Tropical Journal of Pharmaceutical Research October 2013; 12 (5): 761-765
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v12i5.15

Original Research Article

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

Sunil Jawla[1*], Yatendra Kumar[1] and Mohammad Sardar Yar Khan[2]
[1]*Department of Pharmaceutical Chemistry, I.T.S. Paramedical College (Pharmacy), Delhi-Meerut Road, Muradnagar, Ghaziabad, 201206.* [2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, (Jamia Hamdard) Hamdard University, New Delhi, India 110062.*

For correspondence:* ***Email:* *suniljawla@its.edu.in;* ***Tel:*** *+911232-225380;* ***Fax:*** *+911232-225380*

Received: 31 January 2012 *Revised accepted:* 7 July 2013

Abstract

Purpose: *To isolate and identify the constituents of Bougainvillea spectabilis Willd (Nyctaginaceae) stem bark.*
Methods: *The methanol extract of Bougainvillea spectabilis stem bark powder was suspended in water and extracted with dichloromethane (CH_2Cl_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.*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

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 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 ($AlCl_3$), $AlCl_3$/ hydrochloric acid (HCl), sodium acetate (CH_3COONa), CH_3COONa/ boric acid (H_3BO_4) 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 ^{13}C NMR spectra were obtained with a Brucker Avance 400 MHz spectrometer and chemical shifts (ppm) were related to tetramethylsilane (TMS, $(CH_3)_4Si$ as internal standard. Elemental analysis was carried on CHNSO analyser (Thermofinnigan-Flash EA 1112 series). Electrospray ionization (ESI) mass spectra were recorded on Micromass Quattro II while melting point was determined by differential scanning calorimeter (DSC 60, Shimadzu, Japan). Open column chromatography was carried out on silica gel, Sephadex LH-20 and octadecylsilyl (ODS) (Amersham Pharmacia Biotech Co., UK) as packing material and Whatmann no. 1 filter paper and TLC on silica gel 60 F_{254} sheets (Merck Co., Germany). All other chemicals used were of analytical reagent grade (Ranbaxy Fine Chemicals Ltd, India).

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 H_2O (1 L) and extracted with hexane (1 L ×3) to give a hexane–soluble fraction (16 g). The aqueous layer was further extracted with CH_2Cl_2 (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 $CHCl_3$: MeOH, and H_2O 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 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 ($CHCl_3$: MeOH, 9:1), Sephadex LH-20 ($CHCl_3$: MeOH, 9:1) and ODS column (MeOH: H_2O, 1:1) afforded compound **1** (107 mg) and **2** (133 mg). Repeated column chromatography of fraction C on Sepohadex LH-20 ($CHCl_3$: MeOH, 9:1) and ODS column (MeOH: H_2O, 2:8) afforded compound **3** (109 mg) and **4** (103 mg) (Fig 1).

(+)-Pinitol (1)

Colour: White powder, R_f value: 0.62 (EtOAc: MeOH, 3:2), m.p.; 182-185˚C, Anal Calcd for $C_7H_{14}O_6$ (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 (CD_3OD) δ: δ 3.92 (2H, d, *J* = 9.6 Hz, H-1&-6), 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, OCH_3). ^{13}C NMR (CD_3OD) 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 (OCH_3). MS (m/z): 194 (M^{+1}).

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

Colour: White powder, R_f value: 0.39 (MeOH: H_2O: $CHCl_3$, 100:10:7.5), m.p.; 136-138˚C, Anal

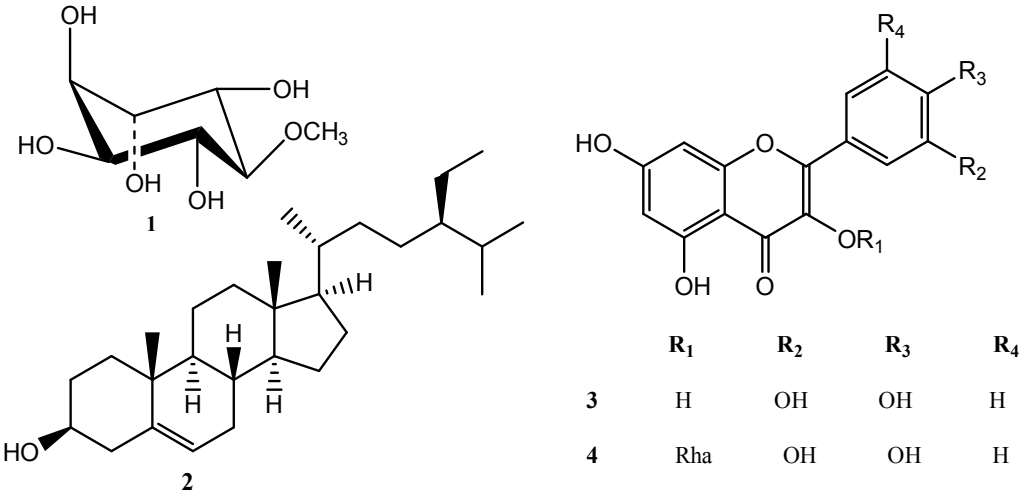

Fig 1: Compounds **1 - 4** (pinitol, β-sitosterol, quercetin, quercetin 3-*O*-α-L-rhamnopyranoside, respectively) isolated from the stem bark of *B. spectabilis.*

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-H bend alkane); 1063 (C-O str). ^{1}H NMR ($CDCl_3$) 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*=6.0 Hz, H-27, 29), 0.84 (3H, d, *J*=6.2Hz, H-26), 0.77 (3H, s, H-18). ^{13}C NMR ($CDCl_3$) 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^{+1}).

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

Colour: Yellow crystals, R_f value: 0.83 (n-butanol: acetic acid: water, 4:1:5), m.p.; 315-317˚C, Anal Calcd for $C_{15}H_{10}O_7$ (302.24): C, 59.55; H, 3.31; O, 37.06 %. Found C, 58.52; H, 3.29; O, 38.19 %. UV (λ_{max} MeOH) nm: 256, 268(sh), 301(sh), 374 and with shifting reagent NaOMe: 327 (dec), 248 (sh); $AlCl_3$: 457, 331, 272; $AlCl_3$/HCl: 426, 364, 301(sh), 267; NaOAc: 389 (dec), 327, 290, 261; NaOAc/ H_3BO_3: 385, 302, 261. IR (KBr in cm^{-1}): 3409 (O-H str), 1663 (C=O str), 1562 (C=C str), 1260 (C-O str), 1132 (C-O-C str), 843 and 705 (Aromatic system). ^{1}H NMR (CD_3OD) δ: 7.74 (1H, dd, *J* = 8.5, 2.5 Hz, H-6'), 7.65 (1H, dd, *J* = 8.5, 2.5 Hz, H-2'), 6.89 (1H, d, *J* = 8.5Hz, H-5'), 6.43 (1H, d, *J* = 2.1Hz, H-8), 6.25 (1H, d, *J* = 2.1Hz, H-6). ^{13}C NMR (CD_3OD) δ: 94.5(C-8), 99.5(C-6), 104.2(C-10), 116.5(C-5'), 116.1(C-2'), 121.8(C-6'), 123.6(C-2), 136.5(C-3), 137.61(C-1'), 145.7(C-3'), 148.1(C-4'), 156.7(C-9), 161.0(C-5), 166.7(C-7), 176.1(C-4). MS (m/z): 302 (M^{+1}).

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

Colour: Yellow needles, R_f value: 0.24 ($CHCl_3$: MeOH, 9:1), m.p.; 179-180˚C, Anal Calcd for $C_{21}H_{20}O_{11}$ (448.38): C, 56.20; H, 4.46; O, 39.25 %. Found C, 57.05; H, 4.92; O, 39.93 %. UV (λ_{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 ($CDCl_3$) δ: 6.38 (1H, d, *J* = 2.05Hz, H-6), 6.21 (1H, d, *J* = 2.05Hz, H-8), 7.35 (1H, d, *J* = 2.0Hz, H-2'), 6.93 (2H, d, *J* = 8.2Hz, H-5'), 7.32 (1H, dd, *J* = 2.0, 8.2 Hz H-6'). ^{13}C NMR ($CDCl_3$) δ: 157.33 (C-2), 35.066 (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), 121.804 (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^{+1}).

DISCUSSION

Compound **1** was isolated as a white powder having m.p. of 182 – 185 ˚C with a molecular formula of $C_7H_{14}O_6$ 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 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-2 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. ^{13}C NMR spectrum showed signals for 7 carbons including oxygenated carbon at δ 60.75 (OMe),

72.04 (C-4), 72.56 (C-2), 73.47 (C-6), 73.76 (C-3), 74.33(C-5), and 85.93 (C-1). The spectral data obtained were identical to pinitol previously described in the literature.

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=C str), and 1063 (C-O str). EI 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, 289, 273, 255, 231, 213, 199, 173, 159, 145, 119, 95, 81, 69, and 55. ^{1}H 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 δ3.20 and 5.36 while ^{13}C-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 olefinic double bonds at 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 315-137^{o}C, IR spectrum bands appeared at 3409 (O-H str), 1663 (C=O str), 1562 (C=C str) and 1260 (C-O str) cm^{-1}. The UV spectrum of the ethanol solution of **3** exhibited two major absorption bands at 374 nm and 256 nm, which confirmed the flavonol structure. Degradation of **3** in the presence of MeONa and hypsochromic shifts with $AlCl_3$/HCl and AcONa/H_3BO_4 supported the presence of 3, 3, 4' trihydroxy system. Bathochromic shifts with AcONa are related to 7-hydroxyl and the bathochromic shift with $AlCl_3$/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. ^{1}H NMR of this compound showed five signals for methyne groups at δ 6.25, 6.43, 7.65, 6.89, and 7.74. The ^{13}C 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 $C_{21}H_{20}O_{11}$, based on FAB MS (*m/z*=448) as well as ^{13}C NMR data. Its ^{1}H and ^{13}C spectra showed signals for quercitrin. The ^{1}H 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. ^{13}C 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 antihyperglycemic potential of the stem bark of *B. spectabilis* [13].

CONCLUSION

We have isolated antidiabetic 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 ethnomedicinal use of this plant in various herbal formulations for the treatment of diabetes.

ACKNOWLEDGEMENT

The authors are thankful to I.T.S Paramedical College (Pharmacy), Muradnagar, Ghaziabad, Uttar Pradesh, India, for providing the required funds and facilities for carrying out this work.

REFERENCES

1. *WHO information: 1998 Press Releases [Internet]. WHO/63: Global burden of diabetes; [cited 1998 Sept 14]. Available from: http://who.int/inf-pr-1998/en/pr98-63.htm.*
2. *Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes, estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053.*
3. *Soumyanth A, editor. Traditional Medicine for Modern Times: Antidiabetic Plants, London: Taylor and Francis Group; 2006; p 19.*
4. *Nadkarni AK, editor. The Indian Materia Medica. Bombay: Popular Prakashan; 2002. 822 p.*
5. *Balasaraswathi R, Sadasivam S, Ward M, Walker JM. An antiviral protein from Bougainvillea spectabilis roots; Purification and characterization. Phytochem 1998; 47(8): 1561-1565.*
6. *Bolognesi A, Fabiola P, Paola V, Luigi B, Giulia BM, Carusi VM, Benvenuto E, Francesca DVB, Antimo DM, Augusto P, Mario DL, Fiorenzo S. New ribosome-inactivating proteins with polynucleotide: adenosine glycoside and antiviral activities from*

Basella rubra L. and Bougainvillea spectabilis wild. Planta 1997; 203: 422-429.

7. *Bhat M, Zinjarde SS, Bhargava SY, Kumar RA, Joshi BN. Antidiabetic Indian plants: a good source of potent amylase inhibitors, 2008 [cited 2008 July 28]. Available from: http://downloads.hindawi.com/ journals/ecam/aip/810207.v1.pdf.*
8. *Srivastava SK, Krishnan PS. An Oxalic acid oxidase in the leaves of Bougainvillea spectabilis. Biochem J 1962; 85: 33-38.*
9. *Narayanan CR, Joshi DD, Mujumdar AM, Dhekne VV. Pinitol, a new antidiabetic compound from the leaves of Bougainvillea spectabilis. Curr Sci 1987; 56: 139–41.*
10. *Narayanan CR, Joshi DD, Majumdar AM. Hypoglycemic action of Bougainvillea spectabilis leaves. Curr Sci 1984; 53: 579-81.*
11. *Jawla S, Kumar Y, Khan MSY. Hypoglycemic potential of B. spectabilis root bark in normal and alloxan induced diabetic rats. Pharmacologyonline 2011; 3: 73-87.*
12. *Evans WC, Ed. Trease and Evans Pharmacognosy. New Delhi: Sounders - An imprint of Elsevier, 2002.*
13. *Bates H, Jones RB, Bailey CJ. Insulin-like effect of pinitol. Br J Pharmacol 2000; 130 (8): 1944-1948.*