Isolation of Antidiabetic Principle from *Bougainvillea spectabilis* Willd (Nyctaginaceae) Stem Bark

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Abstract

**Purpose:** To isolate and identify the constituents of *Bougainvillea spectabilis* Wild (Nyctaginaceae) stem bark.

**Methods:** The methanol extract of *Bougainvillea spectabilis* stem bark powder was suspended in water and extracted with dichloromethane (CH$_2$Cl$_2$), ethyl acetate (EtOAc), and butanol (BuOH) successively. The ethyl acetate fraction was loaded in a column packed with silica gel and eluted with a gradient of chloroform (CHCl$_3$): methanol (MeOH), and water yielded five fractions (A - E). Chemical constituents were isolated by repeated column chromatography of these fractions.

**Results:** Column chromatography of fractions B and C afforded four compounds identified as pinitol, β-sitosterol, quercetin and quercetin-3-O-α-L-rhamnopyranoside. For the first time, pinitol, β-sitosterol, quercetin and quercetin-3-O-α-L-rhamnopyranoside were isolated from the stem bark of *B. spectabilis* Willd.

**Conclusion:** An antidiabetic principle, pinitol, was successfully isolated from the stem bark of *B. spectabilis* Willd.

**Keywords:** *Bougainvillea spectabilis*, Column chromatography, Pinitol, Quercetin, Quercetin-3-O-α-L-

rhamnopyranoside.

INTRODUCTION

Diabetes is one of the major causes of premature illness and death worldwide. The prevalence of diabetes has reached epidemic proportions. World Health Organization predicts that developing countries will bear the brunt of this epidemic in the present 21st century. Currently available treatments for diabetes are expensive and not easily accessible in developing countries such as India. Therefore, WHO has recommended continuous search for new antidiabetic agents from plants and other natural resources [1]. Herbal products are gaining popularity in developing countries due to their lesser side effects and easy availability [1,2].

*Bougainvillea spectabilis* Wild (Nyctaginaceae) is a potential herbal drug candidate for the treatment of diabetes [3]. *Bougainvillea spectabilis* Willd is commonly known Bougainvillea, Great Bougainvillea with the local Indian names as Booganbel, Cherei, Baganbilas, Booganvel, Bouganvila, Kagithala Puvvu [4]. Phytoconstituents such as flavonoids, phenolic compounds, antiviral [5], ribosome inactivating protein [6], amylase inhibitors [7], oxidase [8] and pinitol [9] have been isolated from *B. spectabilis*. 
The potent antihyperglycemic activity of its leaf, root and bark extracts have been reported [10,11].

EXPERIMENTAL

General experimental procedures

Spectrophotometric (Shimadzu UV 1800) evaluation of each compound was determined in MeOH and after addition of different shift reagents such as aluminum chloride (AlCl₃), AlCl₃/ hydrochloric acid (HCl), sodium acetate (CH₃COONa), CH₃COONa/ boric acid (H₃BO₃) and sodium methoxide (NaOMe). IR spectra were recorded on a Jasco FTIR-4000 spectrometer in KBr pellets and are expressed in cm⁻¹. The ¹H and ¹³C NMR spectra were obtained with a Bruker Avance 400 MHz spectrometer and chemical shifts (ppm) were related to tetramethylsilane (TMS, CH₃)₃Si as internal standard. Elemental analysis was carried on CHNSO analyser (Thermofinnigan-Flash EA 1112 series). Electrospray ionization (ESI) mass spectra were recorded on a Micromass Quattro II spectrometer in KBr pellets and are expressed in mass/charge units. The purity of the compounds was determined by TLC plates using iodine vapors as visualizing agent.

RESULTS

The stem bark extract showed the presence of glycosides, saponins, alkaloids, steroids and tannins. The compounds (1-4) were isolated by column chromatography of the EtOAc fractions (A - E) of the MeOH extract on silica gel, ODS, and Sephadex LH-20. Repeated column chromatography of fraction B on silica gel (CHCl₃: MeOH, 9:1) afforded compound 1 (107 mg) and 2 (133 mg). Repeated column chromatography of fraction C on Sephadex LH-20 (CHCl₃: MeOH, 9:1) and ODS column (MeOH: H₂O, 1:1) afforded compound 3 (109 mg) and 4 (103 mg) (Fig 1).

(+)-Pinitol (1)

Colour: White powder, Rₚ value: 0.62 (EtOAc: MeOH, 3:2), m.p.; 182-185°C, Anal Calcd for C₆H₁₄O₅: C, 37.11; H, 7.22; O, 49.48 %. Found C, 37.22; H, 7.26; O, 49.53 %. IR (KBr in cm⁻¹): 3450 (O-H str, broad); 2950 (C-H str); 1250 (C-O-C str); 1050 (C-O-O str). ¹H NMR (CD₂OD) δ: δ 3.92 (2H, d, J = 9.6 Hz, H-18 & 16), 3.72 (1H, d, J = 9.6 Hz, H-5), 3.72 (1H, d, J = 9.6 Hz, H-2), 3.62 (1H, t, J = 9.6 Hz, H-4), 3.29 (1H, t, J = 9.6 Hz, H-3), 3.65 (3H, s, J = 9.6 Hz, OCH₃). ¹³C NMR (CD₂OD) δ: 85.93 (C-1), 74.32 (C-5), 73.75 (C-3), 73.47 (C-6), 72.56 (C-2), 72.04 (C-4), 60.75 (OCH₃). MS (m/z): 194 (M⁺).

Stigmast-5-en-3β-ol (β-Sitosterol) (2)

Colour: White powder, Rₚ value: 0.39 (MeOH: H₂O: CHCl₃, 100:10:7.5), m.p.; 136-138°C, Anal
Calcd for C_{29}H_{50}O (414.71): C, 83.96; H, 12.06; O, 3.86 %. Found C, 84.03; H, 12.09; O, 3.91 %.

IR (KBr in cm⁻¹): 3549 (O-H str); 2935 (C-H str); 1638 (C=C str); 1460 (C=C str); 1063 (C-O str). ¹H NMR (CDCl₃) δ: δ 5.36 (1H, t, J = 6Hz, H-6), 3.20 (1H, m, H-3), 1.06 (3H, s, H-21), 0.99 (3H, s, H-19), 0.87 (3H, d, =J=6.0Hz, H-27), 0.84 (3H, d, =J=6.2Hz, H-26), 0.77 (3H, s, H-18). ¹³C NMR (CDCl₃) δ: 37.33 (C-1), 31.63 (C-2), 69.51 (C-3), 41.98 (C-4), 140.17 (C-5), 119.94 (C-6), 31.15 (C-7), 31.81 (C-8), 49.57 (C-9), 36.74 (C-10), 21.66 (C-11), 39.80 (C-12), 41.98 (C-13), 56.04 (C-14), 24.19 (C-15), 28.60 (C-16), 55.41 (C-17), 11.36 (C-18), 19.30 (C-19), 36.74 (C-20), 18.75 (C-21), 33.30 (C-22), 25.73 (C-23), 45.14 (C-24), 29.15 (C-25), 20.37 (C-26), 19.30 (C-27), 23.56 (C-28), 11.03 (C-29); MS (m/z): 414 (M⁺).

3, 5, 7, 3', 4' Pentahydroxyflavone (Quercitin) (3)

Colour: Yellow crystals, Rᵣ value: 0.24 (CHCl₃: MeOH, 9:1). m.p.: 179-180 °C. Anal Calcd for C_{13}H_{10}O₇ (302.24): C, 56.20; H, 4.46; O, 39.25 %. Found C, 57.05; H, 4.92; O, 39.33 %. UV (λ max EtOH) nm: 352. IR (KBr in cm⁻¹): 3972 (O-H str, broad), 1656 (C=O str), 1261 (C=O str, broad), 1637 (C=O str), 1135 (C=O str, broad), 843 and 705 (Aromatic system). ¹H NMR (CDCl₃) δ: 6.83 (1H, d, =J=2.0Hz, H-6), 6.21 (1H, d, =J=2.0Hz, H-8), 7.35 (1H, d, =J=2.0Hz, H-2), 6.93 (2H, d, =J=8.2Hz, H-2'), 7.32 (1H, dd, =J=2.0, 8.2 Hz H-6'). ¹³C NMR (CDCl₃) δ: 157.33 (C-2), 35.06 (C-3), 178.64 (C-4), 158.13 (C-5), 98.63 (C-6), 164.67 (C-7), 93.54 (C-8), 104.9 (C-4a), 162.02 (C-8a), 121.80 (C-1'), 115.77 (C-2'), 145.21 (C-3'), 148.60 (C-4'), 115.77 (C-5'), 121.71 (C-6'), 102.37 (C-1''), 72.94 (C-2''), 70.94 (C-3''), 70.85 (C-4''), 70.73 (C-5''), 16.47 (C-6''). MS (m/z): 448 (M⁺).

DISCUSSION

Compound 1 was isolated as a white powder having m.p. of 182 – 185 °C with a molecular formula of C_{13}H_{18}O_{11} based on mass spectral (m/z= 194) and ¹³C NMR data. ¹H and ¹³C NMR spectra showed signals for pinitol. The ¹H NMR spectrum exhibited two doublets at δ 3.92 and 3.78 (2.4 Hz each) for H-1 and H-5. The presence of one doublet at δ 3.72 (9.2 Hz) for H-6 and two triplet at δ 3.62 and 3.29 (9.6 Hz each) were ascribed to H-4 and H-3, respectively. One singlet at δ 3.65 indicates protons of OMe group. ¹³C NMR spectrum showed signals for 7 carbons including oxygenated carbon at δ 60.75 (OMe).
Isolation of compound 1 (pinitol) indicates the antihyperglycemic potential of the stem bark of B. spectabilis [13].

CONCLUSION

We have isolated anti-diabetic principle pinitol from the alcohol extract of Bougainvillea spectabilis stem bark. Pinitol possesses potent antihyperglycemic properties like insulin, as reported earlier. Phytoconstituents pinitol, β-sitosterol, quercetin, quercetin 3-O-α-L-rhamnopyranoside are reported for the first time as constituents of the stem bark of B. spectabilis. Isolation of the anti-diabetic principle, pinitol, from the stem bark of B. spectabilis further strengthens the ethnomedical use of this plant in various herbal formulations for the treatment of diabetes.

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