Anticancer agents from Solanum Surattense

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Abstract: Two steroids glycosides solamargine (1) and dioscine (2) were isolated from methanolic extract of Solanum surattense and evaluated for their anticancer activity. The structure of the isolated compounds was identified through 1D and 2D NMR techniques, mass spectrometry and in comparison, with the literature values of their spectroscopic data. Both compounds showed the excellent anticancer activity against NIH-3T3 fibroblast cancer cell line with IC₅₀ value of 7.55±1.5 and 3.3±1.9µg/ml respectively which were comparable with control Cyclohexamide which showed the IC₅₀ value of 0.8± 0.2 µg/ml.

Keywords: anticancer activity, steroids glycosides, Solanum surattense, structure elucidation, anticancer 3T3 cell line

Introduction

The importance of plant based anti-cancer drugs has inspired the researchers to discover more potent natural products showing anticancer and anti-tumor activities as the level of toxicity is different in most of the drugs already in market. Many of marketed drugs do not differentiate between normal and cancer cells causing harm to normal body cells. So, more efficient chemotherapeutic agents are the need of hour. On the other hand folk medicinal system hasreported the anti-cancer as well as anti-inflammatory plants which are needed to be explored for their phytochemicals. Solanum surattense, is one of them, as it is known to exhibit antibacterial [1], antihyperlipidemic [2], anti-inflammatory [3], anti-urithliatic and natriuretic [4] and cytotoxic activities [5]. These properties are due to presence of steroids, terpenes, phenolics, saponins, fatty acids, alkaloids [6] steroidal alkaloids and steroidal saponins [5]. Herein we report the isolation of alkaloidal steroid glycosides, solamargine (1) and a steroidal saponin, dioscine (2) as anticancer agents. Dioscin, a plant steroidal saponin, is known to have antiproliferating effect against a number of human cancer cells and induce apoptosis in a variety of tumor cells [6]. Recently dioscin is investigated for the potential against crystalline silica-induced pulmonary fibrosis in a mouse model of silicosis and the possible mechanism is explained [7]. Similarly, Steroidal glyco alkald, Solamargineis known to show the anti-cancer activity against various cancer cells lines, (Hep3B, HepG2, A549,MCF-7, and K562)[8]. Therefore, two important isolated steroids were studied for their NIH-3T3 fibroblast cancer cell line activity[7].
and another quaternary carbon resonate at δc 99.1 indicated the alkaloidal steroid nature of compound I. The 13C-NMR spectrum of I (Table 1) showed altogether 45 carbon signals which comprises of 24 methine, 11 methylene, 6 methyl and 4 quaternary carbon atoms, separated through DEPT 90, 135 experiment. Signals of olefinic carbon were present at δc 141.1 and 121.9 and anomic methine were resonated at δc 99.6, 101.2 and 101.9. The six methyl carbon displayed their position at δc 18.6, 17.7, 16.7, 16.6, 15.2 and 13.4. Four methyl signals were attested to steroidal skeleton, while, two were identified for rhamnose moieties. After hydrolysis, sugars were identified as L-rhamnose and D-glucose, in a ratio of 2:1. On the basis of above mentioned spectroscopic evidences and chemical analysis and comparison with the literature data compound I was identified as (22R, 25R)-16α-H-22α-N-spirosol-3β-ol-5-ene-O-(3)β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside-(1→4)-α L-rhamnopyranoside-solasodine. [9-12]. Compound 2 was isolated as white crystals. The IR absorption spectra showed absorption bands for hydroxyl and olefinic system at 3320 and 1622 cm⁻¹ respectively. The (−ve) HR-FAB-MS of compound 2 showed the molecular formula as C45H71NO13 due to a quasi-molecular ion at m/z 869.0436 [M-H]. Most of the 1H-NMR and 13C-NMR data for aglycone part in 3 was almost similar to that of compound I, except few carbons which showed downfield chemical shift at δc 110.0 (C-22), 20.7 (C-23), 30.75 (C-24) as compared to compound 1 signals at these carbons. The downfield protons attested the presence of oxygen at C-22 (δc 110.0) which was comparable with the literature values of steroid skeleton of dicosene. [10] Moreover, the signals for three anomic protons and carbon confirmed the presence of three sugar units (Table 1). Further their acid hydrolysis confirmed that three sugar moieties were designated as two rhamnosyl and one glucosyl. By the comparison of spectroscopic data of compound 2 with literature values of dicosene, compound 2 was confirmed as (22R, 25R)-16α-H-22α-spirosol-3β-ol-5-ene-O-(3)β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside-(1→4)-α-L-rhamnopyranoside-β-D-glucopyranosyl] diosgenin [12]. Compounds Solamargine I and
Dioscine 2 were evaluated against NIH-3T3 fibroblast cancer cell line, which is widely used to investigate role of fibroblast in fibrogenesis [7] using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) colorimetric assay. Compounds 1 and 2 showed strong cytotoxicity against 3T3 cell line with IC50 value 7.55±1.5 and 3.3±1.9 µg/ml respectively which was comparable with control Cyclohexamide which showed the IC50 value of 0.8±0.2.

Table 1: 3T3 cell line and hela cell line anticancer

<table>
<thead>
<tr>
<th>Sample(conc.)</th>
<th>%inhibition</th>
<th>IC50 ± SD</th>
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<tbody>
<tr>
<td>1 (30 µg/ml)</td>
<td>93%</td>
<td>7.55±1.5</td>
</tr>
<tr>
<td>2 (30 µg/ml)</td>
<td>91%</td>
<td>3.3±1.9</td>
</tr>
<tr>
<td>Cyclohexamide (30 µg/ml)</td>
<td>71%</td>
<td>0.8±0.2</td>
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Experimental

General Experimental Procedures:

Cytotoxicity assay: Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, 3T3 (mouse fibroblast) cells were cultured in Dulbecco's Modified Eagle Medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm2 flasks, and kept in 5% CO2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of 5x104cells/ml was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-30µM). After 48 hrs, 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC50) for 3T3 cells. The percent inhibition was calculated by using the following formula:

\[ \text{% inhibition} = 100 - \frac{\text{mean of O.D of test compound}}{\text{mean of O.D of negative control}}/ \frac{\text{mean of O.D of positive control}}{\text{mean of O.D of negative control}} \times 100 \]

The results (% inhibition) were processed by using Soft-Max Pro software (Molecular Device, USA) [11].

Equipments

JASCO-320-A (Duisburg, Germany) spectrophotometer was used for IR spectra. HR-FAB-MS spectra were obtained using Finnigan (Varian MAT, Waldbronn, Germany) JMS HX110 with data system and JMSA 500 mass spectrometers. The 1H-NMR (600 MHz) and 13C-NMR (150 MHz) spectra were measured on Bruker AV-600 spectrometers (Zurich, Switzerland), with tetramethylsilane (TMS) as internal standard. 2D-NMR (COSY, NOESY, HSQC and HMBC) spectra were also recorded on the same machine operating at 600 MHz Silica gel (Kieselgel 70-230, E. Merck) and Sephadex LH-20 (20–100 µm, Pharmacia) were used for column chromatography. Various fractions were monitored by pre-coated TLC plates silica gel (Kieselgel 60 F254, thickness 0.25 mm E. Merck, Darmstadt, Germany). The chromatograms were visualized under UV lamp using wavelength of 254 and 366 nm or by spraying with ceric sulphate followed by heating. Final purification of the metabolites was done on HPLC system, Sykam GmbH, S2100 Solvent/Sample delivery system and S3210 UV/Visible detector system (Eresing, Germany).

Plant Material and Isolation

The plant Solanum suratense (voucher specimen no. SS-12-2010) was collected from Akhtar Abad, Bahawalpur city, Pakistan in July 2010, identified by Dr. Muhammad Arshad (Late), Ex-plant Taxonomist at Cholistan Institute for
Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan.

Air-dried whole plant material of Solanum surattense (10.0 Kg) were extracted (8.0 L), with MeOH, which was removed to give a concentrated extract (160 g) that was suspended in H2O and extracted successively with n-hexane, EtOAc and n-butanol. The solvents were removed in vacuum to produce n-hexane fraction (40 g), and (35 g) and n-butanol fraction (27 g). EtOAc fraction was separated over a column of silica gel using n-hexane–chloroform gradient (100:0-0:100) to produce 13 chromatographic fractions (E1-E13). Fraction E5 (6 g) was rechromatographed over silica gel using n-hexane–EtOAc. Subsequent purification of the obtained fractions over Sephadex LH-20 using MeOH–CH2Cl2 (9:1) isolated I (14 mg).

Five sub-fractions (B1-B5) were obtained from n-butanol fraction, when chromatographed on a silica gel by eluting with a gradient of methanol in chloroform (0 to 50%). Fraction B2 (430 mg) was further purified on HPLC system Sykam, equipped with S 1521 solvent delivery system and UV/Visible detector (S 3210), RP-18 (Machery Nagel MN) 5 µm, 10x250 mm column. A gradient of water/methanol [90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 20:80, 90:10] at a flow rate of 5.0 ml/min was used to get compound 2 (18.0 mg, Rr 12 min).

Solamargine (I): White amorphous solid; IR (KBr) vmax 3432-3310 and 1624 cm⁻¹; ¹H-NMR: δ 1.10, 1.90 (m, H-1); 1.97, 1.64, (m, H-2), 3.92, (m, H-3), 2.48, (d, J = 11.5, H-4), 2.33 (d, J = 11.0, H-4), 5.39 (d, J = 5.4, H-6), 2.09, 1.57, (m, H-7), 1.73, (m, H-8), 1.04, (m, H-9), 1.35, (m, H-11), 1.85, 1.25, (m, H-12), 1.24, (m, H-14), 1.93, (br, d, J = 12.0, H-15), 1.42, (m, H-15), 4.57, (m, H-16), 1.94, (m, H-17), 0.87, (s, H-18), 1.05, (s, H-19), 2.37, (m, H-20), 1.14, (d, J = 6.2, H-21), 1.86, (m, H-23), 1.75, 1.55, (m, H-24), 1.44, (m, H-25), 2.78, 3.03 (d, J = 10.2, H-26), 1.03, (d, J = 6.0, H-27), 4.51, (d, J = 7.8, H-1'), 3.61, (m, H-2'), 3.32, (m, H-3'), 3.82, (m, H-4'), 3.75, (m, H-5'), 3.81, (dd, J = 11.6, 4.3, H-6'), 4.88, (br, s, H-7') 3.40, (m, H-8'), 3.52, (m, H-9'), 3.69, (m, H-10'), 1.26, (d, J = 6.3, H-11'), 5.19, (s, H-11'), 3.86, (m, H-12'), 3.60, (m, H-13'), 3.35, (m, H-14'), 4.19, (m, H-15'), 1.29, (d, J = 6.2, H-16'); ¹³C-NMR: δ 37.2 (C-1), 29.1 (C-2), 77.0, (C-3), 38.1 (C-4), 141.1 (C-5), 121.9 (C-6), 31.9 (C-7), 31.2 (C-8), 50.4 (C-9), 36.6 (C-10), 20.4 (C-11), 39.1 (C-12), 40.7 (C-13), 56.3 (C-14), 36.8 (C-15), 83.3 (C-16), 61.8 (C-17), 15.2 (C-18), 18.6 (C-19), 41.4 (C-20), 13.4 (C-21), 99.1 (C-22), 28.0 (C-23), 27.5 (C-24), 31.5 (C-25), 45.5 (C-26), 17.7 (C-27), 99.6 (C-1'), 76.4 (C-2'), 75.1 (C-3'), 78.6 (C-4'), 76.6 (C-5'), 60.4 (C-6'), 101.2 (C-7'), 70.7 (C-2''), 79.5 (C-3''), 72.7 (C-4''), 73.5 (C-5''), 16.7 (C-5''), 101.9 (C-1'''), 71.9 (C-2'''), 72.0 (C-3'''), 73.1 (C-4'''), 70.3 (C-5''') and 16.6 (C-1'''); HR-FAB-MS 867.498 [M-H] (calcd. 868.5088 for C₃₇H₇₄N₂O₁₅ attested for C₃₇H₇₃N₂O₁₅).

Dioscine (2): white crystals; IR (KBr) vmax 3320 and 1622 cm⁻¹; ¹H-NMR: δ 0.98, 1.57, (m, H-1), 1.97, 1.41, (m, H-2), 3.57, (m, H-3), 2.45, 2.28, (d, J = 6.0, H-4), 5.37 (m, H-6), 1.97, 1.39, (m, H-7), 1.64, (m, H-8), 0.94 (m, H-9), 1.53, 0.81 (m, H-11), 1.56, 1.88 (m, H-12), 1.28, (m, H-14), 1.64, 1.42 (m, H-15), 4.39 (m, H-16), 1.72 (m, H-17), 0.81 (s, H-18), 1.03 (s, H-19), 1.39 (m, H-20), 0.96 (d, J = 6.0, H-21), 1.75, (m, H-23), 1.28, 1.50 (m, H-24), 1.91 (m, H-25), 2.64 (d, J = 10.2, H-26), 0.77, (d, J = 6.0, H-27), 4.49, (d, J = 7.8, H-1'), 3.51 (m, H-2'), 3.57 (m, H-3'), 3.58 (m, H-4'), 3.31 (m, H-5'), 3.77 (d, J = 4.5, H-6'), 4.83 (d, J = 1.4, H-1''), 3.81 (m, H-2''), 3.39 (m, H-3''), 3.6 (m, H-4''), 4.14 (m, H-5''), 1.23 (d, J = 6.0, H-6''), 5.19 (d, J = 1.4, H-1''), 3.82 (m, H-2''), 3.37 (m, H-3''), 3.90 (m, H-4''), 3.91 (m, H-5''), 0.79 (d, J = 6.0, H-6''), ¹³C-NMR: δ 32.5 (C-1), 29.7 (C-2), 78.0 (C-3), 39.5 (C-5), 141.3 (C-6), 122.2 (C-7), 33.0 (C-8), 32.6 (C-9), 51.5 (C-10), 38.0 (C-11), 21.8 (C-12), 38.4 (C-13), 41.7 (C-14), 57.6 (C-15), 32.2 (C-16), 82.0 (C-17), 63.5 (C-18), 17.3 (C-18), 19.6 (C-19), 42.7 (C-20), 14.7 (C-21), 110.0 (C-22), 40.7 (C-23), 30.7 (C-24), 31.1 (C-24), 67.8 (C-25), 17.7 (C-26), 100.2 (C-1'), 79.9 (C-2'), 79.3 (C-3'), 79.2 (C-4'), 76.4 (C-5'), 61.9 (C-6'), 102.8 (C-1''), 72.2 (C-2''), 73.7 (C-3''), 72.0 (C-4''), 69.6 (C-5''), 17.8 (C-6''), 102.1 (C-1'''), 72.3 (C-2'''), 73.5 (C-3'''), 72.0 (C-4'''), 70.5 (C-5'') and 16.5 (C-6''). HR-FAB-MS, [M-H] - 868.482
Conclusion:

Natural products has proven to be potent anticancer and anti-tumor agents as the level of toxicity is different in most of the drugs being safer in terms of toxicity level. Steroid glycosides isolated from methanolic extract of _Solanum_ showed encouraging anticancer activity against fibroblast cancer cell line with IC$_{50}$ value comparable with control drug.

References


