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 (CH₂Cl₂), 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 (CHCl₃): 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* Willd (Nyctaginaceae) is a potential herbal drug candidate for the treatment of diabetes [3]. *Bougainvillea spectabilis* Willd is commonly known as 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.*
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 (H₂BO₃) 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)₄Si 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 and chemical shifts (ppm) were obtained with a Brucker Avance 400 MHz spectrometer in KBr pellets and are expressed in ppm.

Plant material

The stem bark of B. spectabilis Willd (Nyctaginaceae), was collected in October 2010 from Mahoba district, UP, India. The plant was identified by a plant taxonomist, Dr AK Sharma, Department of Botany, Multanimal Modi (PG) College, Modinagar, Ghaziabad, India, and a voucher specimen (no. MMCM/02/013) deposited in the herbarium of Department of Botany, Multanimal Modi (PG) College, Modinagar, for future reference.

Extraction and isolation

Stem bark of B. spectabilis was air-dried under a shade and pulverized in electric grinder. The powdered bark (1.2 kg) was soaked in methanol, placed on a shaker for 24 h, filtered and concentrated at 45 °C. The weight of the crude extract obtained was 122.5 g (=10 % w/w yield). Phytochemical investigations of stem bark extract was carried out for the presence of alkaloids, flavonoids, tannins, glycosides, anthraquinones, saponins, reducing sugars, triterpenes and steroids according to standard quantitative and qualitative methods [12].

The MeOH extract (100 g) was suspended in H₂O (1 L) and extracted with hexane (1 L ×3) to give a hexane-soluble fraction (16 g). The aqueous layer was further extracted with CH₂Cl₂ (1 L ×3), EtOAc (1 L ×3), and BuOH (1 L ×3) successively. The yield of ethyl acetate fraction was 67 g which was higher than that of the other fractions, and hence was selected for column chromatography.

The column was packed with silica gel and eluted with a gradient of CHCl₃: MeOH, and H₂O to give five fractions, A – E, with yields of 1.2, 7.9, 15.4, 1.8 and, 0.9 g, respectively. Repeated column chromatography of fractions B and C afforded the compounds 1-4 (Fig. 1). 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 glycospirides, 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), Sephadex LH-20 (CHCl₃: MeOH, 9:1) and ODS column (MeOH: H₂O, 1:1) 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, 2:8) afforded compound 3 (109 mg) and 4 (103 mg) (Fig 1).

(+)-Pinitol (1)

Colour: White powder, Rf value: 0.62 (EtOAc: MeOH, 3:2), m.p.; 182-185°C, Anal Calcd for C₁₉H₁₄O₁₀ (194): 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 str). ¹H NMR (CD2OD) δ: δ 3.92 (2H, d, J = 9.6 Hz, H-16&); 3.79 (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⁺).

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

Colour: White powder, Rf value: 0.39 (MeOH: H₂O: CHCl₃, 100:10:7.5), m.p.; 136-138°C, Anal Calcd for C₂₃H₄₈O₃ (360): C, 79.28; H, 11.14; O, 9.58 %. Found C, 79.24; H, 11.13; O, 9.81 %.

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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}): 3349 (O-H str); 2935 (C-H str); 1638 (C=C str); 1460 (C-H bend alkane); 1063 (C-O str). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) = 5.36 (1H, t, \(J = 6\) Hz, H-6); 3.20 (1H, m, H-3); 1.06 (3H, s, H-21), 0.99 (3H, s, H-19), 0.87 (3H, d, \(J=6.0\) Hz, H-27), 29.55; 137.61(C-20), 18.75 (C-13), 24.19 (C-14), 26.60 (C-16), 55.41 (C-17), 11.36 (C-18), 19.30 (C-19), 19.30 (C-26), 19.30 (C-27), 23.56 (C-28), 11.03 (C-29); MS (m/z): 414 (M\(^+\)).

**DISCUSSION**

**Quercetin-3-O-α-L-rhamnopyranoside (Quercitin) (4)**

Colour: Yellow needles, \(R_f\) value: 0.24 (CHCl\(_3\); MeOH, 9:1), m.p.; 179-180°C. Anal Calcd for C_{42}H_{52}O_{11} (448.38): C, 56.20; H, 4.46; O, 39.25 %. Found C, 57.05; H, 4.92; O, 39.35 %. UV (\(\lambda_{max}\) EtOH) nm: 259, 314 (sh) and 352. IR (KBr in cm\(^{-1}\)): 3380 (O-H str, broad), 1656 (C=O conjugated), 1498 (C-C str), 1202 (C-O), 641-937 (aromatic systems). \(^1\)H NMR (CDCl\(_3\)) \(\delta\) = 6.38 (1H, \(J = 2.0\) Hz, H-6); 6.21 (1H, d, \(J = 2.0\) Hz, H-8), 7.35 (1H, d, \(J = 2.0\) Hz, H-2'), 6.93 (2H, dd, \(J = 8.2\) Hz, H-5'), 7.32 (1H, dd, \(J = 2.0, 8.2\) Hz H-6'). \(^13\)C NMR (CDCl\(_3\)) \(\delta\): 157.33 (C-2), 35.066 (C-3), 187.64 (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), 121.804 (C-1'), 115.773 (C-2'), 145.217 (C-3'), 148.601 (C-4'), 115.773 (C-5'), 121.713 (C-6'), 102.367 (C-1''), 72.949 (C-2''), 70.949 (C-3''), 70.851 (C-4''), 70.737 (C-5''), 16.475 (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_{29}H_{50}O based on mass spectral (m/z= 194) and \(^13\)C NMR data. \(^1\)H and \(^13\)C NMR spectra showed signals for pinitol. The \(^1\)H NMR spectrum exhibited two doublets at \(\delta\) 3.92 and 3.78 (2.4 Hz each) for H-1 and H-5. The presence of one doublet at \(\delta\) 3.72 (9.2 Hz) for H-2 and two triplet at \(\delta\) 3.62 and 3.29 (9.6 Hz each) were ascribed to H-4 and H-3, respectively. One singlet at \(\delta\) 3.65 indicates protons of OMe group. \(^13\)C NMR spectrum showed signals for 7 carbons including oxygenated carbon at \(\delta\) 60.75 (OMe),
Compound 2 is a white powder with a m.p. of 136 – 138 °C and IR absorptions bands (cm⁻¹) appeared at 3549 (O-H str), 2935.73 (C-H str), 1638 (C=O str), and 1063 (C-O str). El mass spectrum exhibited molecular ion peak as a base peak at m/z 414. The other prominent fragments were observed at m/z m/z: 396, 381, 329, 303, 268, 273, 255, 231, 199, 173, 159, 145, 119, 95, 81, 69, and 55. ¹H NMR spectrum showed methyl signals at 0.77 (H-18), 0.84 (H-26), 0.87 (H-27, 29), 0.99 (H-19) and 1.06 (H-21) ppm. The signals for H-3β and H-6 were found at 3.20 and 5.36 while ¹³C-NMR spectrum exhibited the presences of 29 carbon signals which were assigned as six methyl, eleven methylene, eight methyne, two quaternary carbons (36.74, 41.98) and two signals for methylene, eight methyne, two quaternary carbons including oxygenated aromatic carbons (140.17 and 119.94 ppm). The spectral data obtained are identical to those of β-sitosterol as described in the literature.

The compound 3 was identified as quercetin with a melting point of 315-317°C, IR spectrum bands was a characteristic feature of flavones. The UV spectrum of the ethanol solution of 3 exhibited two major absorption bands at 278 and 260 nm, which confirmed the flavonol structure. Degradation of 3 in the presence of MeONa and hypsochromic shifts with AlCl₃/HCl and AcONa/H₂O₄ supported the presence of 3, 3', 4' trihydroxy system. Bathochromic shifts with AcONa are related to 7-hydroxyl and the bathochromic shift with AlCl₃/HCl to 5-hydroxyl. MS spectrum showed molecular ion peak at m/z 302 which was also observed as a base peak which is a characteristic feature of flavones. The other prominent peaks were observed at 285, 274, 257, 245, 229, 217, 200, 153, 137, and 69. ¹H NMR of this compound showed five signals for methyne groups at δ 6.25, 6.43, 7.65, 6.89, and 7.74. The ¹³C NMR spectrum showed 15 signals which were assigned as five methyne and ten quaternary carbons. The spectral data obtained were identical to those of quercetin as previously described in the literature.

Compound 4 was isolated as a yellow powder (m.p. 179 – 180 °C) with a molecular formula of C₇₂H₁₂₀O₁₁, based on FAB MS (m/z=448) as well as ¹³C NMR data. Its ¹H and ¹³C spectra showed signals for quercitin. The ¹H NMR spectrum of this compound showed two peaks at 6.25 (1H, d, J = 2 Hz) and 6.43 δ (1H, d, J = 2 Hz) corresponding to H-6 and H-8 on ring A. Similarly peaks at δ 7.65 (1H, d, J = 2.5 Hz, H-2'), 7.74 (1H, dd, J = 8.5, 2.5 Hz, H-6' and 6.89 (1H, d, J = 8.5 Hz, H-5') were appeared due to the catechol protons on ring-B. The presence of a methyl doublet at δ 0.89 (J = 6.0 Hz) along with the anomeric proton doublet at δ 5.42 (J = 1.0 Hz) was indication of a rhamnopyranose moiety. ¹³C NMR spectrum showed resonance for 21 carbons including oxygenated aromatic carbons at δ 134.4 (C-3), 160.8 (C-5), 164.4 (C-7), 144.9 (C-3'), 148.1 (C-4') and 180.2 (C-4). The spectral data obtained are identical to quercitrin as previously described in the literature.

Isolation of compound 1 (pinitol) indicates the anthyperglycemic 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 antidiabetic 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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