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Isolation, characterization and chemobiological quantification of α-glucosidase enzyme inhibitory and free radical scavenging constituents from *Derris scandens* Benth

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Abstract

The hexane and chloroform extracts of *Derris scandens* have displayed potent α-glucosidase inhibitory and moderate free radical scavenging activities. Phytochemical investigation of the active extracts led to the isolation of three new prenylated isoflavones, isoscandinone, scandenin A and scandenin B in addition to scandenone, scandinone and 4′, 5′, 7-trihydroxybiprenylisoflavone as the main constituents, having α-glucosidase enzyme inhibitory and free radical scavenging properties. A reversed-phase HPLC method is developed to quantify these active principles in the plant material, which can serve as an effective quality control method for standardization of *D. scandens*.

Keywords: *Derris scandens*; Isoflavones; α-Glucosidase inhibitors; High-performance liquid chromatography; Chemobiological standardization

1. Introduction

Derris scandens (Fabaceae) is known as *Gonj* (Hindi) in India. This plant is reported to possess anti-inflammatory, free radical scavenging [1], antibacterial, antihypertensive [2], immunomodulatory and anti-HIV properties [3]. Coumarins, prenylated isoflavones, and isoflavone glycosides have been previously reported from the stem of this plant [4–9]. In the course of our ongoing efforts to identify intestinal α-glucosidase inhibitors and free-radical scavengers from medicinal plants, we observed that the hexane and chloroform extracts of *D. scandens* plant powder displayed potent intestinal α-glucosidase inhibitory activity. Extensive chromatography of the chloroform extract led to the isolation of a new isoflavone and two new prenylated isoflavones along with several new α-glucosidase inhibitors and free radical scavengers. In continuation to our earlier efforts in preparing chemobiological standardization protocols [10] of Indian medicinal plants, we herein report the chemobiological standardization of α-glucosidase enzyme inhibitory and free radical scavenging constituents from *D. scandens*.

2. Experimental

2.1. Reagents and chemicals

HPLC grade acetonitrile, methanol, chloroform and water were supplied by Merck (Germany). The whole plant material of *D. scandens* was collected from the forest of Tirumala in Chitoor Dist. (Andhra Pradesh, India) in the month of January in 2005 and identification was done by Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupathi, India. Voucher specimen (DS-01-06) of the plant is deposited at the herbarium of the S.V. University, Tirupathi, India.

2.2. Extraction and isolation procedures

The shade dried plant material (2 kg) was powdered and extracted with hexane in a Soxhlet apparatus for 24 h. The solvent was evaporated under reduced pressure in a rotary flash

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evaporator to obtain a residue (20 g). The residue was adsorbed on silica gel and subjected to column chromatography over silica gel (100 cm × 40 mm, diametre). The column was subjected to elution with hexane first followed by mixture containing increasing amounts of acetone. The fractions eluted at 2% (1.5 L), 4% (3.75 L), 6% (1.5 L) and 10% (3.0 L) were collected separately and concentrated to obtain residues A (0.310 g), B (13.10 g), C (0.645 g) and D (4.72 g). The residue A (0.31 g) was loaded on a silica gel column (60–120 mesh, 50 cm × 10 mm, diametre) and eluted with 2% ethylacetate in hexane to obtain compound **1** (0.20 g). The residue B (13.10 g) was loaded on a silica gel column (60–120 mesh, 100 cm × 25 mm, diametre) and eluted with 3% ethylacetate in hexane to obtain compound **2** (12.0 g). The residue C (0.645 g) was loaded on a silica gel column (60–120 mesh, 30 cm × 10 mm, diametre) and eluted with 5% ethylacetate in hexane to obtain compound **3** (0.490 g). The residue D (4.72 g) was loaded on a silica gel column (60–120 mesh, 60 cm × 20 mm, diametre) and eluted with 6% ethylacetate in hexane to obtain compound **4** (3.50 g). On completion of the hexane extraction, the powdered plant material was extracted with chloroform to obtain 100 g of residue. The extract was dissolved in a 1:1 mixture of chloroform and methanol. The residue was adsorbed on silica gel and subjected to column chromatography over silica gel (60–120 mesh, 150 cm × 40 cm, diametre). The column was subjected to elution with hexane first followed by increasing polarities of hexane/acetone. The fractions eluted at 15% (4.5 L), 30% (2.75 L), 40% (1.5 L) and 50% (5.0 L) were collected separately and concentrated to obtain residues E (15.90 g), F (6.25 g), G (0.78 g) and H (23.0 g). The residue E (15.90 g) was loaded on a silica gel column (60–120 mesh, 100 cm × 35 mm, diametre) and eluted with 10% ethylacetate in hexane to obtain compound **5** (0.80 g), elution with 12% ethylacetate afforded compound **6** (6.0 g) and elution with 15% afforded compound **7** (7.0 g). The residue F (6.25 g) was loaded on a silica gel column (60–120 mesh, 50 cm × 20 mm, diametre) and eluted with 15% ethylacetate in hexane to obtain compound **8** (2.0 g) and compound **9** (1.5 g). Further elution of the column with 17% ethylacetate afforded compound **10** (0.50 g). The residue G (0.78 g) was loaded on a silica gel column (60–120 mesh, 30 cm × 15 mm, diametre) and eluted with 18% ethylacetate in hexane to obtain compound **11** (0.295 g). Further elution of the column with 22% ethylacetate led to the isolation of compound **12** (0.185). The residue H (23.0 g) was loaded on a silica gel column (60–120 mesh, 150 cm × 40 mm, diametre) and eluted with 25% ethylacetate in hexane to obtain compound **13** (20.0 g). Further elution of the column with 35% afforded compound **14** (0.245 g).

2.3. Bioassay procedures

2.3.1. α-Glucosidase inhibitory activity

Estimation of intestinal α-glucosidase inhibitory activity was carried out as reported earlier [11]. Rat intestinal acetone powder (Sigma Chemicals, USA) was sonicated properly in normal saline (100:1, w/v) and after centrifugation at 3000 rpm × 30 min the supernatant was treated as crude intestinal α-glucosidase. Ten microlitres of test samples dissolved in DMSO (5 mg/mL solution) were mixed and incubated with 50 μL of enzyme in a 96-well microplate for 5 min. Reaction mixture was further incubated for another 10 min with 50 μL substrate [5 mM, *p*-nitrophenyl-α-D-glucopyranoside, prepared in 100 mM phosphate buffer (pH 6.8)] and release of nitrophenol was read at 405 nm spectrophotometrically ($SPECTRA_{MAX}$ $PLUS^{384}$, Molecular Devices, USA). Percent α-glucosidase inhibition was calculated as $(1\text{-}B/A) \times 100$, where A was the absorbance of reactants without test compound and B was the absorbance of reactants with test samples. All the samples were run in triplicate and acarbose was taken as standard reference compound. Several dilutions of primary solution (5 mg/mL DMSO) were made and assayed accordingly to obtain concentration of the test sample required to inhibit 50% activity (IC_{50}) of the enzyme applying suitable regression analysis.

2.4. Free-radical scavenging activity

Free-radical (DPPH) scavenging activity assay procedure was adopted from previous report [12]. In a 96-well microplates, 25-μL test sample (1 mg/mL DMSO), 125 μL of 0.1M Tris–HCl buffer (pH 7.4) and 125 μL of 0.5mM DPPH (1, 1-diphenyl-2-picrylhydrazyl, Sigma Chemicals, USA, dissolved in absolute ethyl alcohol) were mixed and shaken well. After incubating 20 min in dark, absorbance was recorded spectrophotometrically ($SPECTRA_{MAX}$ $PLUS^{384}$, Molecular Devices, USA) at 517 nm. The free radical scavenging potential was determined as the percent decolourisation of DPPH due to the test samples and calculated as $(1 - B/A) \times 100$, where A is absorbance of DPPH control with solvent, and B is absorbance of decolorized DPPH in the presence of test compound. All the analysis was done in duplicate; trolox was taken as reference compound. Several dilutions of primary solution (1 mg/mL DMSO) were made and assayed accordingly to obtain concentration of the sample required to scavenge 50% (SC_{50}) of DPPH free radical applying suitable regression analysis.

2.5. HPLC analysis

In order to carry out exhaustive extraction of the bioactive isolates, the plant material (25 g) was extracted with methanol thrice (3 × 150 mL) by sonicating for a period of 15 min each time and the combined extract is concentrated under vacuum to get a residue of 3.8 g. The analysis of the residue was performed on an Agilent 1100series instrument (Waldbronn, Germany) equipped with a binary pump, auto injector G1329A ALS with a loop of 100 μL capacity and a PDA detector. The active molecules isolated from *D. scandens* were separated on a phenomenex aqua 250 mm × 4.6 mm i.d., 5 μ particle size. A gradient mixture of (A) water and (B) acetonitrile was used as mobile phase. The flow rate was maintained at 1.0 mL/min during the run. The gradient profile of solvent A and solvent B is given in Table 1. The detection of the compounds was performed using a photo diode array detector with a resolution of 2 nm. All the analyses were carried out at room temperature and the run time was 35 min. The individual bioactive isolates were detected at their respective

Table 1
Mobile phase gradient pattern

Time (min)	Solvent A water	Solvent B acetonitrile	Flow rate (mL/min)
3.0	30	70	1.0
6.0	25	75	1.0
18.0	20	80	1.0
22.0	20	80	1.0
25.0	30	70	1.0

absorption wavelengths (Table 3). Agilent Chemstation software was used for data acquisition and data processing.

2.5.1. Calibration standards

Individual stock solutions of bioactive compounds were prepared by dissolving appropriate amounts of each individual compound in acetonitrile to obtain the concentration of 0.5 mg/mL. These solutions were further diluted with mobile phase so as to obtain the concentration range from 5–100 μg/mL. Twenty microlitres of these solutions were injected thrice to draw the calibration curve.

2.6. Test sample

5.7 mg of methanol extract of *D. scandens* was dissolved in 10 mL mobile phase to obtain a concentration of 0.57 mg/mL. Twenty microlitres of this test sample was injected to obtain the HPLC chromatogram of crude extract.

3. Results and discussion

Phytochemical investigation of the whole plant of *D. scandens* led to the isolation of several compounds. Repeated chromatography of the bioactive hexane extract over silica gel led to the isolation of several known compounds which include, 4′-*O*-methylosajin (**1**) [13], osajin (**2**) [14], 4′-*O*-methylscandinone (**3**) [15] and 4′, 5′, 7-trihydroxybiprenylisoflavone (**4**) [8]. On the other hand, extensive chromatography of active chloroform extract led to the isolation of a new naturally occurring isoflavone isoscandinone (**5**) whose structure was confirmed by X-ray crystallography, two new prenylated isoflavones, scandenin A (**6**) and scandenin B (**7**) along with seven known compounds, scandenone (**8**) [15], scandinone (**9**) [15], lupalbigenin (**10**) [16], derrisisoflavone A (**11**) [7], derrisisoflavone C (**12**) [7], 4′,4-*O*-dimethylscandenin (**13**) [17] and scandenin (**14**) [18]. Structures of all the isolated compounds are depicted in Fig. 1.

Compound **5** was isolated as a pale yellow solid whose molecular formula was determined as $C_{26}H_{26}O_5$ from its FABMS [M^+ + H] *m/z* 419.0. The IR spectrum exhibited hydroxyl-stretching frequency at 3352 cm^{-1} and carbonyl stretching frequency at 1637 cm^{-1}. Its ^{13}C NMR spectrum indicated the presence of a carbonyl carbon at δ 177.0. It also displayed 15 signals due to the isoflavone skeleton including 2 intense signals at δ 115.5 and 130.0 assigned to two equivalent aromatic carbons of the symmetrical *para*-sustituted B-ring. The signal at δ 62.5 was assigned to a methoxy group based on DEPT and HSQC experiments. The ^1H NMR spectrum showed the characteristic signal of H-2 of an isoflavone at δ 7.85 (1H, s, H-2) and AA′ BB′ type signals at δ 7.35 (2H, d, *J* = 8.7 Hz), 6.85 (2H, d, *J* = 8.7 Hz), assignable to H-2′, 6′ and H-3′, 5′, respectively of the B-ring together with typical signals due to 3,3-dimethylallyl group [δ 3.43 (2H, d, *J* = 7.0 Hz, H-1‴), 5.10 (1H, t, *J* = 7.0 Hz, H-2‴), 1.73 (3H, s, H − 4‴) and 1.53 (3H, s, H − 5‴)]. The chromene methyl groups appeared as a singlet integrating for six protons at δ 1.45 (6H, s, H-4″ and H-5″). Thus compound **5** was identified as 5-methoxyscandenone. Compound **5** was found to be isomeric with scandinone [7]. However, in scandinone (**9**), the chromene ring is angular in position where as in compound **5**, the chromene ring is found to be linear. Hence, compound **5** is named as isoscandinone. Crystallography studies (Cambridge Crystallography Data Center no.275210) confirmed the structure assigned to isoscandinone (**5**)

Compound **6** is a white amorphous solid whose molecular formula was determined to be $C_{27}H_{28}O_6$ from FABMS [M^+ + H] *m/z* 449. Its IR spectrum showed the presence of hydroxyl and carbonyl groups at 3298 and 1688 cm^{-1}, respectively. The 200 MHz ^1H-and ^{13}C NMR spectra of this compound suggested it to contain a 3-phenylcoumarin system. The ^{13}C NMR and DEPT experiments indicated the presence of 27 carbons of which 4 methyls, 2 methylenes, 7 methines and 13 quaternary carbons are present. ^{13}C NMR spectrum also showed 13 signals between δ 110.5 and 158.5 and a carbonyl group at δ 160.5 indicating the general nature of a 3-phenylcoumarin skeleton. Intense signals at δ 113.1 (C-3′ and C-5′) and 131.2 (C-2′ and C-6′) were assigned to two equivalent aromatic carbons of a symmetrical B-ring, and two signals at δ 55.5 and 64.5 were assigned to two methoxyl groups. The remaining five signals were identified to be signals of a prenyl group. The ^1H NMR spectrum showed two doublets integrating for one proton each at δ 6.50 and δ 5.75 indicating a chromene ring system. AA′ BB′ type signals at δ 7.43 (2H, d, *J* = 8.7 Hz) and 6.95 (2H, d, *J* = 8.7 Hz) were assignable to H-2′, 6′ and H-3′ 5′ of the B-ring, respectively, together with typical signals due to a 3,3-dimethylallyl group [δ 3.43 (2H, d, *J* = 7.0 Hz, H − 1‴), 5.10 (1H, apparent triplet, *J* = 7.0 Hz, H − 2‴), 1.73 (3H, s, H − 4‴) and 1.53 (3H, s, H − 5‴)]. In addition, the ^1H NMR spectrum showed two methoxy groups at δ 3.90 (3H, s, H-4′) and 3.98 (3H, s, H-5) along with enolic hydroxyl group at δ 9.82 [1H, s, C-4 (OH)]. Based on HSQC and HMBC correlations it was evident that chromene ring and prenyl group were fused to the parent structure at C-7, 8 and C-6, respectively. Complete HMBC data confirmed the structure of compound **6** as 4′-*O*-methylscandenin and was named as scandenin A. Though this compound was previously synthesized [9], its natural occurrence and complete spectral data are being presented for the first time in this study.

Compound **7** was isolated as a pale yellow amorphous solid with fluorescence under UV at 365 nm. The molecular formula was determined as $C_{26}H_{26}O_7$ from FABMS [M^+ + H] *m/z* 451.0. The IR spectrum indicated absorption bands at 3410, 3250, 2926, 1682, 1515, 1219 and 834 cm^{-1} suggesting the presence of hydroxyl, carbonyl and general nature of an aromatic compound. The ^1H NMR spectrum showed hydroxyl signal at δ 9.81 (1H, s, Ar–OH) and δ 7.80 (1H, s, Ar–OH), and

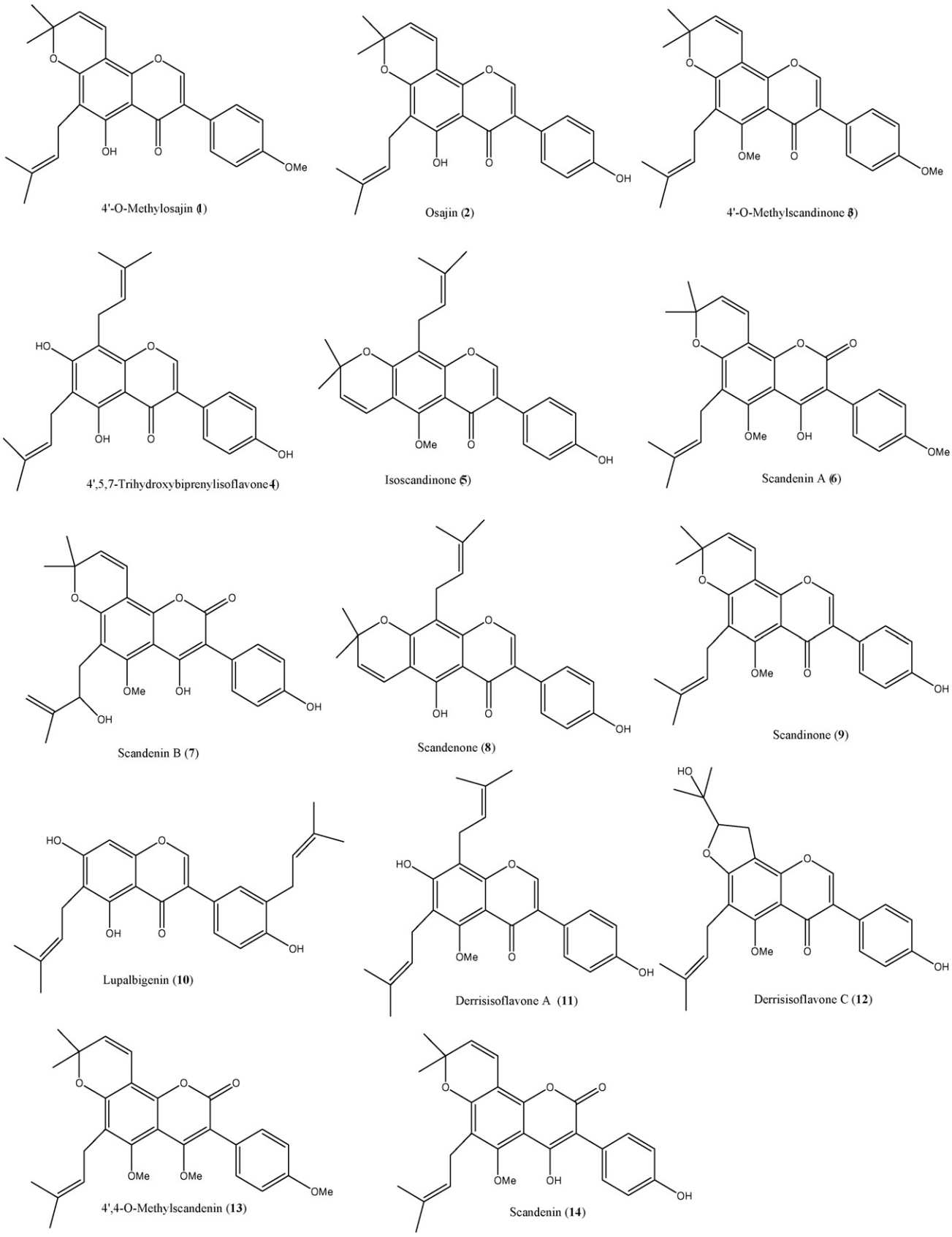

Fig. 1. Compounds isolated from *D. scandens*.

AA′ BB′ type signals at δ 7.23 (2H, d, J = 8.5 Hz) and 6.78 (2H, d, J = 8.5 Hz) assignable to H-2′, 6′ and H-3′, 5′ of the B-ring, respectively. Typical signals due to a chromene system at δ 6.50 (1H, d, J = 9.8 Hz, H − 1‴), 5.60 (1H, d, J = 9.8 Hz, H − 2‴), two methyl groups at δ 1.44 (6H, s, H − 4‴, 5‴) and an aromatic methoxy group at 3.98 (3H, s, 5-OMe) are present in the same spectrum. In addition, the ^{1}H NMR spectrum showed ABX-type signals at δ 3.01 (1H, dd, J = 14.5 and 8.6 Hz, Ha-1″), 3.10 (1H, dd, J = 14.6 and 2.6 Hz, Hb-1″) and 4.32 (1H, dd, J = 8.6 Hz and 2.6 Hz, H-2″) along with three signals at δ 1.83 (3H, s, H-4″), 4.82 (1H, s, Ha-5″) and 4.98 (1H, s, Hb-5″) derived from a 2-hydroxy-3-methyl-3-butenyl group. The ^{13}C NMR spectrum suggested that the structure of compound **7** is similar to that of scandenin A but the former contains an additional terminal double bond and a hydroxyl group. The DEPT spectrum indicated the presence of 4 methyls, 2 methylenes, 7 methines and 13 quaternary carbons. The HMBC and HSQC experiments confirmed the position of chromene and prenyl groups to be in an angular position. From above studies, compound **7** was confirmed as (4-hydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-3-(4-hydroxyphenyl)-5-methoxy-8,-8-dimethyl-2H,-8H-pyrano-[2,3-f]-chromen-2-one, and was named scandenin B.

3.1. Isoscandinone (*5*)

Pale yellow solid, m.p. 143 °C, IR (KBr) γ_{max}: 3352, 3250, 1637, 1610, 1380, 1360 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.60 (1H, s, 4′-OH), 7.85 (1H, s, H-2), 7.35 (2H, d, J = 8.7 Hz, H-2′, 6′), 6.85 (2H, d, J = 8.7 Hz, H-3′, 5′), 6.75 (1H, d, J = 10 Hz, H-4″), 5.65 (1H,d, J = 10 Hz, H-3″), 3.43 (2H, d, J = 7 Hz, H − 1‴), 5.10 (1H, t, J = 7.0 Hz, H − 2‴), 1.73 (3H, s, H − 4‴), 1.53 (3H, s, H − 5‴), 1.45 (6H, s, H-4″ and H-5″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.3 (C-2), 125.4 (C-3), 177.0 (C-4), 158.0 (C-5), 121.5 (C-6), 155.3 (C-7), 112.0 (C-8), 153.0 (C-9), 112.2 (C-10), 123.6 (C-1′), 130.2 (C-2′), 115.6 (C-3′), 156.2 (C-4′), 115.7 (C-5′), 130.0 (C-6′), 21 (C-1″), 122.2 (C-2″), 131.5 (C-3″), 17.8 (C-4″), 25.7 (C-5″), 115.7 (C − 1‴), 130.5 (C − 2‴), 77.5 (C − 3‴), 29.0 (C − 5‴), 63.5 (C-OMe). FABMS: m/z 419 [M^+ + H]; HRESIMS: [M^+ + H] 419.1862 (calculated 419.1858). Elemental analysis: found: C, 71.82; H, 5.93; $C_{26}H_{26}O_5$ requires: C, 71.75; H, 5.91%

3.2. Scandenin A (*6*)

A white amorphous solid, m.p. 190 °C, IR (KBr) γ_{max}: 3298, 1688, 1605, 1340 and 1310 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.0 (1H, s, Ar–OH), 7.43 (2H, d, J = 8.7 Hz, H-2′,6′), 6.95 (2H, d, J = 8.7 Hz, H-3′,5′), 6.50 (1H, d, J = 9.8 Hz, H − 1‴), 5.75 (1H, d, J = 9.8Hz, H − 2‴), 5.23 (1H, t, J = 7.2 Hz, H-2″), 5.10 (1H, t, J = 7.2 Hz, H − 2‴), 3.98 (3H, s, C5-OMe), 3.90 (3H, s, OMe), 3.43 (2H, d, J = 7.0 Hz, H-1″), 1.73 (3H, s, H − 4‴), 1.53 (3H, s, H − 5‴), 1.50 (6H, s, 4″, 5″-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.5 (C-2), 101.0 (C-3), 160.5 (C-4), 151.0 (C-5), 113.0 (C-6), 154.5 (C-7), 110.0 (C-8), 151.0 (C-9), 101.0 (C-10), 132.0 (C-1′), 131.2 (C-2′), 113.1.0 (C-3′), 158.5 (C-4′), 113.1 (C-5′), 131.2 (C-6′), 22.0 (C-1″), 131.5 (C-2″), 17.9 (C-4″), 25.7 (C-5″), 115.5 (C − 1‴), 131.0 (C − 2‴), 77.0 (C − 3‴), 28.1 (C − 4‴), 28.1 (C − 5‴), 64.5 (-OMe), 55.5 (-OMe). FABMS: m/z [M^+ + H] 449; HRESIMS: 449.1992 (calculated: 449.1964).

3.3. Scandenin B (*7*)

Pale yellow amorphous solid, m.p. 129–130 °C, $[\alpha]_D^{25} = -20.4$ (c 1.0, $CHCl_3$); IR (KBr) γ_{max}: 3410, 3250, 2926, 1682, 1515, 1219 and 834 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.80 (1H, s, Ar–OH), 7.80 (1H, s, Ar–OH), 7.23 (2H, d, J = 8.8.5 Hz, H-2′, 6′), 6.78 (2H, d, J = 8.5 Hz, H-3′, 5′), 6.50 (1H, d, J = 9.8 Hz, H − 1‴), 5.60 (1H, d, J = 9.8 Hz, H − 2‴), 4.82 (1H, s, Ha-5″), 4.98 (1H, s, Hb-5″), 4.32 (1H, dd, J = 8.6 Hz and 2.6 Hz, H-2″), 3.98 (3H, s, 5-OMe), 3.01 (1H, dd, J = 14.6 Hz and 2.6 Hz, Ha-1″), 3.10 (1H, dd, J = 14.6 Hz and 8.3 Hz, Hb-1″), 1.83 (3H, s, 4″-Me) 1.44 (6H, s, 4‴, 5‴-Me). FABMS: [M^+ + H] m/z 451.0; HRESIMS: 451.4892 (calculated: 451.4884).

3.4. Crystal data for compound (*5*)

Crystal data: $C_{26}H_{26}O_5$: MW = 418.47, colourless needle crystal 0.19 × 0.11 × 0.09 mm, a = 9.2608(5) Å, b = 21.9760(12) Å, c = 11.4746(6) Å, β = 109.384(1)°, V = 2202.9(2) Å3, monoclinic, space group $P2_1/c$, ρ_{calc} = 1.262 mg m^{-3}, λ = 0.71073 Å, μ(Mo Kα) = 0.087 mm^{-1}, F_{000} = 888, T = 273(2) K. Data collection yielded 18844 reflection resulting in 5155 unique, averaged reflection, 4277 with $I > 2\sigma(I)$, θ range: 1.85–28.00°. Full-matrix least-squares refinement led to a final R = 0.0499, wR = 0.1318 and GOF = 1.028. Intensity data were measured on Bruker Smart Apex with CCD area detector. CCDC 275210 contains supplementary crystallographic data for the structure.

3.5. Biological activities

3.5.1. Intestinal α-glucosidase enzyme inhibition (AGI)

Both hexane and chloroform extracts displayed potent α-glucosidase inhibitory activity at primary screening concentration (99.42 ± 6.34% and 91.95 ± 3.20% activity respectively). Compounds isolated from these extracts were assayed for their α-glucosidase inhibitory potential. Compounds displaying more than 50% inhibitory activity at primary screening concentration were further assayed to find out their IC_{50} values. From among the compounds isolated from the hexane extract, namely, 4′-O-methylosajin (**1**), osajin (**2**), 4′-*O*-methylscandinone (**3**) and 4′, 5′, 7-trihydroxybiprenylisoflavone (**4**), only compound **4** could display potent α-glucosidase inhibitory activity (IC_{50}: 45.14 ± 1.13 μg/mL). However, from the chloroform extract, scandenone and scandinone have displayed strong enzyme inhibitory activity. It was observed that the chloroform extract (IC_{50}: 6.28 ± 1.02 μg/mL) was more potent than the hexane extract (IC_{50}:10.63 ± 0.319 μg/mL). It appears therefore that synergistic action of other mild active compounds in hexane

Table 2
AGI and DPPH radical scavenging levels of bioactive isolates from *D. scandens*

Compound number	Compound name	AGI[a] IC_{50} (μg/mL)	DPPH radical scavenging[a] SC_{50} (μg/mL)
4	4′,5′,7-Trihydoxybiprenyl isoflavone	45.14 ± 1.13	9.21 ± 0.98
6	Scandenin A	25.17 ± 0.61	4.98 ± 0.21
7	Scandenin B	–	6.18 ± 0.116
8	Scandenone	34.74 ± 0.60	–
9	Scandinone	33.83 ± 1.32	–
	Chloroform extract	6.28 ± 1.02	7.15 ± 0.118
	Hexane extract	10.63 ± 0.319	–
	Trolox	–	1.48 ± 0.04
	Acarbose	4.1 ± 0.91	–

[a] Values expressed as mean ± S.D.

extract may be responsible for better activity of the extract. However, possibility of more potent compounds in hexane extract cannot be ruled out. The reference compound acarbose displayed IC_{50} value of 4.1 ± 0.91 μg/mL. The IC_{50} values for α-glucosidase inhibition for the isolated compounds are indicated in Table 2.

3.5.2. Free-radical (DPPH) scavenging activity

The new compounds scandenin B (SC_{50}; 6.18 mg/mL) and scandenin A (SC_{50}; 4.98 mg/mL) have displayed potent DPPH free radical scavenging activity. The reference compound trolox displayed SC_{50} value of 1.48 μg/mL. The activity levels of the test compounds are indicated in Table 2.

3.5.3. HPLC method development

All the isolates were subjected to biological screening and scandenone (**8**), scandinone (**9**), scandenin A (**6**), scandenin B (**7**) and 4′, 5′, 7-trihydroxybiprenyl isoflavone (**4**) were identified as the bioactive molecules having moderate free radical scavenging and α-glucosidase inhibitory activity. Good separation of these bioactive isolates was achieved by the newly developed reversed-phase HPLC method. Our efforts towards developing the present method included, an isocratic mobile phase with 80% acetonitrile and 20% water, which gave good separation of all the isolates except scandenin A and scandenone which are eluting with very low retention difference. Changing the mobile phase to 70% acetonitrile and 30% water brought good separation of scandenin A and scandinone but scandenone was not eluting out. Hence, the mobile phase was changed to methanol and water mixture (80/20) which also gave good separation of these five compounds but the other compounds present in the extract were not getting resolved properly. Therefore a gradient mixture comprising of acetonitrile and water (Table 1) was found to be the correct mobile phase for quantification of these compounds in crude extract of *D. scandens*. Retention times of scandinone, scadenone, scandenin A, scandenin B and 4′, 5′, 7-trihydroxybiprenyl isoflavone are15.81, 22.01, 17.05, 4.33 and 12.52 min, respectively. Calibration graphs pertaining to the active isolates were drawn for each compound and estimated with respect to the dried plant material. HPLC chromatogram of methanol extract of *D. scandens* is shown in Fig. 2.

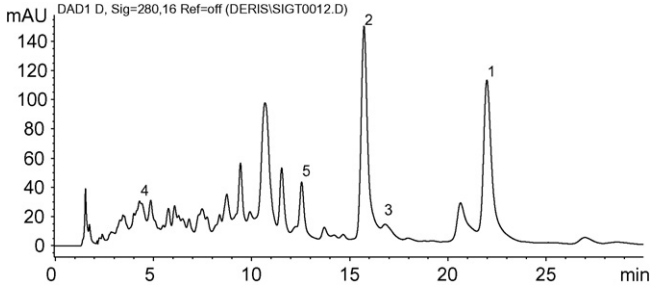

Fig. 2. Chromatogram of the crude extract of *D. scandens*.
1 = Scandenone (**8**); 2 = Scandinone (**9**); 3 = Scandenin A (**6**); 4 = Scandenin B (**7**); 5 = 4′, 5′, 7- trihydoxybiprenyl isoflavone (**4**).

3.5.4. Linearity

The calibration curve was drawn for each isolate using five calibration standards in the concentration range from 5 to 100 μg/mL and was found to be linear. The correlation coefficients from the graph were found to be in the range from 0.9996 to 0.9999. Linearity equation was expressed as $y = mx \pm c$ where, 'y' is area in milli absorbance units, and 'x' is concentration in μg/mL. The regression analysis was performed using Agilent chemstation software. Calibration curve was drawn with con-

Table 3
Parameters for quantification of bioactive isolates from *D. scandens*

Compound number	Name of compound	λ_{max} (nm)	Retention time	Regression equation	r^2	LOD (mg/mL)	LOQ (mg/mL)	% Abundance in dry plant powder
4	4′,5′,7-Trihydoxybiprenyl isoflavone	268	12.52	$y = 847.74x \pm 19.26$	0.9998	0.0005	0.001	0.0212
6	Scandenin A	262	17.05	$y = 352.76x \pm 9.04$	0.9996	0.000175	0.0005	0.0072
7	Scandenin B	254	4.33	$y = 377.47x - 3.59$	0.9995	0.0005	0.001	0.0067
8	Scandenone	286	22.02	$y = 2731.06x - 112.25$	0.9998	0.00005	0.00010	0.041
9	Scandinone	274	15.81	$y = 756.93x - 33.56$	0.9997	0.00065	0.0025	0.048

centration on *x*-axis and peak areas on *y*-axis. Limits of detection (LOD) (S/N=3) and limits of quantification (LOQ) (S/N-10) for all the bioactive isolates are depicted in Table 3.

3.6. Method validation

The reproducibility of the retention time of the bioactive isolates under optimum HPLC conditions was investigated by doing repeated injections ($n = 6$) of a mixture of the standards at a concentration of 5 μg/mL. The relative standard deviation (R.S.D) of retention times (min) for the isolates was 0.45%. The good reproducibility in retention time indicated that this method is accurate, robust and would probably be reliable for screening the active bioactive constituents in the plant sample.

4. Conclusion

In our effort to provide quality control tools for plant materials used in Ayurvedic system of medicine, we have undertaken the task of developing chemobiological standardization methods for Indian medicinal plants. In that process, scandinone, scadenone, scandenin A, scandenin B and 4′, 5′, 7-trihydroxybiprenyl isoflavone were identified as the main compounds showing intestinal α-glucosidase inhibitory and free radical scavenging activities from *D. scandens*. Intestinal α-glucosidase inhibitors effectively manage postprandial hyperglycemia and control the development of diabetic complications. Therefore, a combination of free radical scavengers and α-glucosidase inhibitors has become novel therapeutic approach to holistically manage diabetes mellitus. Presence of both these actives in significant proportions in *D. scandens*, hence opens novel avenues for its use and application in diabetes. All the bioactive isolates are exhaustively extracted in to methanol and a reversed-phase HPLC method has been developed to separate them, which can be used as an effective quality control tool in the herbal industry.

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