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

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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 hyderabadensis*, 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 specifies 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. hyderabadensis* 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. hyderabadensis. 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 $(IC)_{50}$ values 9.59 and 11.32 µg/ml respectively when compared with herb methanol extract (IC_{50} values 36.21 and 44.86 µg/ml respectively).

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INTRODUCTION

Ledebouria is a genus of deciduous or weakly evergreen bulbs in the Hyacinthaceae family. The genus is now regarded as distinct from *Scilla,* which is a genus of the Northern Hemisphere. There are 60 + species distributed in India and Madagascar. It is found mostly in areas of summer rainfall (there are a few winter rainfall species) in subtropical savannas and grasslands in the eastern and northeastern parts of southern Africa.[1] This is recognized as the first collection made of the new taxon *Ledebouria hyderabadgensis*,[2] exist in the Hyderabad city of Andhra Pradesh (AP), India. In spite of its presence in the city localities, the new taxon is confined to rocky habitats and is not very frequent. The new taxon is currently known only from Hyderabad in contrast to its most common other species in the same genera, *L. revolute,* which is widespread throughout India. *L. hyderabadensis* is being a new plant any kind of previously published reports are not available and hence, this prompted us to look into its phytochemistry and biological activity. Our phytochemical investigation resulted in the isolation of a rare homoisoflavone, which was also exhibited significant cytotoxic activity. Different types of homoisoflavones were also reported *Ledbouria graminifolia* which also showed potential cytotoxic activity.[3]

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MATERIALS AND METHODS

Plant material collection

The underground bulb part of *L. hyderabadensis* collected at Osmania University Campus, Hyderabad, AP, India during mid-rainy season, and was identified by taxonomist, Dr. Venkat Ramana, Assistant Professor, Department of Botany, Nizam College, Osmania University, AP, India. A voucher specimen (CIMAP-SH/11) was deposited at Central Institute of Medicinal and Aromatic Plants Research Center, Hyderabad.

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, R_f 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 CO_2 incubator at 37°C with a 90% humidified atmosphere and 5% CO_2.

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

Test compounds methanol extract and compound 1 were taken in 10 mg/ml of DMSO and various dilutions were made with sterile phosphate buffered saline (PBS) (×1) to get desired concentrations. All formulations were filtered with 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 CO_2 incubator at 370°C with a 90% humidified atmosphere and 5% CO_2. 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 ^{1}H-/^{13}C-/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 $C_{17}H_{12}O_6$. In ^{1}H-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 dioxy methylene protons. Rest all the protons resonated as reported. The ^{13}C-NMR spectrum coupled with distortionless enhancement by polarization transfer (DEPT)-135 showed total 17 carbons in the compound 1 with three CH_2, four CH, 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

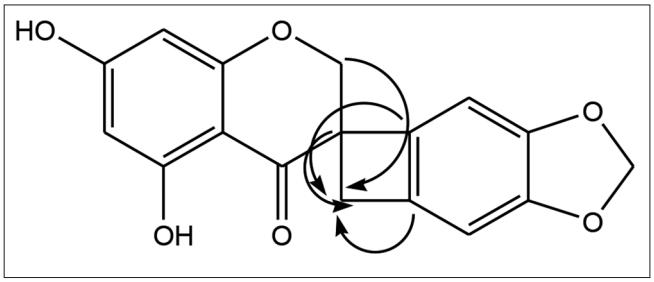

Figure 1: Important 1H-13C heteronuclear multiple-bond correlation correlations of Scillascillin

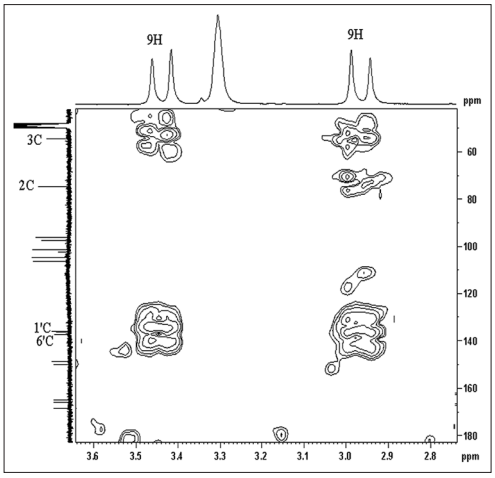

Figure 2: Important 1H-13C (heteronuclear multiple-bond correlation) long range correlations of 1

Table 1: NMR spectral data of compound 1

Carbon position	Chemical shift values δ (ppm)			
	^{1}H NMR	^{13}C NMR	Multiplicity (DEPT)	HMBC
2	3.39 (s)	74.68	CH_2	2.98
3		54.59	Q	3.41, 2.98
4		197.75	Q	
4a		102.50	Q	-
5		165.86	Q	-
6	5.95 (s)	97.25	CH	
7	4.56 (OH)	168.46	Q	-
8	5.96 (s)	96.03	CH	-
8a		164.97	Q	-
9	3.49-3.47 (d) 3.02-2.99 (d)	35.66	CH2	-
1^1		136.18	Q	3.41, 2.98
2^1	6.59 (s)	106.56	CH	-
3^1		148.56	Q	-
4^1		149.88	Q	-
5^1	6.76 (s)	104.75	CH	-
6^1		137.29	Q	3.41, 2.98
O–CH_2–O	5.90 (s)	101.57	CH_2	-

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	IC_{50} in µg/ml	
	MCF-7	DU-145
Methanol extract	36.211±0.003	44.862±0.024
Compound 1	9.592±0.010	11.320±0.035
Doxorubicin	1.856±0.003	13.707±0.020

IC=Inhibitory concentration

structure of compound 1 was confirmed as Scillascillin[5,6] [Figure 3 and Table 1].

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 IC_{50} 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).

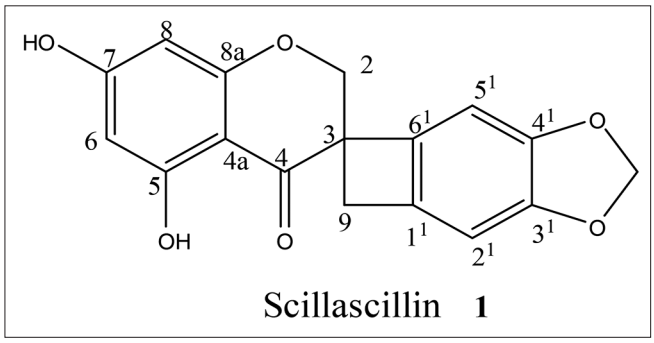

Figure 3: Structure of Scillascillin

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 IC_{50} values 9.59 and 11.32 µg/ml, respectively.

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