractions were systematically analyzed by TLC and fractions with similar TLC patterns were combined to give three major fractions (F₁, F₂ and F₃). Repurified fraction of F₂ on silica gel (100-200 mesh & 230-400 mesh) with the elution of CHCl₃: MeOH (90:10) yielded compound 1 (16 mg). Similarly, fraction F₃ subjected to CC by the gradient elution of CHCl₃ and MeOH to give two sub fractions A1 and A2. The sub fraction A1 was purified by flash chromatography with the elution of CHCl₃: MeOH (90:10) to yield compound 2 (20 mg). Sub fraction A2 was purified by the flash chromatography using CHCl₃: MeOH (80:20) as an eluent to give compound 3 (20 mg).

3.4. Spectral data
3.4.1. chrysophanol (1) [10]
Yellow powder. ESI-MS 253.4 [M+H]+. FT-IR (KBr), ν, cm⁻¹: 3469, 1719, 1633, 1525 and 995. 1H NMR (MeOH-d₄, 500 MHz): 12.04 (1H, s, OH-I), 11.97 (1H, s, OH-I), 7.76 (1H, dd, J = 0.76 and 7.52, H-5), 7.60 (1H, d, J = 8.1, H-6), 7.59 (1H, d, J = 0.4, H-4), 7.23 (1H, dd, J = 0.74 and 8.4, H-...
7. 7.04 (1H, d, J = 0.4, H-2), 2.40 (3H, s, H-3). 13C NMR (MeOH-d4, 125 MHz: 162.1(C-1), 124.3(C-2), 148.9 (C-3), 121.4 (C-4), 119.3 (C-5), 124.5 (C-6), 136.7 (C-7), 162.1 (C-8), 192.1 (C-9), 181.3 (C-10), 22.0 (C-11), 115.2 (C-4'), 115.5 (C-8'), 107.7 (C-9'), 134.9 (C-9').

3.4.2: Compound 2
Yellow gum. [α] D25 = -11.640 (c 0.05 (CH₃)₂CO). FT-IR (KBr), ν, cm⁻¹: 3444, 2926, 1633, 1248, 1111 and 762. HRMS (ESI +) m/z 453.2564 [M + H]⁺, C₂₉H₃₂O; calcd. ----. 1H NMR (MeOH-d4, 500 MHz): 4.87 (1H, brs, H-2), 4.42-4.48 (1H, m, H-3), 2.94 (2H, dd, J = 16.9, 5.0, H-4), 6.25 (1H, s, H-7), 6.80 (1H, s, H-2'), 6.98 (1H, s, H-4'), 6.80 (1H, s, H-6'), 3.09 (2H, dd, J = 7.3, 16.9 Hz, H-2''), 4.45 (3H, s, H-3''), 6.61 (1H, H-5''), 6.67 (1H, d, J = 8.1 Hz, H-8''), 6.43 (1H, dd, J = 8.1, 1.8 Hz, H-9''). 13C NMR (MeOH-d4, 125 MHz): 79.2(C-2), 66.2(C-3), 29.6(C-4), 105.6(C-5), 151.5(C-6), 96.2(C-7), 156.8(C-8), 104.8(C-9) , 152.9(C-10), 131.3(C-1'), 118.8(C-2'), 145.5(C-3'), 114.6(C-4'), 145.3(C-5'), 115.7(C-6'), 170.5(C-1''), 38.2(C-2''), 34.9(C-3''), 134.9(C-4''), 114.7(C-5''), 145.7(C-6''), 144.6(C-7''), 116.2(C-8''), 118.9(C-9'').

3.4.3. Compound 3
Pale yellow solid. [α] D25 = -22.540 (c 0.08 (CH₃)₂CO). FT-IR (KBr), ν, cm⁻¹: 3428, 3065, 2556, 1764, 1701, 1637, 1491 and 894. HRMS (ESI +) m/z 465.1013 [M + H]⁺, C₂₂H₂₃O; calcd. --. 1H NMR (MeOH-d4, 500 MHz): 6.20 (1H, d, J = 1.78 Hz, H-2), 6.40 (1H, d, J = 1.78, Hz, H-4), 7.59 (1H, m, H-6), 7.58 (1H, m, H-7), 6.80 (1H, s, J = 8.73Hz, H-8), 5.45 (1H, d, J = 7.3 Hz, H-1'), 3.22 (3H, s,H-2'), 3.12(H-3'), 3.22(H-4'), 3.12(H-5'), 3.58 (1H, d, J = 11.58 Hz, H-6') 13C NMR (MeOH-d4, 125 MHz): 121.05(C-1a), 161.12(C-1), 98.61(C-2), 103.81(C-3), 93.47(C-4), 156.23(C-4a), 156.23(C-5), 148.39(C-5a), 116.08(C-6), 121.5(C-7), 115.11(C-8), 144.72(C-8a), 133.20(C-9), 177.31(C-10), 164.21(C-11), 100.77(C-1'), 69.83(C-2'), 76.40(C-3'), 77.48(C-4'), 73.94(C-5'), 60.87(C-6').

4. Conclusions
In this paper, we have described the isolation of two new phenolic constituents (2 and 3) along with known compound, chrysophanol (1) from the *walsura trifoliata*. The structures were elucidated on the basis of extensive NMR spectroscopic and MASS analyses.

5. References