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From Antimicrobial Action to Antiproliferative Potential: Functional Characterization of *Scadoxus multiflorus* Bulb Lectin (SmL)

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KEYWORDS
Scadoxus multiflorus, bulb lectin, antibacterial activity, antifungal activity, anticancer activity, HeLa cells, HEK-293

ABSTRACT:

Introduction

Lectins are plant-derived proteins known for their wide range of biological activities, particularly antimicrobial and antiproliferative properties. Scadoxus multiflorus is a medicinal plant reported to contain lectins with potential therapeutic value. However, limited information is available regarding their biological efficacy.

Objectives

This study aimed to evaluate the antimicrobial and Antiproliferative properties of the bulb lectin (SmL) isolated and purified from S. multiflorus.

Methods

The antibacterial activity of SmL was assessed using the agar well diffusion method against Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Antifungal activity was evaluated against Aspergillus flavus, Aspergillus niger, and Candida albicans using standard antifungal assays, with clotrimazole as the reference drug. Cytotoxic effects were determined by MTT assay on HeLa (human cervical cancer) and HEK-293 (normal human embryonic kidney) cell lines, and IC_{50} values were calculated.

Results

SmL exhibited strong antibacterial activity against S. aureus (20 mm) and S. pyogenes (22 mm), with moderate inhibition of K. pneumoniae (15 mm) and P. aeruginosa (16 mm). Antifungal activity was comparatively weak, with inhibition zones of 9 mm for A. flavus, 8 mm for A. niger, and 8 mm for C. albicans, compared with clotrimazole. Cytotoxicity analysis demonstrated potent inhibition of HeLa cell proliferation, with an IC_{50} of 0.0095 µg/100 µL after 48 h, while HEK-293 cells showed negligible toxicity even at concentrations up to 50 µg/500 µL.

Conclusion

The findings demonstrate the dual bioactivity of SmL as an effective antibacterial agent and a selective cytotoxic molecule. These results suggest that SmL may represent a promising candidate for further investigation into its mechanisms of action and potential therapeutic applications.

Introduction

Lectins are a diverse group of carbohydrate-binding proteins of non-immune origin, found in plants, animals, and microorganisms. Lectins are widely studied due to their capacity to specific recognition and reversible binding to specific glycan moieties on cell surfaces without altering their covalent structure.[1] Plant lectin have been classified into various families such as legume lectins, jacalin-related lectins, and mannose-binding lectins, each with distinct structural and functional characteristics.[2] In plants, lectins function as defence proteins against herbivores and pathogens by binding to glycoconjugates, thereby interfering with

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microbial adhesion or insect digestion.[3] Beyond their ecological roles, lectins have attracted considerable biomedical interest due to their potential as antimicrobial, antiviral, immunomodulatory, and anticancer agents.[4] The emergence of antimicrobial resistance has become a major global health threat, limiting the effectiveness of conventional antibiotics and creating an urgent need for alternative therapeutic strategies.[5] Natural products, particularly proteins from plants, are being extensively investigated as potential antimicrobial agents due to their structural diversity and novel mechanisms of action.[6] Numerous plant lectins exhibit antimicrobial effects against bacteria, fungi, and viruses. These effects are mediated through binding to microbial surface carbohydrates, thereby disrupting cell wall integrity or blocking adhesion to host tissues.[7]

Among the plant lectins, lectins isolated from underground storage organs such as bulbs and tubers are of increasing interest due to their abundance, stability, novel carbohydrate specificity and potent biological activity. Bulb and tuber lectins have been reported to possess antimicrobial, antifungal, and antiviral properties, in addition to their roles in plant defence.[8] For instance tuber-derived lectins are particularly abundant in species like *Dioscorea* (yam) and *Colocasia esculenta* (taro). *Dioscorea batatas* lectin has demonstrated antimicrobial activity against both Gram-positive and Gram-negative bacteria, attributed to its mannose-binding ability that interferes with bacterial adhesion.[9] Similarly, *Colocasia esculenta* tuber lectin exhibits antifungal activity against *Candida albicans* and *Aspergillus* species, likely through binding to cell wall mannans.[10] These studies suggest that tuber lectins could act as natural antimicrobial agents. Cancer remains one of the leading causes of morbidity and mortality worldwide, and the limitations of current chemotherapeutic agents—including toxicity, resistance, and lack of specificity—necessitate the search for novel therapeutic biomolecules.[11] Lectins exploit the aberrant glycosylation of cancer cells, binding to glycoconjugates such as sialylated or fucosylated residues, which are often up regulated during tumour progression.[12] This selective recognition provides a basis for lectins to trigger downstream signalling pathways that lead to apoptosis, autophagy, or growth inhibition.[13] Plant-derived lectins, particularly those isolated from underground storage organs such as tubers and bulbs, have gained attention for their potential anticancer activities. Bulbous plants, particularly those in the Amaryllidaceae and Alliaceae families, are rich in lectins with documented anticancer effects. Garlic (*Allium sativum*) bulb lectin demonstrated inhibitory effects on colon and breast cancer cell lines by inducing apoptosis and modulating ROS generation.[14] Onion (*Allium cepa*) lectin has shown selective growth suppression of human tumour cells, possibly through glycan-mediated signalling pathways.[8] Despite these promising findings, many bulb and tuber-derived lectins remain underexplored, warranting systematic investigation into their antimicrobial potential and cancer potential.

Scadoxus multiflorus (family: Amaryllidaceae), commonly known as the blood lily, is an ornamental and medicinal plant distributed in tropical and subtropical regions of Africa and Asia. Traditionally, its bulbs and leaves have been used to treat fever, infections, and inflammatory disorders.[15] Phytochemical studies on *S. multiflorus* have revealed the presence of alkaloids, flavonoids, glycosides, and lectins, contributing to its pharmacological profile.[16] Despite this, limited research has been directed toward isolating and characterizing the bulb lectin (SmL) and exploring its biological potential. Given the increasing prevalence of antimicrobial resistance and the rising global burden of cancer, natural products such as lectins are gaining attention as potential therapeutic leads. The present study investigates the dual therapeutic potential of SmL, aiming to establish its relevance in drug discovery from natural sources.

Materials and Methods

Plant Material and Lectin Preparation

Fresh bulbs of *Scadoxus multiflorus* (family: Amaryllidaceae) were collected during May–June from Shivamogga district, Karnataka, India. The plant material was authenticated, and bulbs were thoroughly washed with distilled water to remove soil and surface impurities. Clean bulbs were stored at –20 °C until further use. The lectin (SmL) purified from *S. multiflorus* bulbs in our previous work was utilized in the present study to evaluate its antimicrobial and anticancer properties.

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Cell Culture and Maintenance

HeLa (human cervical cancer) and HEK-293 (human embryonic kidney) cell lines were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained under aseptic conditions at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere containing 5% CO_2. For experimental assays, cells were seeded in suitable culture plates and allowed to adhere overnight before treatment with varying concentrations of *Scadoxus multiflorus* lectin (SmL) for 48 h. Culture medium (90% DMEM/F12 with 10% FBS) served as the untreated control, Dulbecco's phosphate-buffered saline (DPBS) was used as a negative control, and Cisplatin was included as a positive control. All chemicals and reagents employed in this study were of analytical grade and procured from SRL (India), HiMedia (India), and Sigma-Aldrich (USA).

Collection and Maintenance of Bacterial and Fungal Cultures

For antimicrobial assays, bacterial strains *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96), and *Streptococcus pyogenes* (MTCC 442), along with fungal strains *Aspergillus flavus* (MTCC 277), *Aspergillus niger* (MTCC 404), and *Candida albicans* (MTCC 227), were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Bacterial cultures were maintained on Nutrient Agar (NA) slants at 4 °C and sub-cultured every 10–12 days to ensure viability. Fungal strains were maintained on Potato Dextrose Agar (PDA) slants at 4 °C and transferred every 15 days. For experimental assays, working fungal cultures were prepared in Potato Dextrose Broth (PDB) and incubated at 28 ± 2 °C for 48–72 h.

MTT Assay for Cytotoxicity Evaluation

The cytotoxic potential of *Scadoxus multiflorus* lectin (SmL) was evaluated on HeLa (human cervical carcinoma) and HEK-293 (human embryonic kidney, normal) cell lines using the colorimetric MTT assay as originally described by Mosmann [17], with minor modifications.

Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2 to allow cell attachment. After incubation, the culture medium was replaced with fresh medium containing different concentrations of SmL (0.00018, 0.0018, 0.018, 0.18, 1.8, and 18 µg/100 µL) and maintained for 24 h. Each concentration was tested in triplicate.

Following treatment, the medium was aspirated, and cells were gently washed with phosphate-buffered saline (PBS). Thereafter, 100 µL of MTT solution (0.5 mg/mL in culture medium) was added to each well and incubated for 4 h. The formazan crystals formed were solubilized by adding 20 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells (considered 100% viable). The IC_{50} value, defined as the concentration of SmL that reduced cell viability by 50%, was calculated from the dose–response curve. The percentage of cell viability was calculated using the following equation:

Cell viability (%) = (Absorbance of control cells / Absorbance of treated cells) × 100

Antibacterial Assay (Agar Well Diffusion Method)

The antibacterial activity of purified *Scadoxus multiflorus* lectin (SmL) was assessed using the agar well diffusion method [18]. Briefly, Nutrient Agar plates were seeded with 100 µL of standardized bacterial inoculum (18 h culture, OD adjusted to 0.6) and evenly spread using a sterile glass spreader. Wells of 6 mm diameter were bored into the agar and filled with 50 µL of SmL solution at concentrations ranging from 50–200 µg/mL. Azithromycin (30 µg/mL) served as the positive control. The plates were incubated at 37 °C for 24 h, and antibacterial activity was determined by measuring the diameter of inhibition zones (mm) around the wells using an antibiotic zone scale (HiMedia, Mumbai, India).

Antifungal Assay (Cup Plate Method)

Antifungal activity of SmL was evaluated by the cup plate method with slight modifications [19]. Potato Dextrose Agar (25 mL) was poured into sterile Petri dishes (HiMedia, Mumbai, India) and allowed to solidify. A 100 µL suspension of fungal spores (3-day-

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old cultures) or yeast cells (24 h culture) was uniformly swabbed over the agar surface using a sterile cotton swab. Wells of 6 mm diameter were filled with 50 µL of SmL solution at concentrations of 50–200 µg/mL. Clotrimazole (30 µg/mL) was used as the positive control, while sterile water served as the negative control. Plates were incubated at 28 °C for 48–72 h, and antifungal activity was recorded by measuring inhibition zones (mm) around the wells using an antibiotic zone scale (HiMedia, Mumbai, India).

Statistical Analysis

All assays were performed in triplicate ($n \geq 3$), and results were expressed as mean ± SD. Data were analysed by one-way ANOVA followed by t- test. P-value < 0.05 was considered statistically significant.

Results and Discussion

Antiproliferative Effect of SmL on HeLa and HEK-293

In this study, we investigated the anticancer activity of SmL on HeLa (human cervical cancer) and HEK-293 (Human embryonic kidney normal) cell lines by MTT assay. Fig 1A and 1B represents the percentage of cell viability shown by HeLa and HEK-293 on lectin treatment in a concentration-dependent manner after 48 h of exposure respectively. The cytotoxicity of Scadoxus multiflorus bulb lectin (SmL) was first evaluated on non-cancerous human embryonic kidney cells (HEK-293) to assess its safety profile. SmL exhibited no significant cytotoxic effect on HEK-293 cells, even at the highest tested concentration of 50 µg/500 µL. This observation suggests that SmL maintains a favourable therapeutic index**,** selectively targeting malignant cells while sparing normal counterparts. Such selective toxicity is an essential characteristic for the development of novel anticancer therapeutics derived from plant lectins. In contrast, SmL demonstrated pronounced cytotoxic activity against HeLa cells in a concentration- and time-dependent manner. After 48 h of treatment, the IC_{50} value was 0.0095 µg/100 µL**,** indicating strong antiproliferative potential at remarkably low concentrations. This high potency suggests that SmL may interact with cancer cell-specific surface glycoconjugates, leading to disruption of cellular signalling and induction of cell death pathways, consistent with the known mechanism of lectins. The anticancer potential of SmL is in agreement with previous reports on bulb-derived lectins. For instance, a mannose-specific lectin purified from Hyacinth bulbs inhibited Caco-2 and HeLa cells with IC_{50} values of 127µg/mL and 158 µg/mL, respectively, which is several orders of magnitude less potent than SmL.[20].Similarly, extracts from *Hymenocallis littoralis* bulbs demonstrated growth inhibition in HepG-2 cells with IC_{50} values around 0.8µg/mL, while showing no apparent effect on non-tumour cells.[21, 22] These findings indicate that although bulb lectins are generally cytotoxic to tumour cells, SmL displays superior potency and selectivity**,** distinguishing it from previously studied lectins. A major challenge in cancer therapeutics is avoiding toxicity toward normal cells. SmL's lack of cytotoxicity in HEK-293 cells, combined with its strong activity against HeLa cells, suggests it selectively targets malignant cells. This selectivity likely arises from SmL's ability to recognize altered glycosylation signatures on tumour cell membranes, a mechanism previously reported for other plant lectins.[23]

Antibacterial Activity

The antibacterial activity of *Scadoxus multiflorus* bulb lectin (SmL) was evaluated against four pathogenic bacterial strains, namely *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96), and *Streptococcus pyogenes* (MTCC 442), using the agar well diffusion method. SmL exhibited significant inhibitory effects against all tested strains, as reflected by the zones of inhibition (Table 1). Among the tested bacteria, SmL showed remarkably higher activity against Gram-positive strains**.** The maximum zones of inhibition were recorded against *S. aureus* (20 mm) and *S. pyogenes* (22 mm) at a concentration of 200µg. In comparison, relatively lower inhibition zones were observed against *K. pneumoniae* (15mm) and *P. aeruginosa* (16mm) at the same concentration. These findings indicate that SmL displays a broader antibacterial spectrum, with pronounced efficacy against Gram-positive pathogens. The present study demonstrates that *Scadoxus multiflorus* bulb lectin (SmL) possesses broad-spectrum antibacterial activity, with greater efficacy against Gram-positive bacteria (*S. aureus* and *S. pyogenes*) compared to Gram-negative strains (*K. pneumoniae* and *P. aeruginosa*). The stronger inhibition zones observed

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in Gram-positive bacteria (20–22 mm) may be attributed to the structural differences in their cell walls. Unlike Gram-negative bacteria, which possess an outer membrane rich in lipopolysaccharides that acts as a permeability barrier, Gram-positive bacteria lack this protective layer, making them more susceptible to lectin-mediated interactions.[24] Lectins are known to bind specific carbohydrate moieties on microbial cell surfaces, leading to agglutination, disruption of cell membrane integrity, and inhibition of microbial growth.[23] Similar antibacterial activities have been reported for lectins from other bulbous plants. For instance, a lectin purified from *Hymenocallis littoralis* bulbs displayed inhibitory effects against both Gram-positive and Gram-negative bacteria, though with moderate zones of inhibition.[22] Likewise, mannose-binding lectins from *Hyacinthus orientalis* bulbs have been shown to interfere with bacterial adhesion and colonization.[20]

The relatively stronger antibacterial effect of SmL against *S. aureus* and *S. pyogenes* suggests that this lectin may have potential therapeutic applications in combating infections caused by Gram-positive pathogens, which remain a major concern due to increasing antibiotic resistance.

Antifungal Activity

The antifungal activity of *Scadoxus multiflorus* bulb lectin (SmL) was evaluated against *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans* using the agar well diffusion method. SmL exhibited very low antifungal activity, with inhibition zones of 9 mm, 8 mm, and 8 mm, respectively (Table 2). In contrast, the positive control Clotrimazole (30µg/mL) demonstrated significantly higher activity, producing inhibition zones greater than 20 mm for all tested fungi. These results indicate that SmL possesses limited antifungal potential under the tested conditions. Although plant lectins are widely reported to exhibit antifungal properties, the *S. multiflorus* bulb lectin displayed comparatively weak activity against the tested fungal strains. The observed inhibition zones (8–9 mm) were significantly smaller than those of the standard antifungal drug clotrimazole, suggesting that SmL has minimal direct inhibitory effects on fungal growth. Several studies have highlighted variability in the antifungal efficacy of lectins depending on their carbohydrate-binding specificity and the nature of fungal cell wall glycans. For example, mannose-binding lectins from *Hippeastrum hybrid* bulbs demonstrated moderate antifungal activity against *Candida* spp.[25], whereas lectins from *Allium sativum* exhibited stronger inhibition due to their affinity for mannose/glucose residues on fungal surfaces.[26] The comparatively weak antifungal action of SmL may be explained by its limited binding to the predominant carbohydrates in fungal cell walls such as chitin and β-glucans, thereby reducing its capacity to interfere with fungal growth and viability. Moreover, differences in fungal susceptibility have been reported, with *Candida* species being more resistant to lectins than filamentous fungi.[27] The minimal inhibition observed against *C. albicans* in this study aligns with such findings. Thus, while SmL shows promising antibacterial and anticancer activities, its antifungal activity appears negligible, warranting further investigation into its structural carbohydrate specificity and potential synergistic effects when combined with antifungal agents.

Conclusion

The present study demonstrates that *Scadoxus multiflorus* bulb lectin (SmL) exhibits promising antibacterial and anticancer activities, while displaying limited antifungal potential. SmL showed broad-spectrum antibacterial activity, with greater efficacy against Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* compared to Gram-negative strains. In cytotoxicity studies, SmL displayed significant selectivity toward cancer cells, with potent growth inhibition of HeLa cells while sparing normal HEK-293 cells, indicating its potential as a safe anticancer agent. Conversely, its antifungal activity was minimal when compared to the standard antifungal drug clotrimazole, suggesting a narrower antimicrobial spectrum.

Overall, these findings highlight *S. multiflorus* bulb lectin as a bioactive protein with dual antibacterial and anticancer potential**,** meriting further investigation into its carbohydrate-binding specificity, structure–function relationship, and mechanism of action. Future studies focusing on in vivo validation, molecular docking, and protein engineering approaches may strengthen its applicability in the development of novel therapeutic agents.

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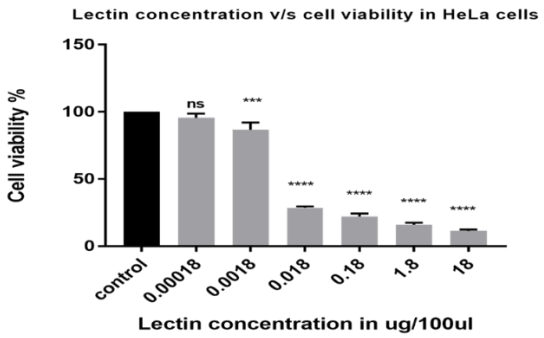

Fig 1 A: Effect of SmL on the viability of HeLa cells. HeLa cells were treated with increasing concentrations of SmL (0.00018, 0.0018, 0.018, 0.18, 1.8, and 18 µg/100 µL) for 48 h, and cell viability was evaluated using the MTT assay. Untreated controls were considered 100% viable. Data are expressed as mean ± SD from two independent experiments performed in triplicate.

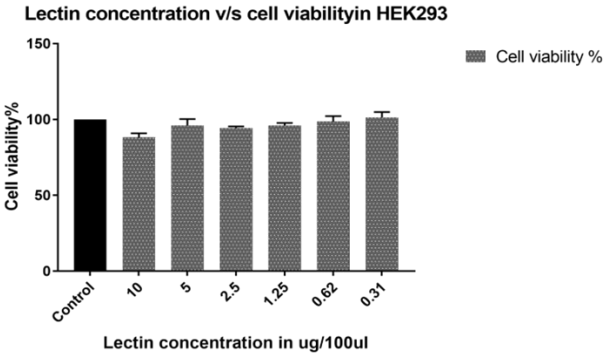

Fig 2 A: Effect of SmL on the viability of HEK-293 cells. HEK-293 cells were treated with increasing concentrations of SmL (0.31, 0.62, 1.25, 5, and 10 µg/100 µL) for 48 h, and cell viability was assessed using the MTT assay. Untreated cells were considered as 100% viable. Data are presented as mean ± SD from two independent experiments performed in triplicate.

Name of the organism	**Zone of Inhibition (mm)**					**ZOI (mm) standard** (Azithromycin)
	0µg	**50 µg**	**100 µg**	**150 µg**	**200 µg**	**30 µg**
Klebsiella pneumoniae	-	8	9	12	15	16
Pseudomonas aeruginosa	-	10	12	14	16	24
Staphylococcus aureus	-	13	15	17	20	22
Streptococcus pyogenes	-	12	14	18	22	24

Table 1: Antibacterial activity of *Scadoxus multiflorus* bulb lectin (SmL) against selected bacterial strains by agar well diffusion assay.

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Values are expressed as mean ± SE from three independent experiments performed in triplicate. Zone of inhibition is expressed in millimetres (mm). Azithromycin (30µg) served as the positive control.

Name of the organism	**Zone of Inhibition (mm)**					**ZOI (mm) standard** (Azithromycin)
	0µg	**50 µg**	**100 µg**	**150 µg**	**200 µg**	**30 µg**
Aspergillus flavus	-	-	-	8	9	25
Aspergillus niger	-	-	7	7	8	19
Candida albicans	-	7	7	7	8	21

Table 2: Antifungal activity of *Scadoxus multiflorus* bulb lectin (SmL) against selected fungal strains by agar well diffusion assay.

Values are expressed as mean ± SE from three independent experiments performed in triplicate. Zone of inhibition is expressed in millimetres (mm). Clotrimazole (30 µg/mL) served as the positive control.

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