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Chemical constituents and mushroom tyrosinase inhibition activity of *Chloroxylon swietenia* leaves

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From the leaves of *Chloroxylon swietenia* DC., 5 known secondary metabolites, namely 6, 8-diprenylumbelliferone (**1**), bergaptan (**2**), isopimpinellin (**3**), tritriacontanol (**4**), and isoquercetrin (**5**), were isolated from a folklore medicinal plant. Three compounds were isolated for the first time from this genus. The structures of these compounds were elucidated by UV, IR, 1D and 2D NMR techniques. Different fractions from the leaves of *C. swietenia* were investigated for their tyrosinase inhibition activity.

Key Words: *Chloroxylon swietenia*, coumarins, fatty alcohol, flavonoid glycoside, tyrosinase inhibition activity.

Introduction

Chloroxylon swietenia (Roxb) belongs to the family Rutaceae and is known as Bherul and Bhirra Giriya in Hindi and satinwood in English. It is a moderate sized tree, 9-15 m in height and 1.0-1.2 m in girth and is common in dry, deciduous forests throughout peninsular India.¹ The decoction of the leaves is used as a lotion for ulcers and for healing abrasions of the skin and the smoke from burning leaves is used to drive ticks out of stables. The decoction of the bark is astringent and used in contusions and for painful joints.¹ The leaves are also prescribed for rheumatism.^{1,2} The plant contains an alkaloid, a powerful irritant, and causes dermatitis when applied to the skin.² Previous reports on this plant occurring in different regions of India yielded various secondary metabolites such as mono and sesqui terpenoids, phenolic derivatives, alkaloids, and lignans. The

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bark samples of *Chloroxylon swietenia* collected from 3 different sources yielded 9 new coumarins.³ Thirty-one coumarins were reported from *Citrus* plants, which showed anti-tumor promoting activity.⁴ Two cytotoxic coumarins, bergaptan, and isopimpinellins were reported from the roots of *Angelica dahurica*.⁵ Coumarins were reported from the roots of *Clausena indica* collected from India.⁶ Recently, one member of our group compiled and published the total chemical constituents and biological activities of the plant *C. swietenia*.⁷

Based on our continuing interest on the isolation of bioactive secondary metabolites from plants for personal care applications,⁸⁻¹¹ we undertook a chemical examination of the leaves of *Chloroxylon swietenia* DC. We report here the isolation and structure elucidation of 5 known compounds, **1-5**, of which compounds **1**, **4**, and **5** were isolated for the first time from this plant. The structures of these compounds were deduced by comparison of their spectral data with those reported in the literature. The present paper describes the tyrosinase inhibition activity and isolation of chemical constituents from active fractions.

Experimental

General experimental procedures: Melting points were reported are uncorrected. ¹H-NMR, ¹³C-NMR, HMQC, and HMBC spectra were recorded in CDCl₃ and CD₃OD on a Bruker spectrometer, operating at 400 MHz for ¹H-NMR and 100 MHz for ¹³C-NMR. ESI mass was recorded on Jeol SX 102/DA 600 mass spectrometer. IR spectra were recorded on a Shimadzu IR Prestige 21. UV spectra were recorded on Shimadzu UV spectrophotometer. Column chromatography (CC) was carried on a silica gel column (100-200 mesh) and Sephadex LH-20 (Amersham Biosciences) column. Purity of the samples was checked by TLC on pre-coated aluminium sheets, silica gel 60 F₂₅₄ (20 × 20 cm, 0.2 mm thickness, Merck) and compounds were detected under UV light (254 and 366 nm) and spraying with 5% sulphuric acid in methanol followed by heating the plates at 110 °C for 5 min. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. For the enzyme inhibition assay, all chemicals and kojic acid were purchased from Sigma (USA).

Plant Material: The leaves of *Chloroxylon swietenia* (2.5 kg) were collected in April 2006 from Krishnagiri, Tamil Nadu (India), and were dried in the shade. The plant was identified by Dr. P. Santhan, Plant Taxonomist, Durva Herbal Centre, Chennai, India. A voucher specimen of this plant was deposited in Cavinkare Research Centre, Chennai, India.

Extraction and Isolation of compounds

The shade dried leaves of *Chloroxylon swietenia* (235 g) were exhaustively extracted with methanol (2.5 L) by using a soxhlet apparatus. After evaporation of the solvent under reduced pressure, 70 g of crude extract was obtained. The crude methanolic extract was suspended in methanol:water (2:8), followed by fractionation with hexane, chloroform, ethyl acetate, and saturated n-butanol to get corresponding fractions 21.77 g, 6.87 g, 0.44 g, and 3.20 g, respectively. The hexane and n-butanol fractions showed good tyrosinase inhibition activity, whereas chloroform and ethyl acetate fractions showed moderate activity.

The resulting dark green residue of hexane fraction (21.77 g) was subjected to CC on silica gel (100-200 mesh, 200 g) and the column was eluted with about 7 L of petroleum ether:ethyl acetate mixtures with increasing polarity (from 100: 0 to 10:90, respectively). Combined homogeneous fractions were based on the visualisation of spots on the TLC plate and divided into 4 major fractions (A to D). Compounds **1** (120 mg,

pet. ether: EtOAc, 90:10 from fraction A), **2** (80 mg, pet. ether: EtOAc, 85:15 from fraction B), **3** (20 mg, pet. ether: EtOAc, 70:30 from fraction C), and **4** (90 mg, pet. ether: EtOAc 65:35 from fraction D) were isolated from these column fractions by using repeated column chromatography (silica gel, 100-200 mesh, 500 g) and followed by fractional crystallization techniques. Similarly, an aliquot of n-butanol fraction (3.20 g) was subjected to Sephadex LH-20 by using water:methanol (9:1, 1:1) to methanol and yielded 3 major fractions (E-G). Compound **5** (30 mg, methanol) was isolated from fraction G by repeated purification over Sephadex LH-20 column techniques using methanol.

Results

6, 8-Diprenylumbelliferone (1)

mp: 230 °C. UV (CHCl₃)λ_{max}: 335, 263, 253, and 242 nm; IR (KBR) ν_{max}: 1705 cm⁻¹ (C=O), 1690 and 1620 cm⁻¹ (aromatic), EIMS m/z 298 [M]⁺ (calc. for C₁₉H₂₂O₃). ¹H-NMR (CDCl₃, 400 MHz): δ 1.76 (6H, *s*, H-5', H-5''), 1.80 (3H, *s*, H-4'), 1.86 (3H, *s*, H-4''), 3.35 (2H, *d*, *J* = 7.2 Hz, H-1'), 3.62 (2H, *d*, *J* = 7.2 Hz, H-1''), 5.26 (1H, *t*, *J* = 7.2 Hz, H-2'), 5.30 (1H, *t*, *J* = 7.2 Hz, H-2''), 6.22 (1H, *d*, *J* = 9.4 Hz, H-3), 7.07 (1H, *s*, H-5) and 7.59 (1H, *d*, *J* = 9.4 Hz, H-4). ¹³C-NMR (CDCl₃, 100 MHz): δ 161.5 (C-2), 114.2 (C-3), 144.0 (C-4), 112.2 (C-4a), 120.4 (C-5), 124.7 (C-6), 156.5 (C-7), 112.4 (C-8), 151.5 (C-8a), 28.8 (C-1'), 120.9 (C-2'), 135.8 (C-3'), 17.9 (C-4'), 25.7 (C-5'), 29.6 (C-1''), 120.4 (C-2''), 135.1 (C-3''), 17.8 (C-4''), 25.7 (C-5'').

Bergaptan (2)

mp: 188 °C. UV (CHCl₃)λ_{max}: 330, 263, 254, 240 nm, IR (KBR) ν_{max}: 1706 cm⁻¹ (C=O), 1688 and 1621 cm⁻¹ (aromatic), EIMS m/z 204 [M]⁺ (calc. for C₁₁H₈O₄). ¹H-NMR (CDCl₃, 400 MHz): δ 4.38 (3H, *s*, 5-OCH₃), 6.27 (1H, *d*, *J* = 9.8 Hz, H-3), 7.03 (1H, *d*, *J* = 2.3 Hz, H-9), 7.14 (1H, *s*, H-8), 7.60 (1H, *d*, *J* = 2.3 Hz, H-10) and 8.16 (1H, *d*, *J* = 9.8 Hz, H-4). ¹³C-NMR (CDCl₃, 100 MHz): 161.3 (C-2), 112.5 (C-3), 139.3 (C-4), 106.3 (C-4a), 149.5 (C-5), 112.6 (C-6), 158.3 (C-7), 93.8 (C-8) 152.7 (C-8a), 144.8 (C-10), 105.0 (C-9), 60.1 (C-5-OCH₃).

Isopimpinellin (3)

mp: 210 °C. UV (CHCl₃)λ_{max}: 325, 263, 253, 235 nm; IR (KBR) ν_{max}: 1705 cm⁻¹ (C=O), 1690 and 1620 cm⁻¹ (aromatic), EIMS m/z 234 [M]⁺ (calc. for C₁₂H₁₀O₅). ¹H-NMR (CDCl₃, 400 MHz): δ 4.17 (3H, *s*, 5-OCH₃), 4.18 (3H, *s*, 8-OCH₃), 6.29 (1H, *d*, *J* = 9.7 Hz, H-3), 7.01 (1H, *d*, *J* = 2.2 Hz, 3'-H to H-3'), 7.63 (1H, *d*, *J* = 2.2 Hz, 2'-H to H-2'), 8.13 (1H, *d*, *J* = 9.8 Hz, H-4). ¹³C-NMR (CDCl₃, 100 MHz): δ 160.5 (C-2), 112.8 (C-3), 139.4 (C-4), 107.6 (C-4a), 149.5 (C-5), 114.7 (C-6), 145.1 (C-7), 128.1 (C-8), 143.6 (C-8a), 144.3 (C-2'), 105.3 (C-3'), 60.8 (C-5-OCH₃), 61.9 (C-8-OCH₃).

Tritriacontanol (4)

mp: 89-90 °C. UV(CHCl₃)γ_{max}; 209 nm, IR(KBR) ν_{max}: 3450 cm⁻¹ (hydroxyl), ESIMS m/z 480, [M+H⁺] (calc. for C₃₃H₆₈O). ¹H-NMR (CDCl₃, 400 MHz): δ 0.88 (3H, *t*, *J* = 7.0 Hz, H-33), 1.28 (60H, *m*, H-3 to

32), 1.51 (2H, *t*, *J* = 6.9 Hz, H-2), 3.52 (2H, *t*, *J* = 6.7 Hz, H-1).

Isoquercetrin (5)

mp: 230 °C. UV(MeOH) λ_{\max} : 255, 272, 304, 362 nm; IR(KBR) ν_{\max} : 1705 cm^{-1} (C=O), 1690 and 1620 cm^{-1} (aromatic), EIMS *m/z* 464 [M]⁺ (calc. for C₂₁H₂₀O₁₂). ¹H-NMR (CD₃OD, 400 MHz): δ 3.20-3.70 (6H, *m*, H-2'', H-3'', H-4'', H-5'', H-6''), 5.25 (1H, *d*, *J* = 7.5 Hz, H-1''), 6.18 (1H, *d*, *J* = 2.0 Hz, H-6), 6.37 (1H, *d*, *J* = 2.0 Hz, H-8), 6.85 (1H, *d*, *J* = 8.5 Hz, H-5'), 7.58 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-6'), 7.70 (1H, *d*, *J* = 2.0 Hz, H-2'). ¹³C-NMR (CD₃OD, 100 MHz): δ 156.9 (C-2), 134.1 (C-3), 177.9 (C-4), 161.5 (C-5), 98.4 (C-6), 164.5 (C-7), 93.2 (C-8), 157.5 (C-9), 104.2 (C-10), 121.7 (C-1'), 116.0 (C-2'), 144.4 (C-3'), 148.3 (C-4'), 114.5 (C-5'), 121.6 (C-6'), 102.8 (C-1''), 74.2 (C-2''), 76.9 (C-3''), 69.7 (C-4''), 76.6 (C-5''), 61.0 (C-6'').

Mushroom Tyrosinase inhibition activity¹²

The tyrosinase inhibition activities of different fractions of methanolic extract along with its crude extract and kojic acid (control) were studied by enzyme (Mushroom tyrosinase EC1.14.18.1) assay. The assay method is most precise and reliable. The hexane and n-butanol fractions showed significant activity by reducing the formation of dopachrome, whereas chloroform and ethyl acetate fractions showed moderate activity (Table).

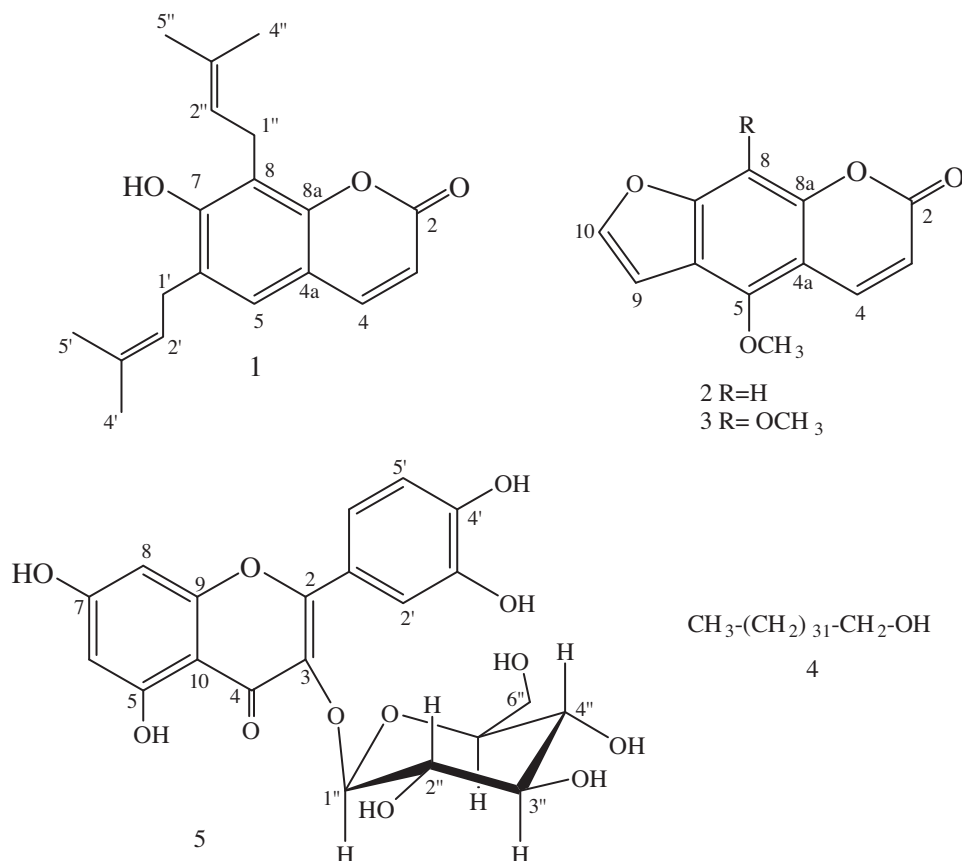


Figure. Structures of compounds 1-5 from *Chloroxylon swietenia*.

Table. In vitro tyrosinase inhibition activity of various fractions of *C. swietenia*.

Compound/fraction	Concentration ($\mu\text{g/mL}$)	% Inhibition
Methanolic extract	69	17
Hexane	69	41
Chloroform	73	25
Ethyl acetate	135	44
n-Butanol	73	45
Kojic acid (Control)	1.7	50

Discussion

The present study on the leaves of *Chloroxylon swietenia* of Indian origin resulted in the isolation and characterisation of compounds **1-5** (Figure). The structure of these compounds were identified on the basis of spectroscopic data and comparison with the literature. They were found to be 6, 8-diprenylumbelliferone (**1**),¹³ bergaptan (**2**),^{5,14} isopimpinellin (**3**),^{5,14,15} tritriacontanol (**4**),¹⁶ and isoquercetrin (**5**).^{17,18} Three of the compounds (**1, 4, 5**) were isolated from this plant for the first time. The crude extract and its fractions showed tyrosinase inhibition activity.¹² The hexane and n-butanol fractions showed more activity than chloroform and ethyl acetate fractions.

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