Anticancer Active Homoisoflavone from the Underground Bulbs of *Ledebouria hyderabadsensis*

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**INTRODUCTION**

*Ledebouria* is a genus of deciduous or weakly evergreen bulbs in the Hyacinthaceae family. This is recognized as the first collection made of the new taxon *Ledebouria hyderabadsensis*, exist in the Hyderabad city of Andhra Pradesh, India. The goal of this work was to investigate the phytochemical constituents present in the new species and also to evaluate the cytotoxic properties of the extracts and pure compounds against human cancer cell lines. **Materials and Methods:** The anticancer activity was evaluated in *in vitro* mode by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. **Results:** Phytochemical investigation of underground bulbs of indigenous, rare, and recently identified herb *L. hyderabadsensis* yielded a bioactive homoisoflavanone, Scillascillin 1. The structure of the compound was established on the basis of various nuclear magnetic resonance and mass spectral data. The compound Scillascillin was isolated for the first time from *L. hyderabadsensis*. **In vitro** anticancer activity, performed using MTT assay, showed compound 1 as significantly active against human cancer cell lines MCF-7 (breast cancer) and DU-145 (prostate cancer) with inhibitory concentration (IC) values 9.59 and 11.32 µg/ml respectively when compared with herb methanol extract (IC values 36.21 and 44.86 µg/ml respectively).

**Key words:** Anticancer activity, Hyacinthaceae, *Ledebouria hyderabadsensis*, Scillascillin

**ABSTRACT**

Background: *Ledebouria* is a genus of deciduous or weakly evergreen bulbs in the Hyacinthaceae family. This is recognized as the first collection made of the new taxon *Ledebouria hyderabadsensis*, exist in the Hyderabad city of Andhra Pradesh, India. **Objective:** The goal of this work was to investigate the phytochemical constituents present in the new species and also to evaluate the cytotoxic properties of the extracts and pure compounds against human cancer cell lines. **Materials and Methods:** The anticancer activity was evaluated in *in vitro* mode by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. **Results:** Phytochemical investigation of underground bulbs of indigenous, rare, and recently identified herb *L. hyderabadsensis* yielded a bioactive homoisoflavanone, Scillascillin 1. The structure of the compound was established on the basis of various nuclear magnetic resonance and mass spectral data. The compound Scillascillin was isolated for the first time from *L. hyderabadsensis*. **In vitro** anticancer activity, performed using MTT assay, showed compound 1 as significantly active against human cancer cell lines MCF-7 (breast cancer) and DU-145 (prostate cancer) with inhibitory concentration (IC) values 9.59 and 11.32 µg/ml respectively when compared with herb methanol extract (IC values 36.21 and 44.86 µg/ml respectively).

**Key words:** Anticancer activity, Hyacinthaceae, *Ledebouria hyderabadsensis*, Scillascillin

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**Extraction and isolation**

Fresh underground bulbs were collected, shade dried and powdered (1.5 kg). The powdered material was extracted with methanol at reflux temperatures using soxhlet apparatus (3 L, 24 siphon cycles). The extract was evaporated at reduced pressures (at 40°C) and then freeze-dried. The viscous gummy methanol extract was washed several times (at room temperature) with n-hexane to remove fats and other coloring impurities. The resulting partially gummy solid was then subjected to column chromatography over silica gel (100-200 mesh) and elution of the column with 10% ethylacetate in hexane solvent mixture yielded a pure pale yellow colored compound 1 (150 mg, thin-layer chromatography 30:70 ethyl acetate: hexane, Rf 0.52).

**Anticancer activity**

**Cell culture**

Human breast cancer cell line (MCF-7) and human prostate cancer cell lines (DU-145) were obtained from American Type Culture Collection (Manassas, VA, USA) the cell lines were grown in Dulbecco's modification of Eagle's medium medium supplemented with 10% fetal bovine serum, 0.3% sodium bicarbonate, 10 mL/L antibiotic anti-mycotic solution (10,000 U/mL penicillin, 10 mg/L streptomycin, and 25 µg/mL amphotericin B), 1 mL/L of 4 mM L-glutamine and 1 mL/L of 100 mM sodium pyruvate culture was maintained in CO2 incubator at 37°C with a 90% humidified atmosphere and 5% CO2.

**Preparation of samples for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay**

Test compounds methanol extract and compound 1 were made with sterile phosphate buffered saline (PBS) (×1) to get desired concentrations. All formulations were filtered with a 0.22 µm sterile filter and 20 min of ultraviolet (UV) eradication before adding to the 96 well plates containing cells.

**Cytotoxicity evaluation (3-(4,5- dimethylthiazol-2-yl),2-5-diphenyltetrazolium bromide assay)**

Cytotoxicity of formulations was assessed using MTT assay to determine the cell viability according to a reported method.[4] The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells into purple formazan crystals, which gets dissolved in dimethyl sulfoxide (DMSO) and read at 570 nm. Briefly, 1 × 10^4 exponentially growing cells were seeded into each 96 well plate (counted by Trypan blue exclusion dye method) allowed to grow until 60-70% confluence then compounds (name of the compounds if applicable) were added to the culture medium with the final concentrations ranging from of 10, 25, 50, and 100 µg/mL and along with controls [negative (without compound) and positive (doxorubicin)] incubated for 24 h CO2 incubator at 370°C with a 90% humidified atmosphere and 5% CO2. Later, the media of the wells were replaced with 90 µL of fresh serum free media and 10 µL of MTT (5 mg/mL of PBS), plates were incubated at 37°C for 2 h, there after the above media was discarded allow to dry for 30 min. Add 100 µL of DMSO in each well at 37°C for 5 min. The purple formazan crystals were dissolved and immediately read absorbance at 570 nm was measured using Spectra Max plus 384 UV-Visible plate reader (Molecular Devices, Sunnyvale, CA, USA). Inhibitory concentration (IC)50 values were determined by probit analysis software package of MS-excel, % cell viability (from control) versus concentration.

**RESULTS AND DISCUSSION**

Earlier reports pertaining to the isolation of compound 1 from different plant sources did not mention the complete spectral characterization like two-dimensional nuclear magnetic resonance (2D-NMR). In the present paper, the structure of the isolated compound 1 was elucidated on the basis of various 1H-/13C-/2D-NMR, and mass spectral data. Compound was obtained as pale yellow needles and its mass spectrum showed molecular ion peak at m/z 313 (M + 1). Basing on the spectroscopy, the molecular formula of 1 was established as C13H12O6. In 1H-NMR spectra, resonances at δ 2.943–2.988 (d, 1H, J = 13.563 Hz) and 3.417–3.462 (d, 1H, J = 13.563 Hz) indicates the presence of geminal protons at C-9 carbon. A singlet at δ 5.90 ppm (s, 2H) is attributed to doxy methylene protons. Rest all the protons resonated as reported. The 13C-NMR spectrum coupled with distortionless enhancement by polarization transfer (DEPT)-135 showed total 17 carbons in the compound 1 with three CH3, four CH2 and rest 10 are quaternary carbons. The heteronuclear multiple-bond correlation spectrum [Figures 1 and 2] showed important 1H-13C long range correlations from carbons at 2, 3, 1’, and 6’ to protons at C-9 carbon establishes a cyclobutane ring moiety in 1. Based on the various spectroscopic data and previously reported literature the
Table 1: NMR spectral data of compound 1

| Carbon position | Chemical shift values δ (ppm) | Multiplicity (DEPT) | HMBC |
|-----------------|--------------------------------|---------------------|------|
| 1               | 3.39 (s)                       | 74.68               | CH₂ 2.98 |
| 2               | 5.95 (s)                       | 97.25               | CH  |
| 3               | 4.56 (OH)                      | 168.46              | Q  |
| 4               | 5.96 (s)                       | 96.03               | CH  |
| 5               | 164.97                         | Q                   |     |
| 6               | 3.49-3.47 (d)                  | 35.66               | CH₂ |
| 7               | 136.18                         | Q                   | 3.41, 2.98 |
| 8               | 136.18                         | Q                   | 3.41, 2.98 |
| 9               | 5.90 (s)                       | 101.57              | CH₂ |

NMR=Nuclear magnetic resonance; HMBC=Heteronuclear multiple-bond correlation; DEPT=Distortionless enhancement by polarization transfer

Table 2: In vitro anticancer activity of Ledebouria hyderabadensis methanolic extract and compound 1

| Sample                  | IC₅₀ in µg/ml |
|-------------------------|--------------|
| MCF-7                   | DU-145       |
| Methanol extract        | 36.21±0.003  | 44.86±0.024   |
| Compound 1              | 9.59±0.010   | 11.32±0.035   |
| Doxorubicin             | 1.85±0.003   | 13.70±0.020   |

IC₅₀=Inhibitory concentration

Anticancer activity

In vitro anticancer activity performed using MTT assay showed both the methanol extract and the isolated compound 1 as potentially active and later being the most with IC₅₀ values of 9.59 µg/ml in MCF-7 and 11.32 µg/ml in DU-145 cell lines [Table 2]. Compound 1 was significantly inhibiting DU-145 cell lines than the standard doxorubicin (13.70 µg/ml).

CONCLUSION

The present phytochemical investigation of the indigenous, rare and unexplored herb L. hyderabadensis lead to the isolation of homoisoflavone, Scillascillin. This the first report of its occurrence in the L. hyderabadensis. Both the methanol extract of the underground bulbs and the isolated compound 1 significantly inhibited the MCF-7 and DU-145 human cancer cell lines with IC₅₀ values 9.59 and 11.32 µg/ml, respectively.

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