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 professors 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 endemic 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 northern 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.

**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 (*Staphylococcus aureus, Bacillus subtilis*) 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 concentrations of samples to bacterial cultures containing test tubes were incubates at 37°C for 24 h and fungal cultures containing test tubes were incubated at 28°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 Hyacinthacea family, homoisoflavonones 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 homoisoflavone 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.

**Spectral Data of the Scillascillin:** IUPAC name of Scillascillin is 5,7-dihydraoxyl-6H-spiro[chromane-3,5’,cyclobuta[4,5]benzo[1,2-\(d\)][1,3]dioxol]-4-one: mp 190-192°C (In lit. 190°C). IR spectrum, ν, cm\(^{-1}\): 3476, 2977, 1672, 1610, 1541, 1371, 1244, 1069. \(^1\)HNMR (400 MHz, DMSO-\(d_6\)) δ 10.26 (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\(_7\)H\(_7\)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*...
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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. subtilis) and gram-negative (P. aeruginosa, K. pneumonia) bacterial strains. The in vitro antibacterial activity results are 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 compared 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      |
| Flavonoids                   | Hager’s test                  | Formation of yellowish precipitate                | Yes     |
| Anthraquinones               | Lead acetate test             | Formation of yellowish precipitate                | Yes     |
| Carbohydrates                | Alkaline Reagent test         | Formation of yellowish solution which becomes colorless on addition of dilute acid | Yes |
| Reducing Sugars              | Boume-Trag reaction           | Formation of white coloured solution              | No      |
| Phenols                      | Molisch’s test                | Formation of violet ring at the at the junction   | Yes     |
| Terpenoids                   | Benedict’s test               | Formation of orange red precipitate               | Yes     |
| Tannins                      | Fehling’s test                | Formation of greenish precipitate with Fehling A reagent | Yes |
| Saponins                     | Ferric chloride test          | Formation of bluish black solution                | Yes     |
| Phytosteroids                | Libermann-Buchard test        | Formation of red violet solution                  | No      |
|                              | Gelatin test                  | Formation of white precipitate                    | Yes     |
|                              | Froth test                    | Appearance of creamy miss of small effervesces    | Yes     |

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 of 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. subtilis            | P. aeruginosa          | K. pneumonia          |
|                |                                | 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                   | 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   |
| Ketakonazole    | 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. subtilis | 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 conclusion, 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

![Spectra of isolated compound](image)

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