**RESEARCH ARTICLE**

# 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 $IC_{50}$ value of 7.55±1.5 and 3.3±1.9µg/ml respectively which were comparable with control Cyclohexamide which showed the $IC_{50}$ 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-urolithiatic and natriuretic [4] andcytotoxic 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 forthe potential against crystalline silica-induced pulmonary fibrosis in a mouse model of silicosis and the possible mechanism is explained [7]. Similarly, Steroidal glyco alkaloid, 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 fibro blast cancer cell line activity[7].

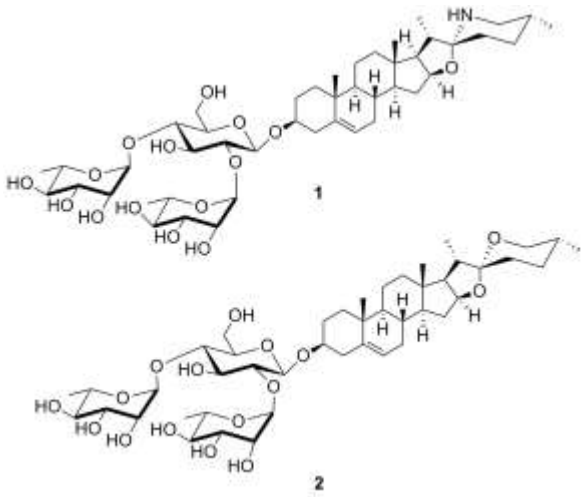

Figure 1: Structures of the secondary metabolites isolated from *Solanum surattense*

## Results and Discussion

Compound **1** was isolated as white amorphous solid, whose molecular formula was calculated through (-ve) HR-FAB-MS that displayed *quasi*-molecular ion at *m/z*867.4985 $[M-H]^-$ (calcd. 868.5088 for $C_{45}H_{73}NO_{15}$). The IR spectrum showed diagnostic absorption broad band between 3432-3310$cm^{-1}$for hydroxyl and secondary amine group and along with absorption band at 1624 $cm^{-1}$for olefinic system. The four methyl groups present at $\delta_H$ 0.87 (s, $CH_3$-18), 1.03 (d, $J$ = 6.0 Hz, $CH_3$-27), 1.05 (s, $CH_3$-19) and 1.14 (d, $J$ = 6.2 Hz, $CH_3$-21)in the upfield region of $^1$H-NMR spectrum, indicated the presence of steroidal skeleton in compound **1**. Further the same spectrum displayed signal for olefinic methine present at $\delta_H$ 5.39 (1H, d, $J$ = 5.4 Hz, H-6), whereas several oxymethine, displayed their resonance at $\delta_H$4.57 (1H, m),4.19 (1H, m), 3.92 (1H, m), 3.86 (1H, m), 3.82 (1H, m), 3.75 (1H, m), 3.69 (1H, m), 3.61 (1H, m), 3.60 (1H, m), 3.52 (1H, m), 3.49 (1H, m),3.42 (1H, m), 3.40 (1H, m), 3.35 (1H, m) and 3.32 (1H, m)along with three anomeric protons showed their resonance at $\delta_H$5.19 (1H, s), 4.88 (1H, br., s) and 4.51 (1H, d). The twodoublet methyl at $\delta_H$ 1.26 (d, $J$ = 6.3 Hz) and $\delta_H$ 1.29 (d, $J$ = 6.2 Hz) were identified as rhamnose methyl of sugar moieties. A signal due to a methylene protonwas appeared at $\delta_H$ 2.78 (2H, d, $J$ = 10.2 Hz, H-26) with its corresponding carbon in $^{13}$C-NMR spectrum (Table 1) resonated at $\delta_C$ 45.5 and another quaternary carbon resonate at $\delta_C$ 99.1 indicated the alkaloidal steroid nature of compound **1.**The $^{13}$C-NMR spectrum of **1** (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 $\delta_C$141.1 and 121.9 and anomeric methine were resonated at $\delta_C$ 99.6, 101.2 and 101.9. The six methyl carbon displayed their position at $\delta_C$ 18.6, 17.7, 16.7, 16.6, 15.2 and 13.4. Four methyl signals were attributed to steroidal skeleton, while, two were identified for rhamnose moieties. After hydrolysis, sugars were identified as L-rhamnose andD-glucose, in a ratio of 2:1. On the basis of above mentioned spectroscopic evidences and chemical analysis and comparsion with the literature data compound **1** was identified as(22*R*, 25*R*)-16*α*-H-22*α*-N-spirosol-3*β*-ol-5-ene-*O*-(3){*β*-D-glucopyranosyl-(1→2)-*α*-$_L$-rhamnopyrano side-(1→4)- *α* rhamnopyranoside}-solasodine. [9-12]. Compound **2** was isolated as white crystals. The IR absorption spectrashowed absorption bands for hydroxal and oleinfinic system at 3320 and 1622$cm^{-1}$respectively.The (-ve) HR-FAB-MS of compound **2** showed the molecular formula as $C_{45}H_{72}O_{16}$ due to a quasi-molecular ion at *m/z* 869.0436 $[M-H]^-$.Most of the $^1$H-NMR and $^{13}$C-NMR data for aglycone part in **3** was almost similar to that of compound **1**, except few carbons which showed downfield chemical shift at $\delta_C$110.0 (C-22), 40.7 (C-23), 30.75 (C-24) as compared to compound **1**signals at these carbons. The downfield protons attested the presence of oxygen at C-22 ($\delta_C$110.0) which was comparable with the literature values of steroid skeleton of dioscine. [10] Moreover, the signals for three anomeric protons and carbon confirmed the presence of three sugar units (Table 1). Further their acid hydrolysis confirmed that three sugar moieties were designated as two rhamnosyl and one glucosyl. By the comparison of spectroscopic data of compound **2** withliterature values of dioscine, compound **2** was confirmed as(22*R*, 25*R*)-16*α*-H-22*α*-spirosol-3*β*-ol-5-ene-*O*-(3){(1→2)-*α*-$_L$-rhamnopyranoside-(1→4)-*α*-$_L$-rhamnopyranoside-*β*-D-glucopyranosyl} diosgenin [12]. Compounds Solamargine**1** 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 cyto-toxicity against 3T3 cell line with IC $_{50}$ value 7.55±1.5 and 3.3±1.9 µg/ml respectively which was comparable with control Cyclohexamide which showed the $IC_{50}$ value of 0.8± 0.2.

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

| Sample(conc.) | %inhibition | $IC_{50}$ ±SD |
|---|---|---|
| **1** (30 µg/ml) | 93% | 7.55±1.5 |
| **2** (30 µg/ml) | 91% | 3.3±1.9 |
| Cyclohexamide (30 µg/ml ) | 71% | 0.8± 0.2 |

## 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 $cm^2$ flasks, and kept in 5% $CO_2$ incubator at 37ºC. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $5x10^4$cells/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 ($IC_{50}$) for 3T3 cells. The percent inhibition was calculated by using the following formula:

% inhibition = 100-(mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100).

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

### Equipments

JASCO-320-A (Duisburg, Germany) spectro photometer was used for IR spectra. HR-FAB-MS spectra were obtained using Finnigan (Varian MAT, Waldbronn, Germany) JMS H×110 with data system and JMSA 500 mass spectrometers. The $^1$H-NMR (600 MHz) and $^{13}$C-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 $F_{254}$, 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

Theplant *Solanum surattense*(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-driedwhole plant material of *Solanum surattense* (10.0 Kg) were extracted (8.0 L), with MeOH, which was removed to give aconcentrated extract (160 g) that was suspended in $H_2O$ and extracted successively with *n*-hexane, EtOAcand*n*-butanol. The solvents were removed in vacuum to produce *n*-hexane fraction (40 g), and (35 g) and *n*-butanol fraction (27g).EtOAc fraction was separatedover 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 ofthe obtained fractions over Sephadex LH-20 using MeOH–$CH_2Cl_2$ (9:1) isolated **1** (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) 5µm, 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, $R_t$ 12 min).

Solamargine (**1**): White amorphous solid; IR (KBr) $\nu_{max}$ 3432-3310 and 1624 $cm^{-1}$; $^1$H-NMR: $\delta$ 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-''),3.40, (m, H-''), 3.52, (m, H-''), 3.69, (m, H-''), 3.49, (m, H-''), 1.26, (d, *J* = 6.3, H-''), 5.19, (s, H-1'''), 3.86, (m, H-2'''), 3.60, (m, H-3'''), 3.35, (m, H-4'''), 4.19, (m, H-5'''), 1.29, (d, *J* = 6.2, H-6'''); $^{13}$C-NMR: $\delta$ 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-'), 70.7 (C-2''), 79.5 (C-3''), 72.7 (C-4''), 73.5 (C-''), 16.7 (C-''), 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-MS867.498 $[M-H]^-$ (calcd. 868.5088 for $C_{45}H_{73}NO_{15}$ attested for $C_{45}H_{73}NO_{15}$).

Dioscine (**2**): white crystals; IR (KBr) $\nu_{max}$ 3320 and 1622$cm^{-1}$; $^1$H-NMR: $\delta$ 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'''), $^{13}$C-NMR: $\delta$ 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 (calcd. for 869.0436 $C_{45}H_{72}O_{16}$ attested for$C_{45}H_{72}O_{16}$).

## 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.

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