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Phytochemical screening and antimicrobial activity studies of underground bulbs of *Ledeboria hyderabadensis*

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ABSTRACT

Plants are good source for the bioactive compounds and are used as traditional medicines. Phytochemical investigation of plants emphasizes in traditional medicines has yielded various bioactive compounds with different pharmacological activities. In the Hyacinthaceae family, *Ledebouria* genus is the weakly evergreen bulbs. *Ledebouria hyderabadensis* is a new toxin exist in the Hyderabad city of Telangana state, India. In the present study, we have carried out isolation of homoisoflavone from the underground bulbs of *L. hyderabadensis* and phytochemical screening of crude extracts of bulbs of *L. hyderabadensis.* The methanol extract of underground bulbs and isolated compound were screened for antimicrobial activity. Both the methanol extract and isolated compound Scillascillin shows significant antimicrobial activity.

Keywords: Phytochemical screening, extraction, antimicrobial activity, Scillascillin, *Ledebouria*.

INTRODUCTION

Plant materials are a wellspring of various medications, for example, antimicrobials, antipyretics, antioxidants, antispasmodics, antitumor, emetics, and antidiarrheals agents. A huge number of the plants are professed to have important properties in customary medication and are additionally utilized broadly by inborn individuals around the world. The plant-based, customary meds keep on assuming an imperative job in social insurance, with about 80% of the world's occupants depending primarily on traditional medications for their essential medicinal services [1]. Plants contain a variety of significant substances valuable as perfumes, cosmetics, food additives, aromas, and for medicinal treatment of different infections [2]. Present day pharmacopeia contains at any rate 25% medications that are gotten from plants, which are synthetic and based on separated compounds from plants [3]. Synthetic drug can cause symptoms and therefore individuals are increasingly ideal to utilize common mixes got from plants. In this way, plants remain a significant source of therapeutic compounds. Phytochemical investigation of plants utilized in traditional medicines has yielded various compounds with different pharmacological activities. Research has underscored the evaluation and interpretation of different plants and plant constituents against various infections. Discovery, estimation and extraction of the bioactive arrangement constituents have dependably been a testing assignment. The WHO (World Health Organisation) [4] characterized medicines from plants as natural arrangements created by extraction, fractionation, cleaning, concentration or other physical or organic procedures which may deliver healthful or therapeutic compounds for as a basis for herbal products. Extraction strategies are the very important initial step to separate the therapeutically active bits of plant constituents from the inactive components. Plant constituents can be gotten from any organ of the plant like roots, bulbs, bark, leaves, flowers, fruits, seeds and so on. Some plant organ may contain more dynamic parts than the others. Crisp or dried plant materials can be utilized for the extraction of secondary metabolites. Plants are normally air dried to a steady weight before extraction. During the years, medicinal herbs, natural medicines were utilized for the cure of a range of diseases.

In view of the importance of isolation and phytochemical investigation of plant materials, the present study was conducted to isolation and phytochemical screening of endomic plant *Ledebouria.* The genus *Ledebouria* Roth belongs to the family Hyacinthaceae [5]. The genus is now regarded as discrete from *Scilla* L, which is genus of the northen South Africa [6]. *Ledebouria* Roth of the Hyacinthaceae family is a genus consisting of approximately 60 species distributed in Madagascar and India. In the Hyacinthaceae family, *Ledebouria* genus is the weakly evergreen bulbs. In 2012, Ramana *et. al.*, [7] reported a new taxon, *Ledebouria hyderabadensis* is recognized as the first collection from the Hyderabad city of Telangana state from India. *L. hyderabadensis* is being a new plant any kind of earlier reports are not available related

to phytochemical analysis and antimicrobial activity and hence, this prompted us to look into its phytochemistry and biological activity.

MATERIALS AND METHODS

Collection and authentication of plant material

For the present study, the underground bulbs of *Ledeboria hyderabadensis* was collected at auditorium premises (17°24'55.8"N 78°31'48.6"E), Osmania University, Hyderabad, Telangana (Figure 1), India during mid-rainy (July-August) season. A voucher specimen (BSI/DRC/2018-19/Tech/348) was deposited in the Herbarium of Botanical Survey of India, Deccan section, Hyderabad, India.

Figure 1: Collected plant material of *Ledeboria hyderabadensis*.

Extraction and isolation

We have isolated the homoisoflavone, Scillascillin from the underground bulbs of *L. hyderabadensis* as per previously reported method. The collected underground bulbs of *L. hyderabadensis* were sterilized by spraying 70% alcohol after cleanly washed with water for three to four times. The freshly sterilized bulbs were dried at room temperature to avoid chemical changes. Then the shade dried bulbs were crushed and powdered. The powdered (700 g) material of bulbs was extracted by using soxhlet apparatus at reflux temperature with methanol. The extract was evaporated under reduced pressures and controlled temperature of 40°C and then freeze dried. To remove fats and other color impurities from the viscous methanol extract washed several times with *n*-hexane. The solid extracts were concentrated under *vacuo*. Then the crude extracts of the bulbs were purified by column chromatography using silica gel as a stationary phase and eluted with 10% ethylacetate in hexane solvent mixture yielded a pure pale yellow colored compound Scillascillin.

Phytochemical screening

The phytochemical screening (qualification tests) of crude extracts of bulbs of *L. hyderabadensis* were carried out by using standard procedures as described by Harborne [8], Sofowara [9], and Parekh [10] to identify the chemical constituents.

Biological assays

The methanol extract and compound Scillascillin were screened for antibacterial activity against Gram-positive (*Staphylococus aureus, Bacillus subtillis*) and Gram-negative (*Pseudomonas aeruginosa, Klebsiella pneumonia*) bacterial strains. The antifungal activities of the methanol extract and Scillascillin were tested against two pathogenic strains *Candida albicans* and *Aspergillus niger*.

Antimicrobial assay

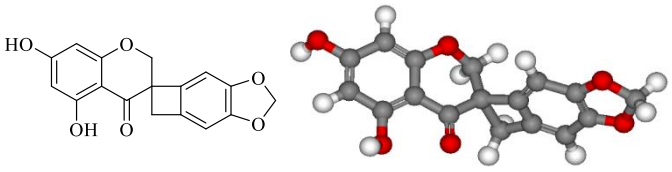
Antimicrobial activity studies of the compounds were tested using previously reported method [11]. 24 h culture medium (100 ml) was used to test the bacterial scrubbing of the nutrient broth plate. The wells (6 mm) were made using a sterile cork borer. The DMSO dissolved compounds with different concentrations at 25 µg/well, 50 µg/well, 100 µg/well were added to the wells using sterile pipette. The standard drugs, Chloramphenicol and Ketoconazole were also tested as positive control for anti-bacterial and anti-fungal respectively. The sample was dissolved in DMSO and DMSO did not show any zone of inhibition region as a negative control. The culture dishes were incubated at 28°C for 48 hours for the fungi and 37°C for 24 h for the bacteria. After proper incubation, the diameter of the inhibition zone was measured. Maintain repeat and calculate the average of final antibacterial activity.

Minimum inhibitory concentration (MIC) assay

To determine MIC (Minimum Inhibitory Concentration) of the isolated compound and extracts, broth dilution method was used [12]. 24 h old culture of the test bacteria and fungi were diluted 100 fold in nutrient broth. The stock solution of the extracts mixes was set up in DMSO by dissolving 5 mg of the compound in 1 ml of DMSO. The concentration are increasing from 6.25 mg to 200 mg of the samples (1.25, 2.5, 5, 10, 20, 40 ml of stock solution contains 6.25, 12.5, 25, 50, 100, 200 mg of the isolated compound and extracts) were added to the microbes containing culture test tubes. After addition of respective concentration of samples to bacterial cultures containing test tubes were incubates at 37 ^{0}C for 24 h and fungal cultures containing test tubes were incubates at 28 ^{0}C for 48 h. The test tubes were inspected for obvious turbidity and utilizing nutrient broth as control. Control without test samples and with dissolvable was examined at the same time. The least concentration that inhibited observable growth of the tested organisms was recorded as Minimum Inhibitory Concentration (MIC).

RESULTS AND DISCUSSION

Our phytochemical investigation resulted in the isolation of a rare homoisoflavone. In previously reported pharmacological investigations in the Hyacinthaceae family, homoisoflavanones are commonly occurring constituents [13, 14]. In literature, some of the reports pertaining to isolation of homoisoflavone, Scillascillin from different plant sources [15, 16, 17]. *L. hyderabadensis* is being a new plant and any kind of earlier reports are not available for photochemical analysis and anti-microbial analysis of underground bulb extracts of *L. hyderabadensis.* We have collected the underground bulb extracts of *L. hyderabadensis* and successfully isolated the homoisflavone from column chromatography by previously reported method [17]. The spectroscopic and other physical data of the isolated compound Scillascillin compared with previous reports. The 2D and 3D structures of isolate compound Scillascillin is represented in Figure 2.



2D structure **3D structure**

Figure 2: 2D and 3D structures of Scillascillin.

Spectral Data of the Scillascillin: IUPAC name of Scillascillin is 5,7-dihydroxy-6'H-spiro[chromane-3,5'-cyclobuta[4,5]benzo[1,2-d][1,3]dioxol]-4-one: mp 190-192 ^{0}C (In lit. 190 ^{0}C). IR spectrum, ν, cm^{-1}: 3476, 2977, 1672, 1610, 1541, 1371, 1244, 1069. ^{1}HNMR (400 MHz, DMSO-d_6) δ 7.19 (s, 1H), 7.02 (s, 1H), 6.76 (s, 1H), 6.09 (s, 1H), 5.88 (s, 1H), 5.74 (s, 2H), 4.01 (s, 1H) 3.51 (d, *J* = 11.75 Hz, 1H), 3.38 (d, *J* = 11.75 Hz, 1H), 3.76 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.1, 168.6, 166.2, 165.5, 150.6, 148.9, 137.1, 135.7, 105.8, 104.1, 102.2, 100.6, 98.1, 96.2, 75.7, 55.3, 35.9. ESI-MS: m/z 313 (M+1) observed for $C_{17}H_{12}O_6$.

Phytochemical screening

The phytochemical screening of crude extracts of bulbs of *L. hyderabadensis* was carried out and the results were tabulated in Table 1. These results indicated that methanolic extracts of *L. hyderabadensis*

bulbs contains glycosides, alkaloids, flavonoids, carbohydrates, reducing sugars, phenols, tannins and saponines. These plant bulbs are void of anthraquinones, terpenoids and phytosteroids.

Antimicrobial activity

The methanol extract and compound Scillascillin were screened for antibacterial activity against gram-positive (*S. aureus, B. subtillis*) and gram-negative (*P. aeruginosa, K. pneumonia*) bacterial strains. The in vitro antibacterial activity results have shown in Table 2. From the activity data, gram-positive bacteria were more susceptible towards than the gram-negative bacteria. This data also revealed that methanol extract shows greater activity than the isolated compound Scillascillin. In fact, the crude compound exhibited almost similar antibacterial activity compare to standard drug Chloramphenicol especially against *Staphylococcus aureus* bacterial strains. The compound Scillascillin exhibited moderate activity towards all bacterial strains.

Table 1: Results of photochemical analysis tests on crude extracts of bulbs of *L. hyderabadensis*.

Type of Secondary metabolite	Method	Observations	Results
Glycosides	Keller Killiani test	Formation of reddish brown colour solution	Yes
Alkaloids	Mayer's test	Formation of yellowish coloured solution	No
	Hager's test	Formation of yellowish precipitate	Yes
Flavonoids	Lead acetate test	Formation of yellowish precipitate	Yes
	Alkaline Reagent test	Formation of yellowish solution which becomes colorless on addition of dilute acid	Yes
Anthraquinones	Boume-Tragr reaction	Formation of white coloured solution	No
Carbohydrates	Molisch's test	Formation of violet ring at the at the junction	Yes
Reducing Sugars	Benedict's test	Formation of orange red precipitate	Yes
	Fehling's test	Formation of greenish precipitate with Fehling A reagent	Yes
		Formation of brownish precipitate with Fehling B reagent	Yes
Phenols	Ferric chloride test	Formation of bluish black solution	Yes
Terpenoids	Libermann-Buchard test	Formation of red violet solution	No
Tannins	Gelatin test	Formation of white precipitate	Yes
Saponins	Froth test	Appearance of creamy miss of small effervesces	Yes
Phytosteroids	Libermann-Buchard test	Formation of green bluish coloured solution	No
	Salkowski's test	Formation of reddish coloured solution	No

The antifungal activities of the methanol extract and Scillascillin were tested against two pathogenic strains, *Candida albicans* and *Aspergillus niger*. Both extract and Scillascillin inhibited spore germination of the tested fungi and exhibited significantly higher antifungal activity towards *Candida albicans* than the *Aspergillus niger*. The anti-fungal activity or the tested compounds compared to standard drug Ketovonazole and the results are shown in Table 3 and the minimum inhibitory concentration (MIC) values were shown in Table 4.

Table 2: The *in vitro* antibacterial activity of the methanol extract and Scillascillin

Compound	Zone of Inhibition(ZOI in mm)											
	Gram-positive bacteria						Gram-negative bacteria					
	S. aureus			*B. subtillis*			*P. aeruginosa*			*K. pneumonia*		
	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg
Methanol extract	24±3	27±2	30±2	22±2	25±3	27±2	16±2	18±1	21±1	21±1	25±2	28±1
Scillascillin	17±3	18±2	21±2	16±2	18±3	21±2	9±2	10±1	13±1	17±1	19±2	22±1
Chloramphenicol	25±2	28±3	30±2	27±3	30±1	33±3	21±2	23±1	27±2	34±3	36±2	38±2
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-

(±) Standard deviation

Table 3: The *in vitro* antifungal activity of the methanol extract and Scillascillin

Compound	Zone of Inhibition(ZOI in mm)					
	C. albicans			*A. niger*		
	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg
Methanol extract	19±1	21±1	25±2	20±2	22±1	23±2
Scillascillin	14±2	15±2	18±1	13±3	15±2	15±2
Ketaconazole	31±1	33±3	36±2	35±1	36±1	38±2
Control (DMSO)	-	-	-	-	-	-

(±) Standard deviation

Table 4: Minimum inhibitory concentration of the methanol extract and Scillascillin

Compound	Minimum inhibitory concentration (MIC in µg/well)					
	S. aureus	***B. subtillis***	***P. aeruginosa***	***K. pneumonia***	***A. niger***	***P. chrysogenum***
Methanol extract	6.25	12.5	50	50	12.5	50
Scillascillin	50	100	>100	100	50	100
Chloramphenicol	6.25	6.25	6.25	12.5	-	-
Ketoconazole	-	-	-	-	6.25	25

CONCLUSION

In concussion, we have successfully isolated homoisoflavone, Scillascillin from the bulbs of indigenous and unexplored herb *L. hyderabadensis.* Phytochemical screening of crude extracts of bulbs of *L. hyderabadensis* was carried out. The methanol extract of underground bulbs and isolated compound Scillascillin were screened for antimicrobial activity. Both the methanol extract and Scillascillin shows significant antimicrobial activity. In fact, it was observed that methanol extract shows greater antibacterial activity than the compound Scillascillin.

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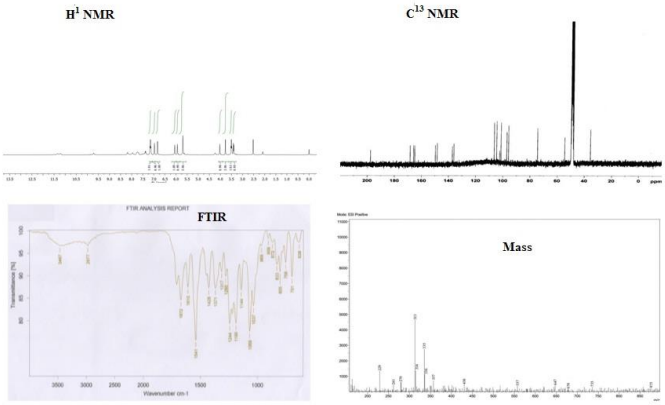
Appendix

Spectra of isolated compound



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