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Phytochemical profiling and evaluation of antioxidant, anticancer, antimicrobial and antibiofilm activities of endophytic fungi isolated from *Lavandula stricta*

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The emergence of multidrug-resistant pathogens underscores the urgent need for novel antimicrobial agents. In this study, ten endophytic fungal isolates (Ls1–Ls10) were isolated for the first time from *Lavandula stricta* and evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella oxytoca*, and *Candida albicans*. The most potent fungal isolate Ls1 was identified as *Sarocladium kiliense* using morphological and molecular techniques. Phytochemical analysis indicated that the *S. kiliense* extract is abundant in bioactive compounds, including phenolics, tannins, flavonoids, and alkaloids. The GC mass analysis proved the presence of 41 active compounds in the *S. kiliense*. Extract including; Benzene, (1-propylnonyl) (9.87%), Hexadecanoic acid (8.05%), Prostaglandin A1-biotin (6.77%), Docosene (6.69%), Octadecenoic acid (5.55%), and 1-Nonadecene (5.16%). The crude extract of *S. kiliense* showed outstanding anticancer activity against cancerous Hep-G2 and MCF-7 cell lines with IC50 of 31.7 and 49.8 µg/ml, respectively. This isolate exhibited significant antimicrobial activity, with inhibition zones ranging from 16.1 ± 0.1 mm to 35.5 mm. MICs varied between 62.5 and 250 µg/mL. *S. kiliense* exhibited antioxidant activity and antibiofilm activities. The *S. kiliense* extract demonstrated concentration-dependent antibiofilm activity. In conclusion, *S. kiliense* as a hopeful home of bioactive combinations with potent antimicrobial, antioxidant, anticancer, and antibiofilm activities, offering the potential for combating multidrug-resistant pathogens and therapeutic applications.

Keywords *Sarocladium*, Antibiofilm, Antioxidant, Antimicrobial, Anticancer, Diseases, Infected, Phytochemical analysis, Endophytes

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The rapid extent of multidrug-resistant microorganisms (MDROs) presents a serious global public health challenge, diminishing the effectiveness of conventional antibiotics and contributing to rising morbidity and mortality rates. Consequently, infections that were once easily treatable are becoming increasingly difficult to manage, leading to prolonged illness, higher healthcare costs, and increased mortality[1]. This alarming trend underscores the urgent need for novel effective antimicrobial agents derived from alternative sources[2]. Biofilm formation by pathogenic microorganisms further complicates treatment strategies, as biofilms confer resistance to antimicrobial agents and host immune responses[3]. The structure of biofilms is a complex, multi-step process involving initial attachment, micro colony formation, maturation, and eventual dispersion of cells to colonize new niches[4]. This mode of growth offers microorganisms several advantages, such as enhanced resistance to antimicrobial agents. The development of agents capable of inhibiting biofilm formation or disrupting established biofilms is a critical area of research[5].

Endophytes, residing asymptomatically within plant tissues, engage in symbiotic relationships and synthesize compounds that can confer protection to their host plants against pathogens. These metabolites exhibit antimicrobial, antioxidant, and antibiofilm potency, positioning endophytic fungi as promising candidates in the search for new therapeutic agents. Endophytic fungi exhibit multiple mechanisms to combat MDROs. One primary approach involves the production of diverse secondary metabolites with potent antimicrobial properties, such as alkaloids, terpenoids, and polyketides, which can inhibit or kill resistant pathogens[6]. Moreover, endophytic fungi may outcompete pathogens within the host environment, effectively suppressing pathogen growth[7]. They can also produce enzymes that degrade pathogenic structures or disrupting biofilm formation and virulence factor expression in MDROs. Fungal endophytes play a significant role in combating cancer by producing bioactive metabolites that induce apoptosis, inhibit tumor cell proliferation, and suppress angiogenesis[8]. These compounds often target cancer cells selectively, minimizing damage to healthy tissues[9]. Endophytes also enhance the production of antioxidants, reducing oxidative stress linked to cancer development[10]. Their ability to modulate immune responses further contributes to their anticancer potential. Research continues to explore these natural sources for novel and effective cancer therapies[11].

Lavandula stricta, a species within the lavender genus (*Lavandula*), is native to arid and semi-arid regions of North Africa, including Egypt[12]. Although specific traditional medicinal uses of *L. stricta* are not extensively recorded, related species within the *Lavandula* genus have a rich history in herbal medicine. *L. stricta* was considered as a promising source of natural antioxidants and bioactive compounds. The essential oil of *L. stricta* was rich in α-pinene, linalool, and other bioactive monoterpenes, while the methanolic extracts showed considerable phenolic content[13]. Isolating and studying endophytes from *L. stricta* could lead to the explore of novel compounds due to the unique environmental adaptations and photochemistry of *L. stricta*, its fungal endophytes may harbor distinct bioactive metabolites worthy of exploration[14].

The objective of our study was to isolate and identify endophytic fungi from *L. stricta*, with a specific focus on characterizing the antimicrobial, antibiofilm, antioxidant and anticancer properties of the most potent isolate. By evaluating its bioactive compounds and assessing its efficacy against multidrug-resistant pathogens, this research aims to explore the potential of endophytic fungi as a novel and alternative source of therapeutic agents.

Materials and methods

Isolation and characterization of endophytic fungi

Lavandula stricta (Ls) plants were collected from Ain Sokhna, the Red Sea, the Suez Governorate in Egypt (29.530865, 32.375677). Experimental research and field studies on plants, including the collection of plants and identification, comply with relevant institutional, national, and international guidelines and legislation by Prof. Dr. Abdou Marie Hamed at the Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt. The plant was kept in the Faculty of Science herbarium, Al-Azhar University (Voucher no. 725).

Healthy plant parts rinsed twice with sterilized distilled water (SDH_2O) and then disinfected using 70% CH_3CH_2OH for 60 S, followed by treatment with 4% NaOCl for another 60 S, finally rinsed with SDH_2O. The Ls sections were placed in sterile Petri dishes (9 cm in diameter) containing sterilized PDA medium. For control, sterile Petri dishes (9 cm in diameter) containing sterilized PDA medium inoculated with the solution of sterilized sections to ensure that fungi are endophytes. The plates were incubated in the dark at 28 °C for 21 days and monitored daily[15]. Emerging mycelium was carefully collected and subcultured. The isolated fungi were then assessed for their antimicrobial activity against pathogenic microorganisms, and the most effective strain was identified based on colony variations, morphological characteristics, and genetic analysis. Finally, the purified fungal isolates were stored at 4 °C for further studies. The molecular identification of the endophytic fungus was conducted by amplifying the internal transcribed spacer (ITS) region. Genomic DNA was extracted and purified using the Quick-DNA Fungal Microprep Kit (Zymo Research, D6007). PCR amplification was performed using ITS-specific primers: ITS1-F (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2-R (5′-TCCTCCGCTTATTG ATATGC-3′). The amplified products were then purified using the Gene JET PCR Purification Kit (Thermo Scientific, K0701). The purified sequences were analyzed through the BLAST tool from NCBI to determine the closest genetic matches. Verified sequences were subsequently submitted to the Gen Bank database, each assigned a unique accession number for global accessibility. To evaluate evolutionary relationships, a phylogenetic tree was constructed using the neighbor-joining method with MEGA software version 5.0[16].

Extraction of active metabolites

The secondary metabolites of *S. kiliense* were extracted by culturing the fungus in 500 mL of PD broth within a 1 L flask, followed by incubation at 28 °C for 15 days. Following incubation, the culture was filtered, and the resulting supernatant was combined with $CH_3COOC_2H_5$ in a 1:1 V/V and stored at 4 °C overnight. The metabolites were subsequently separated, and the extract was evaporated at 40 °C to yield the ethyl acetate crude extract (EACE) and stored at 4 °C for subsequent experimental use[17].

Antimicrobial activity

Muller Hinton agar (MHA, India) was employed to assess the antibacterial activity of *S. kiliense* against bacteria, while PDA was employed to evaluate its antifungal activity against *C. albicans*. The surface of the prepared MHA and PDA was cultured with 24-hour-old cultures of clinical isolates of *S. aureus*, *K. oxytoca*, *B. subtilis*, and *E. coli*, *C. albicans* ATCC10231. All clinical isolates of *S. aureus*, *K. oxytoca*, *B. subtilis*, and *E. coli* were obtained from bacteriology laboratory at Microbiology Department, faculty of science, Al Azhar University, Cairo. Egypt and were identified using standard microbiological methods in previous study[18]. 100 µl of each compound was transferred to each well (6 mm) individually and left at 4 ℃ for 2 h. Amikacin 30 µg was used as a control for bacteria and fluconazole 25 µg for CA. Plates were incubated for 24 h, 48 h at 37 °C, and 28 °C for bacteria and CA. After incubation, inhibitory zones were measured and reported[19].

Determination of MIC

The MIC of *S. kiliense* EACE against *S. aureus*, *K. oxytoca*, *B. subtilis*, and *E. coli*, *C. albicans* ATCC10231 were determined using a broth microdilution assay. Serial dilutions of *S. kiliense* EACE (100 µl) were supplementary to microtiter plate wells inoculated with 100 µl of double-strength MHB, achieving final concentrations of 1000: 31.25 µg/ml. 50 µl bacterial suspension was inoculated to all wells except the -Ve control (SDH_2O + MHB) and + Ve control (The first row used as + Ve control (using MHB + microorganisms) while second row used as -Ve control (using SDH_2O + MHB only without any microorganisms). + Ve control ensured broth adequacy, incubated at 37 °C for 24 h, then addition of 30 µl resazurin (0.02% wt./v) and re-incubation. Color modification from blue to purple indicated bacterial growth. Sterile controls remained unchanged, confirming no contamination. Experiments were performed in duplicate, and mean values were calculated[20].

Anti-biofilm ability

The anti-biofilm activity was assessed using 96-well microtiter plates[21]. Each well of a sterile microtiter plate was filled with 100 µL of MHB for bacteria Sabouraud Dextrose Broth for *Candida* and inoculated with 10 µL of an overnight bacterial and *Candida* culture suspension (OD620 0.05 ± 0.02). The EACE was then added at concentrations of ½, ¼, and 1/8 × MIC, and incubated at 37 °C for 48 h. Then, biofilms were fixed using absolute alcohol, stained with 0.1% (w/v) crystal violet, and incubated for 30 min. After drying, 200 µL of 33% acetic acid was added, and the OD of the stained biofilms was measured at 630 nm. The control used in this experiment involved the growth of microorganisms without any treatment, and the optical density was read. The results were then used in the following equation to calculate the percentage of biofilm inhibition.

$$Biofilm\ inhibition\ (\%) = 1 - \frac{OD630\ of\ cells\ treated\ with\ different\ concentration\ of\ S.\ kiliense}{OD630\ of\ non\ treated\ control} \times 100$$

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Cytotoxicity and anticancer activity of *S. kiliense* EACE

The cytotoxicity experiment was performed according to the MTT procedure established by Van de Loosdrecht, et al.[22]. The MCF-7 and Wi-38, sourced from ATCC, were utilized to evaluate the cytotoxic or anticancer effects of *S. kiliense* EACE, respectively. The measured OD of the cells at 560 nm was utilized to calculate cell viability and inhibition % [23], following Eqs. (1) and (2), respectively:

$$\text{Viability } \% = \frac{\text{Test OD}}{\text{Control OD}} \times 100 \quad (1)$$

$$\text{Inhibition } \% = 100 - \text{Viability } \% \quad (2)$$

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Anti-oxidant activity

The evaluation of the anti-oxidant activity of *S. kiliense* was conducted through the DPPH radical scavenging assay, which was adapted to assess the extract's ability to scavenge free radicals[21]. In the experiment, 100 µL of the DPPH solution was mixed with 100 µL of the sample in a 96-well microplate and allowed to incubate at 25^{O} C for 0.5 h. Ascorbic acid used as + Ve control for comparison. Absorbance was recorded at 490 nm with 100% methanol serving as the control. The DPPH scavenging activity was assessed using the subsequent formula:

$$\text{DPPH scavenging activity} = \frac{\text{control absorbance} - -S.\ kiliense\ \text{absorbance}}{\text{control absorbance}} \times 100$$

To evaluate the anti-oxidant potential, different concentrations of *S. kiliense* EACE (1000: 7.81 µg/mL) were tested. The results were expressed as DPPH scavenging activity (%), and the IC50 value was determined, providing insight into the extract's antioxidant strength. In addition, the ABTS assay used to assess the anti-oxidant activity of *S. kiliense*. This method was conducted following the protocol described by Lee et al.,[23]offering an alternative approach to evaluate the *S. kiliense* EACE ability to neutralize free radicals.

Phytochemical analysis of *S. kiliense*

The phytochemical analysis of *S. kiliense* was conducted following the methodology.

Determination of S. kiliense total flavonoid content (SKTFC)
SKTFC was determined using the $AlCl_3$ method. One mL of *S. kiliense* extract was dissolved in 2 mL methanol. Separate 5% solutions of $NaNO_3$, NaOH, and $AlCl_3$ were prepared. For analysis, 200 µL of the extract was mixed with 75 µL of 5% $NaNO_3$, incubated for 5 min, followed by the addition of 1.25 mL $AlCl_3$ and 0.5 mL NaOH. The mixture was sonicated, incubated for another 5 min, and absorbance was measured at 510 nm[24].

Determination of S. kiliense total phenolic content (SKTPC)
SKTPC of the *S. kiliense* extracts was evaluated using a colorimetric assay with Folin-Ciocalteu reagent. One mL of the extract was dissolved in 2 mL of CH_3OH, and then 500 µL was mixed with 2.5 mL of Folin-Ciocalteu reagent and 2.5 mL of a 75 g/L Na_2CO_3. Absorbance was measured at 765 nm after incubated at 25 °C for 2 h[25].

Determination of S. kiliense total tannin content (SKTTC)
SKTTC was assayed by the vanillin-HCl (VHCl) method, with tannic acid as the standard. A 400 µL aliquot of *S. kiliense* extract was combined with 3 mL vanillin 4% and 1.5 mL of HCl concentrated .Then absorbance measured at 500 nm after incubated at 25 °C for 15 min[25].

Total alkaloid content (TAC) determination
One mL of *S. kiliense* extract was washed three times with chloroform. The pH was adjusted to 7 using 0.1 N NaOH, after which 5 mL of Bromocresol Green solution and 5 mL of phosphate buffer (pH 4.7) were added and shaken vigorously to form a complex, which was then extracted using chloroform. TAC was quantified by measuring absorbance at 470 nm[25].

Gas chromatography-mass spectrometry
Metabolites in *S. kiliense* EACE were analyzed using GC-MS (Trace GC1310-ISQ, Thermo Scientific) with a TG-5MS column (30 m × 0.25 mm × 0.25 µm). The oven temperature started at 50 °C, increasing at 5 °C/min to 230 °C (held for 2 min) and then to 290 °C (held for 2 min). The injector and MS transfer line were set at 250 °C and 260 °C. A 1 µL sample was injected at 250 °C using helium as the carrier gas (split ratio 1:30). The MS operated in EI mode (70 eV, 200 °C) with a 40–1000 m/z scan range. Identification used WILEY 09 and NIST 11 libraries[21].

Statistical analysis
All statistical analyses were performed using Minitab 18.3 with three replicates. Descriptive analysis, including mean and standard error, was conducted.

Results and discussion
Isolation and characterization of endophytic fungi
In the current study, ten fungal isolates were isolated and purified fungal isolates (Ls1 to Ls10), then screened against *S. aureus*, *K. oxytoca*, *B. subtilis*, and *E. coli*, *C. albicans* ATCC10231. The highest effective fungal isolate was Ls1, thus identified as *Sarocladium sp*. *Sarocladium sp* colonies on PDA appear white to cream-colored initially, turning pale yellow with age (Fig. 1A). The texture was cottony, with a dense mycelial growth pattern. The reverse side of the colony is pale yellow to dark (Fig. 1B). Mycelium was septate and hyaline; conidiophores are slender. Conidia were unicellular, ellipsoidal to cylindrical, and typically formed in. Conidia appear smooth-walled and may form in chains or clusters (Fig. 1C). To validate the morphological identification, molecular analysis was conducted for the fungal isolate Ls1. Results revealed that fungal isolate Ls1 was similar to *Sarocladium kiliense* with 99% according to BLAST on gene bank. Then, the sequence of *S. kiliense* was deposited in gene bank with accession number PV248633.1. and phylogenetic tree was created in Fig. 1D.

By isolating these fungi from *L. stricta*, researchers can better understand their ecological roles, interactions with the host plant, and potential use in medicine, agriculture, and biotechnology. The first isolation of endophytic fungi from *L. stricta* marks a significant step in exploring the plant's hidden microbial diversity and its potential applications. *S. kiliense* was reported as an endophytic fungus isolated from a healthy *Aloe dhufarensis Lavranos* desert-adapted plant, highlighting its potential role in plant health and secondary metabolite production. As an endophyte, *S. kiliense* resides within the plant tissues without causing disease, possibly contributing to host defense mechanisms, growth enhancement[26]. The isolation and identification of the fungal strain Ls1 as *S. kiliense* underscore its potential as a prolific source of antimicrobial agents. The morphological characteristics observed white to cream-colored colonies transitioning to pale yellow, cottony texture with dense mycelial growth, and septate, hyaline mycelium were consistent with descriptions in existing literature[27]. The isolation and study of endophytic fungi from stress-resilient plants present a promising avenue for the discovery of novel bioactive compounds with significant medical applications due to the endophytic fungi are shaped by a wide range of factors such as environmental conditions, the type of host tissue, plant evolutionary lineage, geographic region, seasonal variations, and agricultural practices (organic vs. conventional). Additionally, surrounding vegetation and soil characteristics play a significant role[28].

Antimicrobial activity
The Table 1 presents data on the antimicrobial activity of *S. kiliense* EACE against various microbial strains, highlighting its effectiveness. *S. kiliense* EACE exhibits highly activity against both G + ve, G-ve, and *C. albicans*, with varying inhibition zone diameters, as shown in Table 1; Fig. 2. Notably, the inhibition zones for *K. oxytoca*, *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans* were 35.5 mm, 32.3 mm, 30.1 mm, 21 mm, and 16.1 ± 0.1 mm, respectively. The antibacterial activity of *S. kiliense* can be supported the previous study that demonstrated that

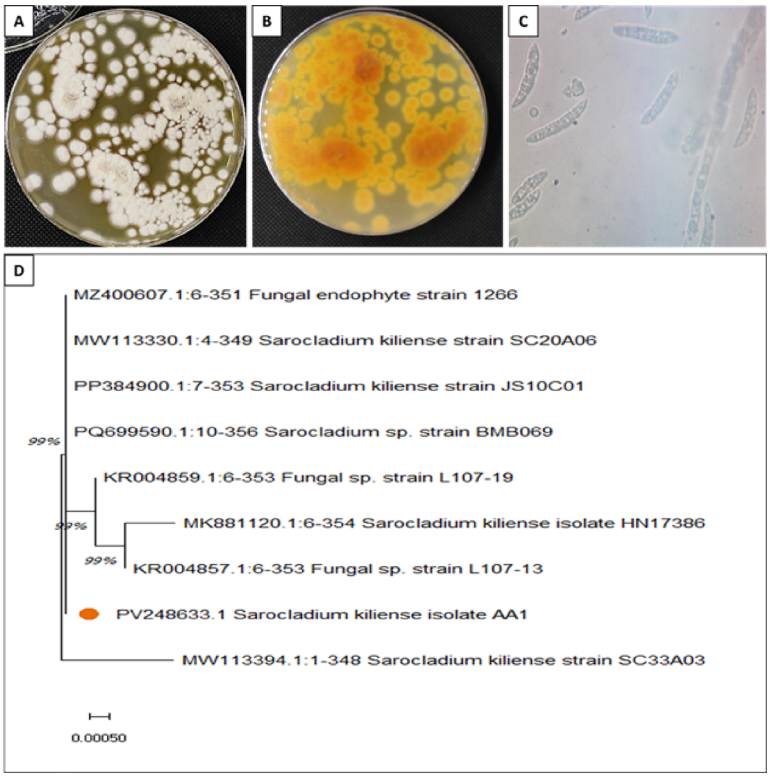

Fig. 1. Morphological identification of *S. kiliense* (**A**–**D**) were Colony surface, Reverse, Conidia, and Phylogenetic tree respectively.

Microbial strain	Inhibition zone (mm) of S. kiliense	DMSO	Amikacin / fluconazole
Staphylococcus aureus	32.3 ± 0.3	0	18 ± 0.5
Klebsiella oxytoca	35.5 ± 0.2	0	19.6 ± 0.3
Bacillus subtilis	30.1 ± 0.7	0	15.8 ± 0.4
Escherichia coli	21 ± 0.5	0	12.3 ± 0.3
Candida albicans ATCC10231	16.1 ± 0.1	0	19 ± 0.5

Table 1. Antimicrobial activity of *S. kiliense* EACE.

antibacterial activity of *L. stricta* essential oil was evaluated against a range of Gram-positive and Gram-negative bacteria including *Salmonella typhi*, *Pseudomonas aeruginosa*, *Listeria innocua*, *S. aureus*, and *E. coli*[29].

Determination of MIC

Table 2 presents the MIC of *S. kiliense* EACE against tested microorganisms. The MIC values ranging from 62.5 to 250 µg/mL, as illustrated in Table 2; Fig. 3. The *S. kiliense* EACE exhibited notable activity, with MICs of 62.5 µg/ml against *B. subtilis* and *K. oxytoca*, 125 µg/ml against *C. albicans* (ATCC10231), and 250 µg/ml against both *S. aureus* and *E. coli*. This broad-spectrum activity may be due to *S. kiliense* EACE-derived bioactive compounds. This is also consistent with many previous studies that have proven the presence of biologically active substances in extracts of endophytic fungi[30]. The MICs further elucidate the potency of *S. kiliense* EACE. MIC values ranging from 62.5 µg/mL to 250 µg/mL against pathogens like *B. subtilis*, *K. oxytoca*, and *C. albicans* demonstrate the extract's efficacy at relatively low concentrations. The mechanism of antimicrobial activity of *S. kiliense* EACE may be due to the disruption of antibiofilm properties. The anti-biofilm activity of *S. kiliense* extract adds another dimension to its antimicrobial profile. The antimicrobial activity could be confirmed by the presence of antimicrobial compounds as Heptacosane, Cyclohexanecarboxy lic acid,2-phenylethyl ester, Dodecanoic acid, Hexadecane, 1-Nonadecene, Octadecane[11,28].

Anti-biofilm ability

The in vitro evaluation of the anti-biofilm activity of *S. kiliense* EACE against the tested pathogens (Fig. 4) revealed a concentration-dependent reduction in biofilm formation across all species. *C. albicans* exhibited the highest inhibition, with biofilm reduction ranging from 67.55 ± 1.13% at ½ MIC to 40.29 ± 0.89% at 1/8 MIC.

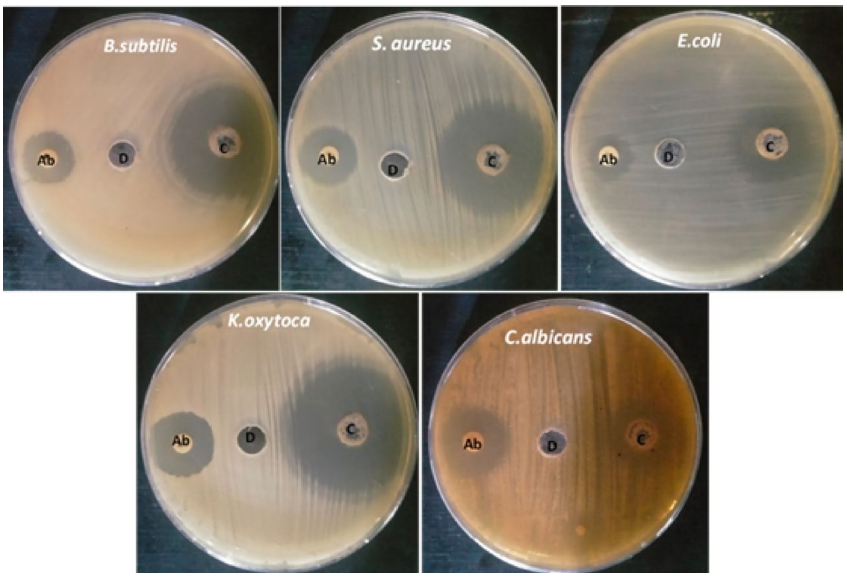

Fig. 2. Antimicrobial activity of *S. kiliense* against microbial pathogens, Ab = Amikacin/Fluconazole (Amikacin 30 µg was used as a control for bacteria and fluconazole for *candida*), D = DMSO.

Microbial strain	MIC of S. kiliense (µg/ml)
S. aureus	250 µg/ml
B. subtilis	62.5 µg/ml
K. oxytoca	62.5 µg/ml
E. coli	250 µg/ml
C. albicans (ATCC10231)	125 µg/ml

Table 2. MIC of *S. kiliense* EACE.

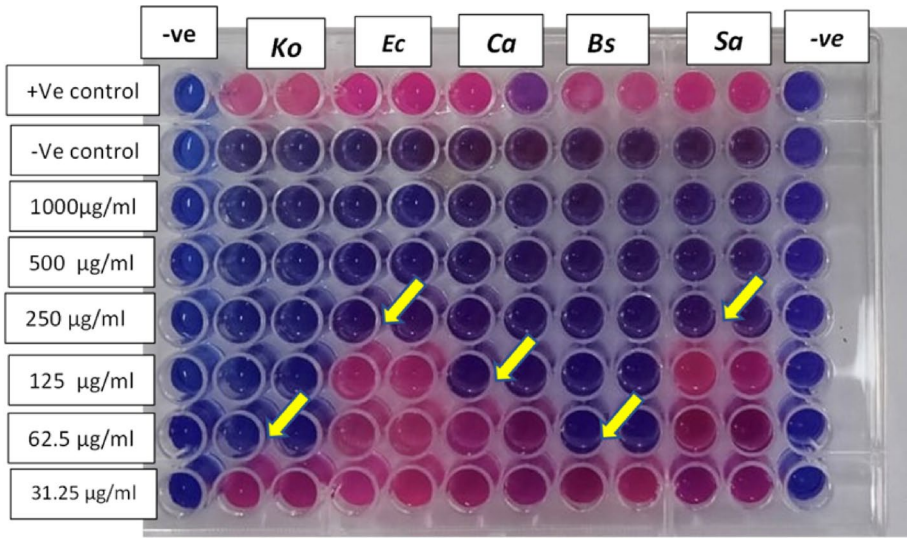

Fig. 3. MIC of *S. kiliense* against *E. coli*, *K. oxytoca*, *S. aureus*, *B. subtilis*, and *C. albicans*. The first row was used as + Ve control (using MHB + microorganisms) while second row used as -Ve control (using SDH_2O + MHB only without any microorganisms).

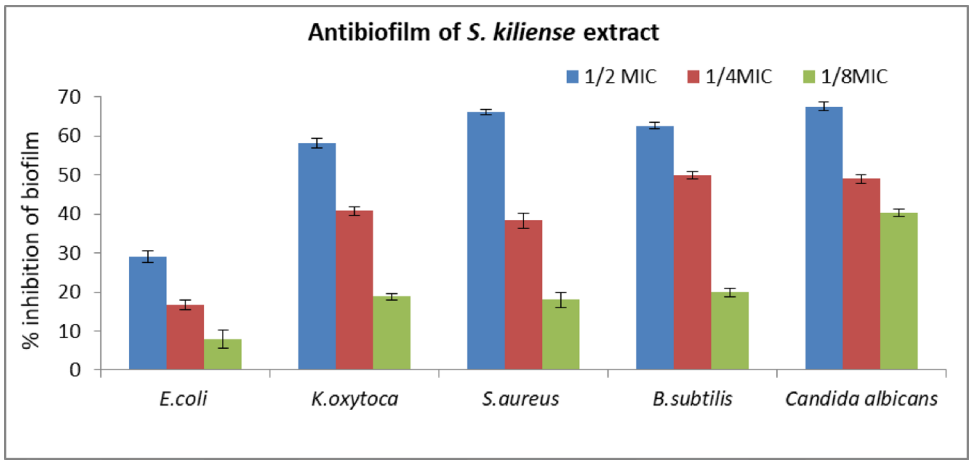

Fig. 4. Antibiofilm activity of *S. kiliense* EACE. The control used in this experiment involved the growth of microorganisms without any treatment.

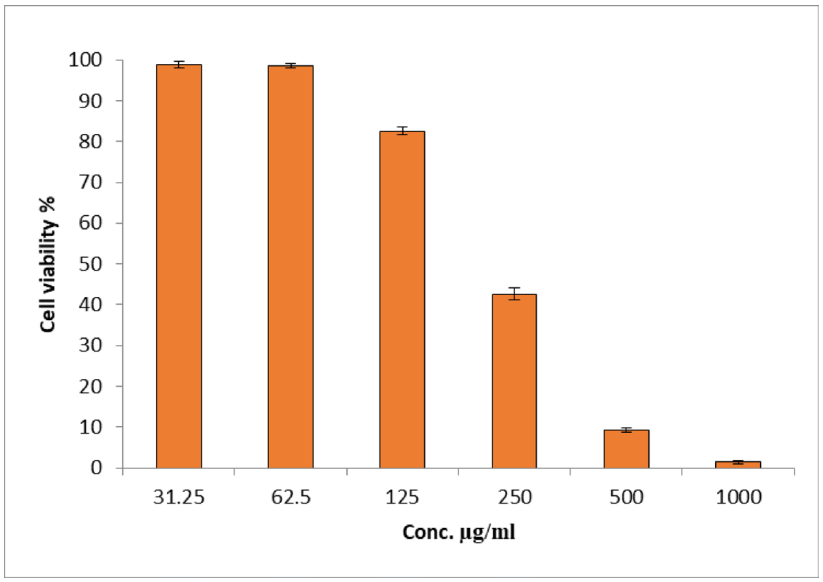

Fig. 5. Cytotoxicity of *S. kiliense* EACE toward Wi 38 normal cell line at different concentrations.

In contrast, *E. coli* showed the lowest reduction, with inhibition percentages varying from 28.99% at ½ MIC to 7.99% at 1/8 MIC. The antibiofilm activity was confirmed by antibiofilm components in *S. kiliense* EACE such as Prostaglandin A1-biotin, Octacosanol, Penta tri acontene, Behenic alcohol, and Eicosane. *Lavandula* essential oil demonstrated potent biofilm degradation activity, effectively reducing bacterial adhesion. The ability of *Lavandula* EACE to significantly impair *Campylobacter jejuni* motility further underscores their impact on biofilm inhibition by downregulating key genes involved in adhesion and biofilm formation[31].

Cytotoxicity and anti-cancer activity

In our study, the EACE of *S. kiliense* was emulated for cytotoxicity toward Wi 38 normal cell line at different concentrations as illustrated in Fig. 5. Results revealed that, IC50 of *S. kiliense* EACE was 226.5 µg/ml. This indicates that at this concentration, *S. kiliense* EACE reduces cell viability by 50%, highlighting its potency in inducing cytotoxicity. Therefore, the *S. kiliense* EACE is considered safe to use. Therefore, the safe and maximum non-toxic concentrations of the EACE were determined and evaluated for their anticancer potential. Evaluating the cytotoxicity of compounds toward normal cell lines involves assessing their effects on cell viability, proliferation, and morphology. This evaluation is crucial for determining the safety profile of potential therapeutic agents before clinical application[16]. Materials with an IC50 value of > 90 µg/mL are often categorized as non-cytotoxic[32]. The safe and optimal non-toxic concentrations of the extract were assessed for anticancer efficacy.

The highest non-toxic concentrations of *S. kiliense* EACE were evaluated for their anticancer effects on Hep-G2 and MCF-7 cancer cell lines (Fig. 6). The results showed that the IC50 values of *S. kiliense* EACE were 31.7 µg/ml for Hep-G2 and 49.8 µg/ml for MCF-7. In comparison, Taxol, a standard anticancer agent, exhibited IC50 values of 10.8 µg/ml for Hep-G2 and 7.9 µg/ml for MCF-7. Our results demonstrate the anticancer potential of *S. kiliense* EACE, an endophytic fungal extract, against Hep-G2 (liver cancer) and MCF-7 (breast cancer) cell lines, with IC50 values of 31.7 µg/ml and 49.8 µg/ml, respectively. These findings indicate that *S. kiliense* EACE is more effective against Hep-G2 cells compared to MCF-7 cells, suggesting a possible selectivity in its mechanism of action toward liver cancer. In comparison, Taxol, a well-established anticancer drug showed significantly lower IC50 values of 10.8 µg/ml for Hep-G2 and 7.9 µg/ml for MCF-7, reflecting its potent and broad-spectrum anticancer activity. These results illustrated the selective toxicity of *S. kiliense* EACE against cancer cells; thus, it may be used as an anticancer agents[33]. Our findings align with a recent study highlighting the anticancer potential of *Lavandula*, which showed notable cytotoxic activity against MCF-7 and MDA-MB-231 breast cancer cell lines and achieved a 43.29% reduction in tumor size in vivo, with complete tumor regression observed in 12.5% of treated mice[34].

Antioxidant activity

In our investigation, we assessed the antioxidant potential of *S. kiliense* EACE across a concentration range of 1000 to 7.81 µg/mL utilizing both DPPH and ABTS assays, as depicted in Fig. 7. The findings indicated that the EACE exhibited an IC50 value of 202.08 µg/mL in the DPPH assay, in contrast to the IC50 of 8.9 µg/mL for ascorbic acid. Similarly, in the ABTS assay, the EACE demonstrated an IC50 of 169.79 µg/mL, whereas ascorbic acid presented an IC50 of 7.61 µg/mL. The antioxidant activity of *S. kiliense* EACE can be explained by the phytochemical analysis that revealing high levels of phenolics, alkaloids, flavonoids, and tannins. The antioxidant activity of *S. kiliense* EACE also proved by the presence of antioxidant compounds that achieved by GC mass analysis as the following; Prostaglandin A1-biotin, Linoleic acid ethyl ester[35]. Our results can be explained by the study reported that *Lavandula* showed the modrate DPPH and ABTS radical scavenging and metal ion-reducing activities, likely due to its high content of oxygenated sesquiterpenes, particularly α-bisabolol and due to synergistic effects of its monoterpene-rich composition[36].

Phytochemical analysis of *S. kiliense* EACE

Total phenolics, alkaloids, flavonoids, and tannins was quantified for *S. kiliense* EACE as shown in (Fig. 8). Results revealed that presence high levels of phenolics, alkaloids, flavonoids, and tannins in the *S. kiliense* EACE. A high phenolic content (809.83 µg/mL) in the *S. kiliense* EACE may exhibit strong antioxidant, antimicrobial, and anti-inflammatory activities. Also playing a crucial role in protecting cells from oxidative damage and free radicals. Tannins are polyphenolic compounds known for their astringent biological properties. The presence of tannins (250.2 µg/mL) indicates that the EACE may contribute to antimicrobial, antifungal, and anti-inflammatory effects. The moderate flavonoid content of 232.23 µg/mL in the *S. kiliense* EACE suggests a potential role in neutralizing free radicals, inhibiting bacterial growth, and modulating immune responses. Alkaloids are bioactive secondary metabolites known for their antimicrobial, antifungal, and medicinal properties. The high alkaloid content (920.5 µg/mL) in this EACE suggests strong antimicrobial potential and possible pharmacological applications, such as anti-bacterial, anti-fungal, and anti-cancer activities. Phenolic compounds are renowned for their anti-oxidant properties, playing a crucial role in neutralizing free radicals and mitigating oxidative stress[37]. The presence of these active components supports the symbiotic relationship between *S. kiliense* and *L. stricta* and explains the *L. stricta* ability to adapt to this difficult environment. It also explains the presence of these substances in the plant when it is analyzed, as previous studies have proven the presence of these compounds in the plant extract[38].

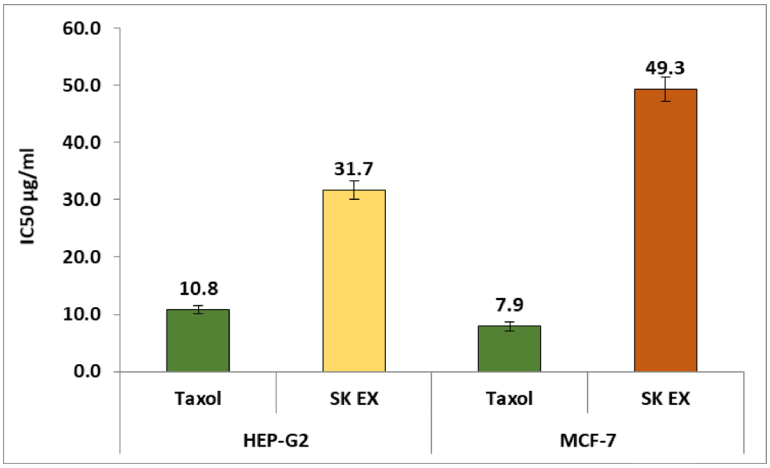

Fig. 6. Anticancer activity of *S. kiliense* EACE.

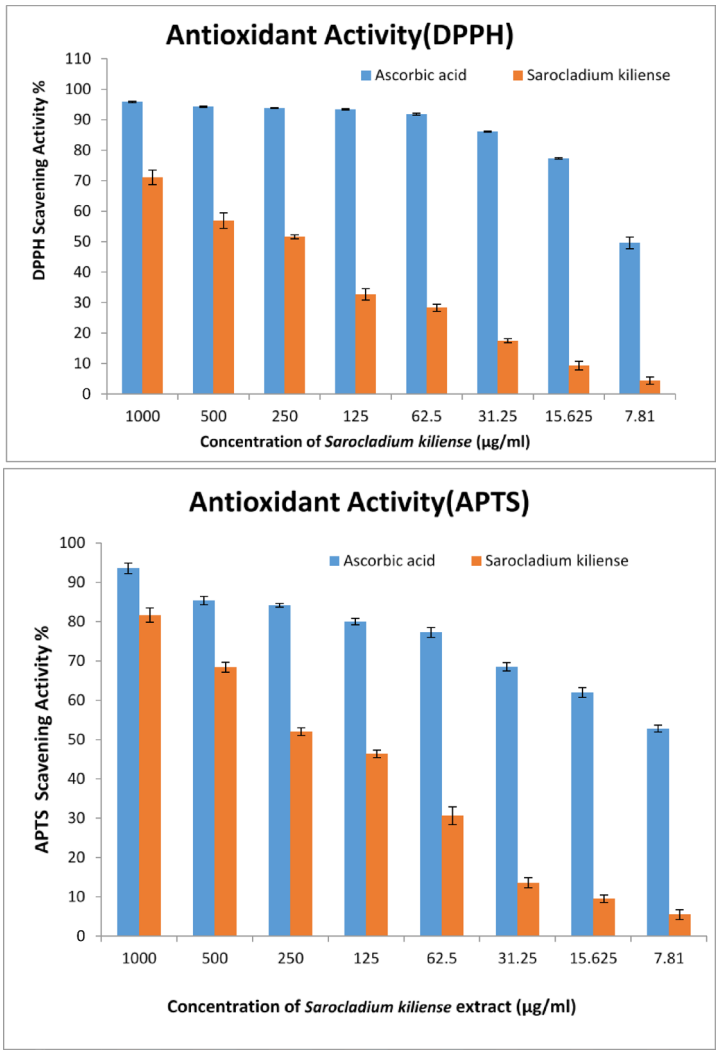

Fig. 7. Antioxidant activity of *S. kiliense* EACE. Ascorbic acid used as + Ve control for comparison.

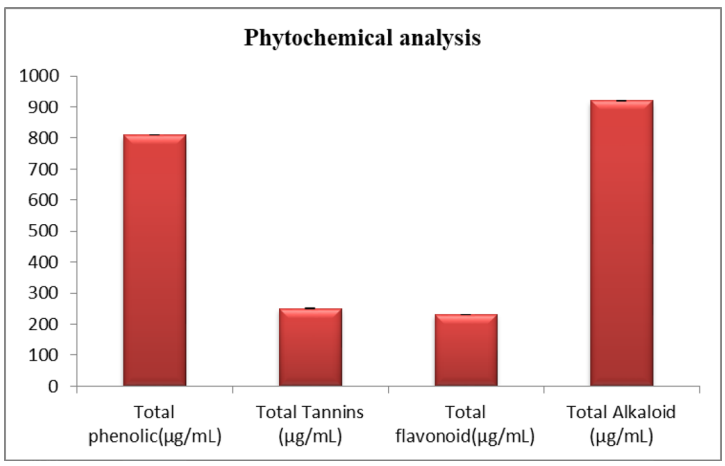

Fig. 8. Determination of total phenolics, alkaloids, flavonoids, and tannins of *S. kiliense* EACE.

GC-MS analysis

Our findings, as presented in Table 3; Fig. 9, identified 41 bioactive compounds in the *S. kiliense* EACE. This EACE contains a diverse mixture of fatty acids, hydrocarbons, and aromatic benzene derivatives, each contributing to distinct biological activities. A significant number of these compounds demonstrate antibacterial and antibiofilm properties, making the EACE highly relevant for pharmaceutical and medical applications. Among the most abundant compounds were Benzene, (1-propylnonyl) (9.87%), Hexadecanoic acid (8.05%), Prostaglandin A1-biotin (6.77%), Docosene (6.69%), Octadecenoic acid (5.55%), and 1-Nonadecene (5.16%). Most of the active compounds present in the EACE belong to the antimicrobial category, which includes Dodecanoic acid, Hexadecane, Oleic Acid, and Eicosane. Several compounds exhibit antibacterial and antibiofilm activity, which is crucial for combating biofilm-related infections that are often resistant to conventional treatments. Notable examples include Linoleic acid ethyl ester, Behenic alcohol, and Octacosanol, which play a vital role in preventing bacterial colonization and persistence. Beyond antimicrobial activity, the EACE contains

No.	Compound	RT (min)	Peak area %	activity	References
1	Trans-2-Decenoic acid	29.08	0.89	Antibiofilm and decreasing bacterial resistance	41
2	Cyclohexanecarboxy lic acid,2-phenylethyl ester	30.81	0.38	Antibacterial and anti-candida	42
3	Diethyltoluamide	33.82	0.20	Antibacterial	43
4	Dodecanoic acid	34.98	1.95	Antimicrobial	44
5	4-Hydroxyvalproic acid	35.16	3.40	Anticonvulsant	45
6	Hexadecane	36.19	0.62	Antimicrobial	25
7	Dodecyl acrylate	39.00	2.52	Antibacterial	46
8	Benfluorex	39.33	0.77	Anti-inflammatory, treat hyperlipidemia and type II diabetes	47
9	Benzene, (1-pentylheptyl)	40.38	1.95	Anticancer; Antiviral	48
10	Benzene, (1-butyloctyl)	40.53	1.76	Anticancer; Antiviral	48
11	Benzene, (1-propylnonyl)	40.93	9.87	Anticancer; Antiviral	48
12	Dotriacontane	41.44	0.12	Anticancer; antibacterial, and antibiofilm	49
13	Benzene, (1-ethyldecyl)	41.65	1.48	Anticancer; Antiviral	48
14	Tetradecanoic acid	41.84	0.54	Anti-insect	50
15	1-Nonadecene	43.0	5.16	Antifungal, antibacterial	51
16	Octadecane	43.33	4.06	Antimicrobial	52
17	Benzene, (1-pentyloctyl)	43.78	4.17	Anticancer; Antiviral	48
18	Benzene, (1-butylnonyl)	43.99	2.41	Anticancer; Antiviral	48
19	Pentadecanoic acid	44.27	0.70	Antimicrobial	53
20	Benzene, (1-propyldecyl)	44.40	1.64	Anticancer; Antiviral	48
21	8à,11-Elemadiol	44.90	0.22	Antibacterial	54
22	Benzene, (1-ethylundecyl)	45.12	1.70	Anticancer; Antiviral	48
23	Hexadecanol,2-methyl	45.48	0.22	Antibacterial	55
24	Benzene, (1-methyldodecyl)	46.37	2.06	Anticancer; Antiviral	48
25	Monobutyl phthalate	47.10	0.28	Antibacterial	56
26	Hexadecanoic acid	47.26	8.05	Antibacterial, anti-inflammatory	57
27	2-Hexadecanol	48.90	0.30	Antibacterial	58
28	Oleic Acid	49.17	1.85	Antimicrobial	17
29	Docosene	49.53	6.69	Antimicrobial	17
30	Eicosane	49.80	2.34	Antibacterial, antibiofilm	59
31	Heptadecenoic acid	50.40	1.53	Antimicrobial	17
32	Octadecenoic acid	51.01	5.55	Antibacterial, antibiofilm	49
33	Linoleic acid ethyl ester	53.32	0.42	Antibacterial, antibiofilm, antioxidant	36b
34	Behenic alcohol	55.19	3.85	Antibacterial, antibiofilm	60
35	Penta tri acontene	58.75	0.14	Antibacterial, antibiofilm	61
36	Octyl palmitoleate	61.10	0.82	Antibacterial	62
37	Octacosanol	62.64	3.61	Antibacterial, antibiofilm	63
38	Heptacosane	62.88	1.52	Antimicrobial	64
39	1,2-benzenedicarboxylic acid	65.85	1.01	Antibacterial	65
40	Flavone (4'-oh,5-oh,7-di-o-) glucoside	85.63	0.21	Antibacterial	66
41	Prostaglandin A1-biotin	92.09	6.77	Antibacterial, antibiofilm, antioxidant	36a

Table 3. The compounds identified by GC–MS of *S. kiliense* EACE.

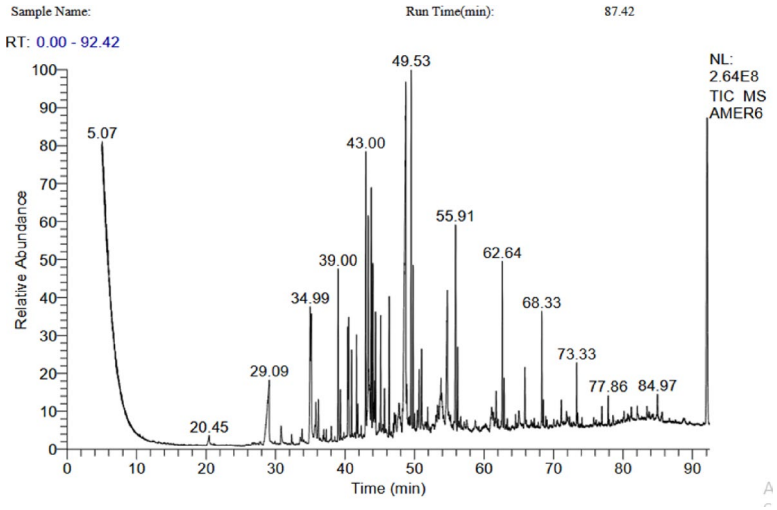

Fig. 9. GC-mass analysis of *S. kiliense* EACE.

anticancer and antiviral compounds, particularly benzene derivatives such as (1-pentylheptyl, 1-butyloctyl, and 1-propylnonyl). Additionally, anti-inflammatory compounds like Hexadecanoic acid and Benfluorex contribute to reducing inflammation, making them beneficial for conditions involving immune responses. The presence of 4-Hydroxyvalproic acid, a known anticonvulsant, further expands the therapeutic potential of this EACE. The identified of 41 bioactive compounds within the *S. kiliense* EACE, including fatty acids such as Hexadecanoic acid and oleic acid have been documented for their antibacterial properties[39]. The detection of compounds with known antibiofilm activity, such as linoleic acid ethyl ester and octacosanol, is particularly significant. The ability of *S. kiliense* EACE to disrupt biofilm formation suggests a potential therapeutic avenue for combating persistent infections[40]. GC-MS analysis results align with previous studies reporting a rich composition of bioactive compounds in *L. stricta*, mainly monoterpenes and sesquiterpenes, which are linked to its pharmacological potential and may be associated with the endophytic fungus *S. kiliense*. Key constituents such as 1,8-cineole, camphor, borneol, and linalool have been identified[13].

Conclusion

This study highlights *S. kiliense* as a highly promising endophytic fungus isolated from *L. stricta*, demonstrating significant potential in addressing the global challenge of multidrug-resistant pathogens. The phytochemical analysis discovered a rich profile of bioactive compounds, including phenolics, tannins, flavonoids, and alkaloids, which contribute to its potent antimicrobial, antioxidant, anticancer, and antibiofilm activities. The EACE exhibited remarkable antimicrobial efficacy against a range of pathogens, along with concentration-dependent antibiofilm properties, making it a strong candidate for developing novel therapeutic agents. Furthermore, the outstanding anticancer activity of *S. kiliense* against Hep-G2 and MCF-7 cell lines, coupled with its antioxidant potential, underscores its multifaceted therapeutic applications. The presence of 41 active compounds, as identified by GC-MS analysis, further validates its pharmacological potential. These findings position *S. kiliense* as a valuable natural resource for combating drug-resistant infections and cancer, paving the way for future research into its clinical applications and developing new bioactive formulations.

Data availability

All data underlying the findings described in our manuscript were inserted in the manuscript.

Received: 28 March 2025; Accepted: 16 June 2025
Published online: 03 July 2025

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Acknowledgements

The author thanks Al-Azhar University, Faculty of Science, Botany and Microbiology Department for supporting this study. The author also thanks Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R454), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, The author also thanks College of Pharmacy, Al-Farahidi University, Baghdad, Iraq for supporting this study.

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Funding

The author also thanks Princess Nourah bint Abdulrahman University Researchers Supporting Project number

(PNURSP2025R454), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Declarations

Competing interests
The authors declare no competing interests.

Consent for publication
All authors have read and agreed to the published version of the manuscript.

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