



Antimicrobial activity of spiculisporic acid isolated from endophytic fungus *Aspergillus cejpaii* of *Hedera helix* against MRSA

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Abstract

The surge in multidrug-resistant pathogens worldwide has jeopardized the clinical efficiency of many current antibiotics. This problem steered many researchers in their quest to discover new effective antimicrobial agents from natural origins including plants or their residing endophytes. In this work, we aimed to identify the endophytic fungi derived from *Hedera helix* L. and investigate their potential antimicrobial activity. Bioguided fractionation approach was conducted to isolate the pure compounds from the most active fungal fraction. Out of a total of six different isolated endophytic fungal strains, only *Aspergillus cejpaii* showed the highest activity against all tested microbial strains. The most active fraction was the dichloromethane/methanol fraction (DCM:MeOH), where it showed significant activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Acinetobacter baumannii*, *Salmonella typhi*, and three drug-resistant clinical isolate strains including Methicillin-resistant *Staphylococcus aureus* (MRSA, H1), *Pseudomonas aeruginosa* (PS 16), and *Acinetobacter baumannii* (ACT 322) using tetracycline and kanamycin as the control antibiotics. Bioguided fractionation of the active fraction led to the isolation of the γ -butenolide, spiculisporic acid. Structure elucidation was carried out using ¹H and ¹³C-NMR spectroscopic analysis. The compound showed good antimicrobial activities with minimum inhibitory concentration (MIC) values ranging from 3.9 to 31.25 μ g/mL against all tested strains. Gas chromatography coupled to mass spectrometry (GC-MS) profiling was also carried out to identify the metabolites in the microbial crude extract. In conclusion, endophytic fungi, *Aspergillus cejpaii*, isolated from *Hedera helix* L. roots showed promising antimicrobial activity which merits further in-depth investigations for potential utilization as a source of new antibiotics in the future. It can also be considered as a novel source for spiculisporic acid.

Keywords *Staphylococcus aureus* · *Pseudomonas aeruginosa* · *Acinetobacter baumannii* · NMR · GC-MS · Bioguided fractionation · Antimicrobial susceptibility · Spiculisporic acid

Introduction

Hedera helix L. (*H. helix* L.), Ivy or English Ivy, is a climbing plant belonging to family Araliaceae [1]. The family is commonly known as the ginseng family. Most members are shrubs or trees, with several climbers and a few herbs. It is distributed in Africa, Asia, Europe, Australasia, and Northern America. Phytochemical screening of *H. helix* L. revealed the presence of different chemical constituents including triterpene saponins, phenolic compounds, flavonoids, polyacetylenes, unsaturated sterols, tannins, terpenoids, alkaloids, and carbohydrates [2–7]. The plant is

well known for its respiratory, anti-inflammatory, analgesic, immunological, anticancer, antimicrobial, and anti-thrombin activities [7–11].

In the continuous search performed by pharmaceutical industry and researchers for discovering novel products, natural sources were found to be superior for their content of novel substances that have the potential to be developed into new industrial products [12, 13]. Hence, their search for new secondary metabolites should be extended to cover the organisms that inhabit the plant kingdom.

Endophytes are microorganisms that inhabit the interior of plants, especially leaves, stems, and roots residing in the internal tissues of the living plants without causing any immediate apparent negative effects. Almost all classes of plants were found to host one or more endophytes. Throughout the last decade, scientists were able to isolate

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approximately 6500 endophytic fungi from herbaceous plants and trees in addition to screening them for biological activities [12]. Endophytes are abundant with rich biodiversity and have been discovered in every plant species examined so far. They act as chemical synthesizers inside plants. Many of them can integrate bioactive compounds that can be used by plants for defense against pathogens, and some of these compounds were proved to be useful for novel drug discovery. Up till now, most of the natural products from endophytes were found to be antimicrobial agents, anticancer, and other bioactive compounds by their various functional parts [14].

Infectious diseases caused by bacteria and fungi affect millions of people worldwide. Throughout history, they have continued to be a major cause of death and disability. Today, infectious diseases account for one-third of all deaths in the world; the World Health Organization (WHO) estimates that nearly 50,000 people die each day/year from infectious diseases [15]. Yet, the upsurge of multidrug-resistant pathogenic microorganisms has threatened the clinical efficiency of many current antibiotics. This global issue has provoked a worldwide search for new antibiotics from numerous sources, and one of these most important sources is the endophytic fungi inhabiting the plant kingdom. Recently, researchers have been directing their efforts towards discovering the hidden treasures of endophytic fungi and their assorted metabolites as they are much easier to grow and were found to be a promising source of novel bioactive compounds including antimicrobials [16]. Accordingly, the objective of this work was to isolate, identify, and characterize active compounds produced by the endophytic fungi associated with *H. helix* L. using a bioguided fractionation scheme, which combines analytical procedures with bioassays, representing a prompt and cost-effective method to discover possible useful fractions and pure compounds.

Materials and methods

Plant material

H. helix L. plant was obtained from Arab Company of Pharmaceuticals and Medicinal Plants (Mepaco-Medifood) El-Sharkya, Egypt, with coordinates near (30.3799° N, 31.4544° E). Plant samples were supplied by Dr. Mokhtar Bishr Technical Director of Mepaco Company.

Isolation of endophytic fungi from *H. helix* L.

Endophytic fungi were isolated by the method described by [17] with some modifications. Briefly, different parts of *H. helix* L. including leaves, stems, and roots were washed with running water and allowed to air dry. For surface

sterilization, the plant parts were dipped twice in 70% ethanol for 2 min., washed multiple times (3 times) with sterile water, then immersed in 0.5% sodium hypochlorite for 1 min., washed again 3 times with sterile water, and allowed to dry. Then, each part was cut into small cubes of 1 mm diameter to release the endophytic fungi and then inoculated aseptically on Potato Dextrose Agar (PDA) (Himedia, India) plates supplemented with 250 µg/mL of both streptomycin and gentamicin to inhibit bacterial growth. Non-inoculated PDA plates were used as negative control in addition to PDA plates inoculated with 1 mL of the last washing water to ensure the inhibition of the surface microorganisms. The plates were incubated for 7 to 21 days at 25 °C. Various mycelia growing out of the pieces were cultured in new PDA plates several times, and the isolated pure fungi were maintained on PDA slants and kept at 4 °C.

Characterization of isolated endophytic fungi

Morphological characterization of the isolated fungi was performed using a standard taxonomic key that included colony characteristics such as texture, shape, and color [18]. For microscopic examination, promising fungus was cultured on PDA for 7 days using the slide culture method, and hyphae, conidiophore, and conidia were observed under a microscope after staining with lactophenol cotton blue. Preliminary fungus identification was based on both the morphological and microscopical characteristics of the fungus [19].

Fungi identification through molecular approach

Genomic DNA was extracted by Quick-DNA™ Fungal/Bacterial Microprep Kit (Zymo research #D6007) following the manufacturer's protocol. ITS 1 and ITS 4 were used as forward and reverse primers, respectively, to amplify internal transcribed spacer ribosomal RNA (ITS rRNA region). PCR was conducted using Maxima Hot Start PCR Master Mix (Thermo; K1051). Thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 1 min, and extension at 72 °C for 1.5 min. The post-cycling expansion was done as one cycle at 72 °C for 10 min. The PCR yields were then purified by GeneJET PCR Purification Kit (Thermo K0701, Waltham, MA, USA) in accordance with the manufacturer's instructions, and the purified DNA was stored at –20 °C. Finally, the refined PCR products were sequenced using an ABI 3730xl DNA sequencer (Applied Biosystems™, ThermoFisher). DNA extraction, PCR, and PCR products purification were carried out by Sigma Scientific Services Company (Egypt), while DNA sequencing was performed by GATC Company (Washington).

Final sequence of the gene product of the fungal isolates was aligned against available sequences in the GenBank

database using NCBI BLAST (Basic Local Alignment Search Tool; <http://blast.ncbi.nlm.nih.gov/>). The neighbor-joining technique was used to build the phylogenetic tree using the MEGA 5 software. The identified isolates' sequences were deposited to the GenBank database and assigned accession numbers [20, 21].

Extraction of the fungi secondary metabolites

Small-scale production was carried out for all isolated fungi for the purpose of preliminary biological screening. Solid rice medium was prepared by adding 100 g of rice mixed with 120 mL of sterilized water in Erlenmeyer flasks (1L) and autoclaved at 121 °C for 20 min. Plugs from PDA pure fungal cultures were used to inoculate three solid rice flasks, and they were allowed to grow for 21 days at room temperature. Fungal metabolites were then extracted using 600 mL of ethyl acetate (EtOAc) for three consecutive times till exhaustion as described by [22], and the extract was evaporated under vacuum yielding a dark brown residue which was reserved for further biological and chemical investigations.

For mass production of the fungal isolate showing the highest antimicrobial activity, fifteen solid rice medium flasks were used following the same procedure.

Determination of antimicrobial activity of fungal crude extracts

Antibacterial activities of secondary metabolites extracted from *H. helix* L. fungal endophytic isolates were determined by screening the fungal crude extracts against Gram-positive and Gram-negative bacterial reference isolates using MIC microtitre plate assay [23].

Five Gram-negative bacteria viz., *Escherichia coli* (*E. coli*, ATCC 25922), *Pseudomonas aeruginosa* (*Ps. aeruginosa*, ATCC 9027), *Serratia marcescens* (*S. marcescens*, ATCC 13880), *Acinetobacter baumannii* (*A. baumannii*, ATCC 19606), and *Salmonella typhi* (*S. typhi*, ATCC 6539) and one Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*, ATCC 6538) were used.

All bacterial isolates were cultured overnight in Tryptone Soya Broth (TSB, Himedia, India) sterile medium at 37 °C. The dried crude extracts of the endophyte fungi were dissolved in DMSO in initial concentration of 500 µg/mL. In a sterile 96-well microtiter plate, 100 µL of sterile TSB were added, followed by a two-fold serial dilution of each fungal extract to reach concentrations ranging from 250 to 1.95 µg/mL, then 10 µL of adjusted bacterial inoculum ($\approx 10^6$ CFU /mL) were added to each well. Tetracycline was used as a positive control. None inoculated wells were used as negative controls, and wells inoculated with the bacteria but without the extracts were used as positive controls. Microtiter plates were incubated overnight at 37 °C. After

incubation, wells were checked for turbidity, and the minimum inhibitory concentration (MIC) values were determined as the lowest concentration that inhibits the growth of bacteria.

Bioguided fractionation of highly active endophyte's secondary metabolites

The endophytic fungal extract demonstrating the highest antimicrobial activity was subjected to bioguided fractionation to isolate the bioactive fractions and compounds. Accordingly, the total fungal ethyl acetate fraction of the promising fungus (35 g) was fractionated on a vacuum liquid chromatography (VLC) packed with silica gel 60 mesh (Merck, Darmstadt, Germany). Elution was performed using a gradient system of *n*-hexane:EtOAc mixture (100:0 v/v – 0:100 v/v) followed by DCM:MeOH (100:0 v/v – 0:100 v/v) to yield 6 collective fractions after investigating by TLC under UV light and spraying with vanillin/sulfuric acid reagent. The most active fraction, AC4 (7 g), was then applied again on VLC packed with silica gel 60 mesh, and elution was performed using a gradient system of DCM:MeOH (100:0 v/v – 0:100 v/v). Subfractions were tested, and the most active subfraction, AC4'-1, was further purified on silica columns using an isocratic system of DCM:MeOH (95:5 v/v) to afford a pure compound (**1**) (150 mg).

Antimicrobial susceptibility of ethyl acetate fractions and purified compound

The antimicrobial activities of the collective fractions, subfractions, and isolated compound were monitored using the six reference strains previously used in this study, in addition to three drug-resistant clinical isolates: *Ps. aeruginosa*, *A. baumannii*, and MRSA. MIC microtitre plate assay was carried out as mentioned previously.

Structural elucidation of spiculisporic acid

Structural identification of compound **1** was achieved using mass spectroscopy, 1D and 2D NMR. NMR spectra were recorded on a Bruker High Performance Digital FT-NMR-Spectrophotometer Avance III HD (1H-NMR: 400 MHz, 13C-NMR: 100 MHz, Bremen, Germany). NMR spectra were recorded in CD₃OD using TMS as an internal standard. The solvent used was 500 µl, and the number of scans (NS) was 200 automatically locked based on the CD₃OD signal at 3.30 ppm. Chemical shift values were recorded in δ ppm.

Mass spectrum was obtained using an XEVO TQD triple quadrupole (Waters Corporation, Milford, MA01757 USA) mass spectrometer. Source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900

L/h. Mass spectra were detected in the ESI between m/z 100 and 1000. The peaks and spectra were processed using the Maslynx 4.1 software and tentatively identified by comparing their retention times (Rt) and mass spectra with reported data.

GC-MS analysis

GC-MS analysis was performed on a Shimadzu GCMS-QP 2010 (Tokyo, Japan) with Rtx-5MS capillary column (30 m \times 0.25 mm i.d. \times 0.25 μ m film thickness) (Restek, USA). The capillary column was coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany). The oven temperature was kept at 45 °C for 2 min (isothermal), programmed to 300 °C at 5 °C/min, and kept constant at 300 °C for 5 min (isothermal); the injector temperature was 250 °C. Helium was utilized as a carrier gas with a constant flow rate set at 1.41 mL/min. Diluted samples (1% v/v) were injected with a split ratio of 15:1, and the injected volume was 1 μ L. The MS parameters were as follows: interface temperature, 280 °C; ion source temperature, 200 °C; EI mode, 70 eV; and scan range, 35–500 amu. The overall run time was about 30 min. Compounds were identified using retention times and mass spectra matching with the National Institute of Standards and Technology (NIST-11) and Wiley library databases.

Statistical analysis

All statistics have been conducted using GraphPad Prism 5 (LaJolla, CA, USA) software. The Kolmogorov-Smirnov test was applied to assess the normal distribution of the collected data. Since the data were found to be normally distributed, a two-way analysis of variance (ANOVA) with Bonferroni post-test was utilized to calculate the significant difference between means. $P < 0.0001$ was considered statistically significant.

Results

Isolation and identification of cultivable endophytic fungi

In the present study, a total of 6 fungal strains were isolated from the different parts of *H. helix* L. Four isolates originated from roots, one from stems, and one from leaves. The isolated fungi were preliminary identified based on morphological and microscopic characters, and the identification was further confirmed using amplification and sequencing of ITS rRNA gene. The ITS rRNA gene sequences were

Table 1 Identified endophytic fungal isolates from different parts of *H. helix* L. with accession numbers

Isolated fungus	Plant organ	Accession number
<i>Aspergillus niger</i>	Leaf	MW142509
<i>Saccharomycopsis fibuligera</i>	Stem	MW165540
<i>Talaromyces assiutensis</i>	Root	MW165542
<i>Aspergillus cejpaii</i>	Root	MW110482
<i>Talaromyces trachyspermus</i>	Root	MW131876
<i>Fusarium solani</i>	Root	MW209717

BLAST aligned and submitted at the GenBank under the accession numbers provided in Table 1.

Preliminary antimicrobial screening

The metabolites produced by the endophytic fungal isolates in the ethyl acetate crude extracts were subjected to preliminary antimicrobial activity against various bacterial strains using tetracycline as a positive control. *Aspergillus cejpaii* (*A. cejpaii*) metabolites showed a promising wide spectrum of antibacterial activity in comparison to other tested endophytic extracts (Table 2). The highest antibacterial activity with the least MIC (62.5 μ g/mL) was detected for the ethyl acetate extract of *A. cejpaii* against *E. coli*, *Ps. aeruginosa*, *S. marcescens*, and *S. typhi* followed by *S. aureus* and *A. baumannii* with MIC values of 125 μ g/mL. Accordingly, *A. cejpaii* was selected for further in-depth investigations.

Characterization of *A. cejpaii*

Complete identification of *A. cejpaii* isolate was performed based on both morphological and microscopical characteristics. Macroscopic description of growing fungi showed fast-growing fungi with white, downy colonies that turned yellowish-white by age, while the reverse side was brownish in color (Fig. 1). Microscopic examination of *A. cejpaii* revealed septated, hyaline hyphae with uniseriate phialides, and small round conidia (Fig. 2). Blast sequence analysis of the endophytic fungal isolate revealed 99% similarity with *A. cejpaii* (Fig. 3).

Bioguided fractionation of *A. cejpaii* extract

A bioassay-guided fractionation was performed for investigation and identification of the active fractions and metabolites in *A. cejpaii* fungus extract (Fig. 1, supplementary material). Fractionation and purification gave 5 subfractions of which fractions AC4-1' and AC4-2' showed the highest antimicrobial activity compared to all other subfractions. The activity was tested against previously mentioned Gram-positive and Gram-negative bacterial strains in addition to three

Table 2 MIC values of endophytic fungal isolates' extracts against different microbial reference strains

Endophytic fungal isolates	Tested bacterial reference strains					
	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 9027	<i>S. aureus</i> ATCC 6538	<i>S. marcescens</i> ATCC 13880	<i>A. baumannii</i> ATCC 19606	<i>S. typhi</i> ATCC 6539
MIC values ($\mu\text{g/mL}$)						
<i>Aspergillus niger</i>	≥ 250	≥ 250	-	≥ 250	-	≥ 250
<i>Saccharomycopsis fibuligera</i>	-	-	-	-	-	≥ 250
<i>Talaromyces assiutensis</i>	≥ 250	-	-	≥ 250	-	-
<i>A. ceipii</i>	62.5 ± 0.94	62.5 ± 0.30	125 ± 0.36	62.5 ± 0.47	125 ± 0.60	62.5 ± 0.72
<i>Talaromyces trachyspermus</i>	≥ 250	≥ 250	-	-	-	-
<i>Fusarium solani</i>	-	-	-	-	-	-

*All measurements were done in triplicates, and values are expressed as mean \pm SD

*(-) No activity detected

Fig. 1 Morphological characters of *A. ceipii* on PDA plate. **a** White downy colony on the surface of the plate, **b** brownish color on the reverse side of the plate

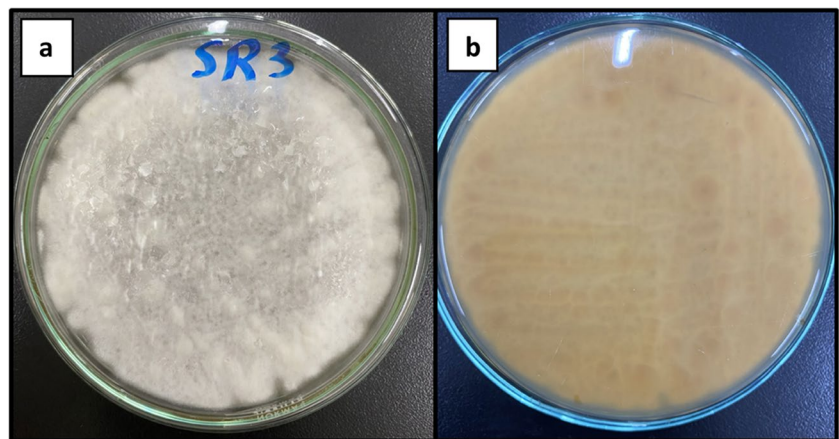
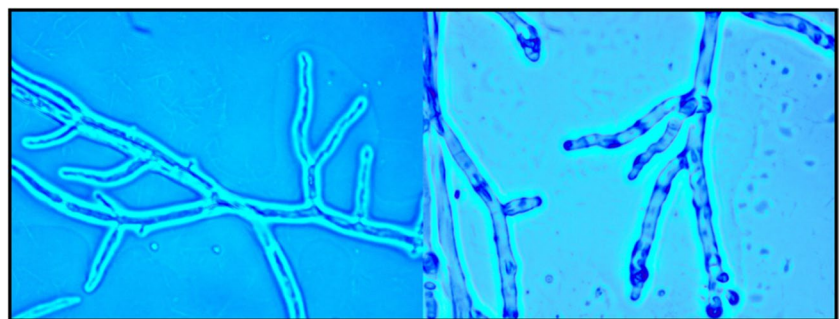


Fig. 2 Microscopic examination of *A. ceipii* revealing hyaline, septate hypha with uniseriate phialides



drug-resistant clinical isolates viz., MRSA-H1, *Ps. aeruginosa* PS 16, and *A.baumannii* ACT 322 using tetracycline and kanamycin as the control antibiotics. Fractions AC4-1' and AC4-2' exhibited similar pattern of activity against *Ps. aeruginosa* and *S. marcescens* with MIC values of $31.25 \mu\text{g/mL}$ and $15.63 \mu\text{g/mL}$, respectively. Both fractions showed similar MIC values of $62.5 \mu\text{g/mL}$ against *A. baumannii* as well as resistant strains MRSA and *Ps. aeruginosa*. On the other hand, AC4-1' showed approximately 50% increase

in its antimicrobial activity against *E. coli*, *S. aureus*, and *A. baumannii* when compared to AC4-2' with MIC value of $31.25 \mu\text{g/mL}$. It also showed very promising activity against *S. typhi* where it exhibited MIC value of $15.63 \mu\text{g/mL}$ (Fig. 4). Upon further fractionation, a major compound was isolated from subfractions AC4-1', compound **1**. The isolated compound proved to be the most active tested sample with the best MIC values against all tested strains including the drug-resistant ones (Fig. 5), with MIC values ranging

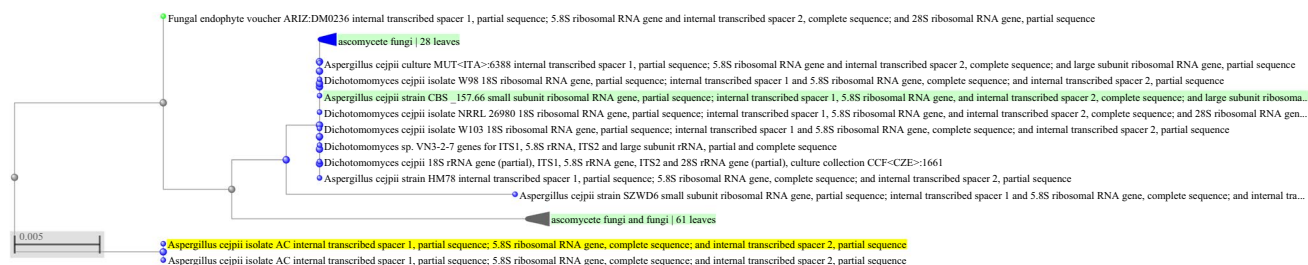


Fig. 3 Phylogenetic tree of the isolated endophyte *A. ceppii* produced using BLAST pairwise alignments, NCBI. The isolate sequence shows 85% Query coverage, E-value = 0, and 96.7% percent identity

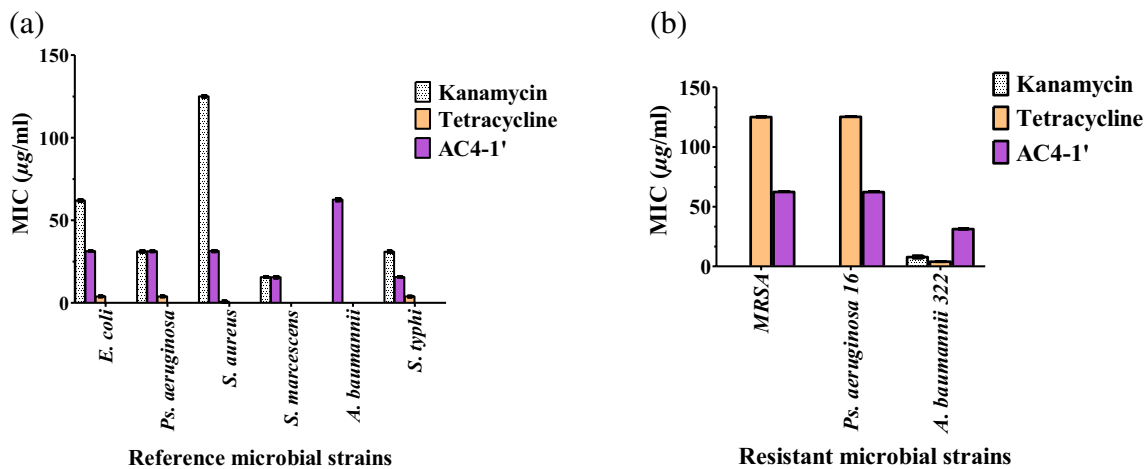


Fig. 4 MIC values of the ethyl acetate subfractions of *A. ceppii* against different microbial reference strains (a) and different resistant strains (b) compared to kanamycin and tetracycline. All measurements were done in triplicates, and values are expressed as means \pm SD

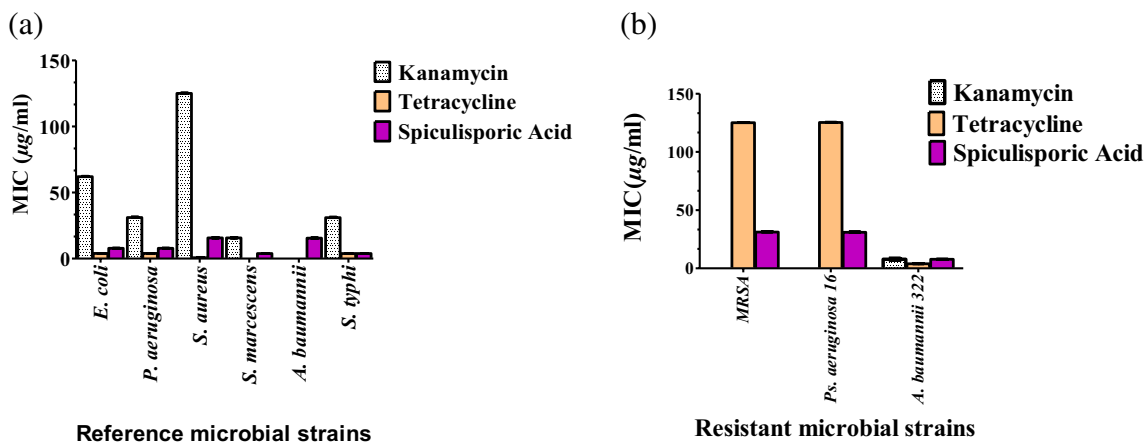


Fig. 5 MIC values of spiculisporic acid isolated from the most active subfractions against different microbial reference strains (a) and against resistant microbial strains (b). All measurements were done in triplicates, and values are expressed as means \pm SD

from 3.9 to 31.25 µg/mL. Compound **1** showed a very promising activity against resistant bacterial strains: MRSA-H1 and *Ps. aeruginosa* PS 16 where it showed MIC value of 31.25 µg/mL compared to standard drugs kanamycin and tetracycline. It is worthy to mention that kanamycin did not show any activity against both strains, while tetracycline showed weak activity (MIC= 125 µg/mL).

Regarding resistant strain *A. baumannii* 322, compound **1** showed very potent activity with MIC value of 7.8 µg/mL which was comparable to the activity achieved by kanamycin.

MIC values of the subfractions and isolated compound against all tested bacterial strains are presented in Table 3 and illustrated in Figs. 4 and 5.

Identification and elucidation of spiculisporic acid

Compound **1** was isolated as white needle crystals. Its molecular formula was determined by ESI mass spectrometry as C₁₇H₂₈O₆ as it gave strong molecular ion at [M + H]⁺ m/z 329.1962 (calc. for C₁₇H₂₉O₆⁺: 329.1986). ESI [M-H]⁻ m/z 327.1809 having four degrees of unsaturation. ¹H- and ¹³C-NMR data assignments of compound **1** were obtained by interpretation of ¹H–¹H COSY, HSQC, and HMBC 2D spectra (Figs. S2–6, supplementary material). The ¹H-NMR spectrum of the compound exhibited a characteristic doublet of doublet signal at δH 3.03 (dd, H-5). It also displayed signals characteristic for three methylenes [δH 2.68–2.47 (m, H-2 and H-3), 1.92–1.81 and 1.58–1.49 (each m, H-6)], and one methyl [δH 0.91 (t, J = 6.7 Hz, CH₃-15)] in addition to 16 aliphatic protons [δH 1.38 – 1.27 (m, H-7–14)] (Table 4). The ¹H–¹H COSY spectrum showed a correlation between

Table 4 ¹H and ¹³C NMR spectral data of spiculisporic acid from *A. cejpaii*

Position	¹³ C-NMR spectral data	¹ H-NMR spectral data
1	176.96	
2	27.45	2.58
3	29.08	2.50
4	86.59	
4- COOH	172.53	
5	50.94	3.03 (dd, J = 10.8, 3.2 Hz)
5- COOH	173.93	
6	27.66	1.86 (m), 1.54 (m)
7	27.45	1.27–1.38 (m) ^(b)
8	29.08	1.27–1.38 (m) ^(b)
9	29.00	1.27–1.38 (m) ^(b)
10	29.17	1.27–1.38 (m) ^(b)
11	29.28	1.27–1.38 (m) ^(b)
12	29.33	1.27–1.38 (m) ^(b)
13	31.68	1.27–1.38 (m) ^(b)
14	22.36	1.27–1.38 (m) ^(b)
15	13.13	0.91 (t)

^{ab}Overlapping signals

Measured in CD₃OD

H-2 (δ H 2.58) and H-3 (δ H 2.50). The methyl protons at H-15 were coupled with the methylene protons at H-14 (δ H 1.38 – 1.27). Correlations between the methylene protons at H-6 (δ H 1.87, 1.54) and H-5 [δ H 3.03 (dd, J = 10.8, 3.2 Hz)] and H-7 (δ H 1.38–1.27) were also observed.

¹³C- NMR spectra revealed 3 carbonyl signals at δC 176.96, 173.93, and 172.53 which suggested that this

Table 3 MIC values of the subfractions and isolated compound against all tested bacterial strains

Tested fraction	Tested bacterial pathogens								
	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 9027	<i>S. aureus</i> ATCC 6538	<i>S. marcescens</i> ATCC 13880	<i>A. baumannii</i> ATCC 19606	<i>S. typhi</i> ATCC 6539	MRSA-H1	<i>Ps. aeruginosa</i> PS 16	<i>A. baumannii</i> ACT 322
	MIC values (µg/mL)								
AC4-1' fraction	31.28 ± 0.055	31.25 ± 0.250	31.35 ± 0.409	15.51 ± 0.461	62.50 ± 1.00	15.61 ± 0.101	62.33 ± 0.764	62.26 ± 0.681	31.25 ± 0.250
Spiculisporic acid	7.80 ± 0.100	7.76 ± 0.252	15.60 ± 0.100	3.90 ± 0.100	15.433 ± 0.208	3.80 ± 0.265	31.133 ± 0.321	30.96 ± 0.451	7.80 ± 0.100
Kanamycin	62 ± 0.500	31 ± 0.557	125 ± 0.500	15.60 ± 0.100	-	31 ± 0.500	-	-	7.800 ± 0.100
Tetracycline	3.893 ± 0.090	3.90 ± 0.100	0.676 ± 0.499	0.050 ± 0.010	-	3.87 ± 0.060	125.06 ± 0.404	125.23 ± 0.493	3.87 ± 0.061

*All measurements were done in triplicates, and values are expressed as mean ± SD

*(-) No activity detected

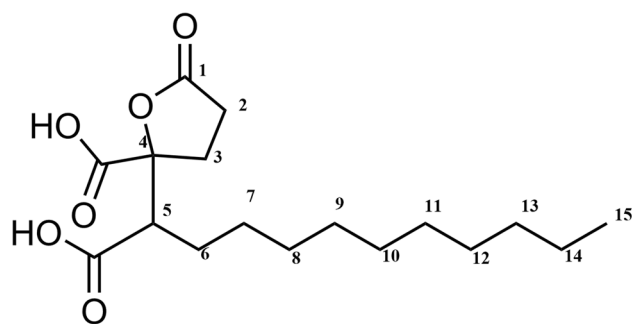


Fig. 6 Structure of spiculisporic acid isolated from *A. cejpii*

compound is a tricarboxylic acid derivative, in addition to signals corresponding to one quaternary carbon (δ^{C} 86.59), one methine (δ^{C} 50.94), eleven methylenes (δ^{C} 31.68–22.36), and one methyl (δ^{C} 13.13) (Table 4). In the HMBC spectrum, the signal at δ^{H} 3.03 revealed a cross-peak with δ^{C} 173.93, also signals at δ^{H} 2.50 and 2.58 showed cross-peaks with δ^{C} 50.94, 172.53, and 176.95 confirming the position of the proposed carbonyls within the chemical structure. From the above data, compound (**1**) was identified as spiculisporic acid with molecular formula $\text{C}_{17}\text{H}_{28}\text{O}_6$ (Fig. 6). The NMR data of the compound was similar with that previously isolated from *T. trachyspermus* [24].

GC-MS analysis

GC-MS analysis of the ethyl acetate extract of *A. cejpii* resulted in the identification of a total of 36 compounds with a total area percentage of 93.94%. The identified compounds belonged to various classes of compounds including aliphatic hydrocarbons, fatty acids, fatty acids esters, and fatty alcohols. The mass spectra of the compounds were compared with data from NIST and Wiley libraries, and the peaks were characterized and identified.

Hydrocarbons were the predominant class of identified compounds representing 40.5% followed by fatty acids and their derivatives including ethyl esters, alcohols, ketones, and ethers, representing 21.94%. The prevailing hydrocarbons were *n*-pentatriacontane (8.93%), *n*-tetratriacontane (6.39%), 17-pentatriacontene (3.06%), and tetracosane (2.83%). Regarding fatty acids and their derivatives, *n*-hexadecanoic acid (4.67%) was the major identified fatty acid. (9Z,12Z)-9,12-Octadecadien-1-ol (4.63%) and its acetate, linoleyl acetate (4.76%), were also found in abundance in the extract. The isolated compound spiculisporic acid was detected in the total ethyl acetate extract with an area percentage of 0.69%. The retention time (Rt) of the identified compounds along with their molecular formula (MF), molecular weight (Mwt), and concentration (Area %) are presented in Table 5.

Discussion

During recent years, a constant increase in global health problems caused by drug-resistant bacteria and fungi has been observed. Different processes are included in antibiotic resistance such as changes in antibiotic permeability, changes in target molecules, and enzymatic breakdown of medicines. Recently, the number of new antibiotics created has dropped considerably leading to the inevitability of a broader search for new and effective antimicrobial agents. Nowadays, traditional medicinal plants are expected to provide more promising antimicrobial agents.

Endophytes have been recognized as useful sources of bioactive secondary metabolites in addition to producing many bioactive compounds beneficial to pharmaceuticals, environment, agriculture, and industries [25]. According to several studies, numerous endophytic fungi have the ability to produce antimicrobial substances with broad-spectrum bioactivity [26–31]. Thus, our goal was to search for compounds of a natural microbial source with promising antimicrobial activities. In this study, we examined all parts of *H. helix* L. plant for isolation of endophytic fungi with antimicrobial activities, where only those belonging to genus *Aspergillus* isolated from the root showed promising antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria.

Genus *Aspergillus* represents a broadly spread genus of fungi that are widely known for possessing potent medicinal potential including antimicrobial, cytotoxic, and antioxidant activities which is highly credited to its rich content of secondary metabolites [32].

Accordingly, a study was designed based on bioguided fractionation to specify the active compound in *A. cejpii* with the best antimicrobial activity. In our study, solid state fermentation medium using complex solid rice was used as standard condition for both small-scale and large-scale production to produce sufficient quantities of the compounds of interest. Selection of solid-state media was based on an earlier study reporting that the cultures grown on solid media generated extracts with masses of one to two orders of magnitude larger than the same fungus grown in any of the liquid media [33].

The ethyl acetate fungal extract of *A. cejpii* showed significant broad-spectrum activity against Gram-positive and Gram-negative bacteria. To the best of our knowledge, this is considered the first report for the isolation of *A. cejpii* from the roots of *H. helix* L. However, several studies have been reported on the isolation and antibacterial activity of different species of endophytic fungi belonging to genus *Aspergillus*, and the results were in accordance with our obtained data regarding antimicrobial activity [34, 35].

Table 5 Compounds identified in ethyl acetate extract of *A. cepjii* via GC-MS

Peak #	Compound	MW	Molecular formula	Rt. time (min)	Area %
1	5-Methyltetradecane	212	C ₁₅ H ₃₂	33.419	0.61
2	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo [8.1.0] undeca-2,6-diene	204	C ₁₅ H ₂₄	34.099	2.46
3	<i>n</i> -Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	34.351	4.67
4	Hexadecanoic acid, ethyl ester	284	C ₁₈ H ₃₆ O ₂	34.982	0.62
5	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	272	C ₂₀ H ₃₂	35.303	1.13
6	<i>n</i> -Eicosane	282	C ₂₀ H ₄₂	35.438	1.15
7	2,4-Dimethylcosane	310	C ₂₂ H ₄₆	37.364	2.38
8	(9Z,12Z)-9,12-Octadecadien-1-ol	266	C ₁₈ H ₃₄ O	37.591	4.63
9	2-Hydroxycyclopentadecanone	240	C ₁₅ H ₂₈ O ₂	37.737	2.31
10	3 E,E,Z-1,3,12-Nonadecatriene-5,14-diol	294	C ₁₉ H ₃₄ O ₂	38.080	1.72
11	Hexadecanenitrile	237	C ₁₆ H ₃₁ N	38.246	0.95
12	1-Heptacosanol	396	C ₂₇ H ₅₆ O	40.335	2.90
13	Hexadecyl cyclohexane	308	C ₂₂ H ₄₄	40.525	1.33
14	2-Octyl-1-dodecanol	298	C ₂₀ H ₄₂ O	40.978	0.50
15	Docosyl ethyl ether	354	C ₂₄ H ₅₀ O	41.169	0.76
16	Carbonic acid, decyl hexadecyl ester	426	C ₂₇ H ₅₄ O ₃	42.213	0.83
17	<i>n</i> -Heptadecylcyclohexane	322	C ₂₃ H ₄₆	42.315	3.35
18	Nonyl tetradecyl ether	340	C ₂₃ H ₄₈ O	42.655	0.71
19	<i>n</i> -Tetracosane	338	C ₂₄ H ₅₀	42.843	2.83
20	1,4-dimethyl-2-octadecyl-Cyclohexane	364	C ₂₆ H ₅₂	43.155	0.75
21	Sulfurous acid, cyclohexylmethyl tridecyl ester	360	C ₂₀ H ₄₀ O ₃ S	43.314	3.13
22	2-Ethylhexadecane	254	C ₁₈ H ₃₈	43.445	1.43
23	<i>n</i> -Tetraatriacontane	478	C ₃₄ H ₇₀	43.733	6.39
24	Unknown	-	-	44.463	3.62
25	Sulfurous acid, cyclohexylmethyl pentadecyl ester	388	C ₂₂ H ₄₄ O ₃ S	44.975	1.71
26	9,12-Octadecadien-1-ol, acetate (Linoleyl acetate)	308	C ₂₀ H ₃₆ O ₂	45.063	4.76
27	<i>n</i> -Pentatriacontane	492	C ₃₅ H ₇₂	45.341	8.93
26	Heneicosylcyclohexane	378	C ₂₇ H ₅₄	45.653	0.99
27	17-Pentatriacontene	490	C ₃₅ H ₇₀	45.978	3.06
28	Spiculisporic acid	328	C ₁₇ H ₂₈ O ₆	46.182	0.69
29	Hexatriacontane	506	C ₃₆ H ₇₄	46.398	1.93
30	Diglycolic acid, decyl phenethyl ester	378	C ₂₂ H ₃₄ O ₅	46.550	5.96
31	Hexatriacontane	506	C ₃₆ H ₇₄	46.845	1.93
32	1,6,10,14,18,22-Tetracosahexaen-3-ol	426	C ₃₀ H ₅₀ O	48.984	1.45
33	Hydratropic acid, tridec-2-yn-1-yl ester	328	C ₂₂ H ₃₂ O ₂	49.084	5.62
34	Glutaric acid, 2-methylpent-3-yl phenethyl ester	320	C ₁₉ H ₂₈ O ₄	49.183	3.49
35	Benzoic acid, 4-amino-, 4-hydroximino-2,2,6,6-tetramethyl-1-piperidinyl ester	305	C ₁₆ H ₂₃ N ₃ O ₃	53.023	3.08
36	Unknown	-	-	55.673	1.16
37	9 β, 10α-Pregn-4-ene-3,20-dione	314	C ₂₁ H ₃₀ O ₂	56.194	2.45

Further investigations were carried out on the ethyl acetate fractions and subfractions to determine the antibacterial activity of *A. cepjii* secondary metabolites against resistant bacterial strains including MRSA, *Ps. aeruginosa* (PS 16), and *A. baumannii* (ACT 322). *S. aureus* is one of the major causes of community- and healthcare-associated infections. It causes various infections ranging from superficial skin and soft tissue infections to invasive infections, sepsis, and

death. MRSA has long been acknowledged as a pathogen associated with healthcare settings. It is considered one of the most dangerous causes of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotics [36]. *Ps. aeruginosa* is naturally resistant to a variety of antimicrobials and can develop resistance during anti-pseudomonal chemotherapy both of which compromise treatment of infections

caused by this organism. It is also a well-known nosocomial pathogen that is considered a serious threat due to its high mortality rate associated with infections. This is attributed to the organism's high resistance to many antimicrobials and the increase of multidrug resistance in healthcare settings [37–39]. *A. baumannii* is an opportunistic pathogen that is responsible for over 10% of infections occurring in hospitals' intensive care units. It has the ability to cause serious respiratory tract infections, necrotizing fasciitis as well as infections associated with intravascular devices, ulcers, surgical sites, and severe wounds. Patients infected with *A. baumannii* have a mortality rate ranging from 20 to 50%, which is attributed to multiple reasons including cellular functions such as capsule production, biofilm formation, the ability to cope with environmental stress, and the acquisition and expression of multidrug resistance (MDR) genes [40].

Our results demonstrated that the crude extract of *A. cejpuii* has a broad-antimicrobial spectrum activity suppressing various bacteria species with MICs ranging from 62.5 to 125 µg/mL against different tested strains compared to other endophytic fungi isolated from the different parts of *H. helix* L. Furthermore, bioguided fractionation of the *A. cejpuii* crude extract resulted in isolation, purification, and identification of spiculisporic acid. The compound or its derivatives were previously isolated from several fungi viz., *Aspergillus* sp. [41] *Aspergillus candidus* [42], and *Talaromyces trachyspermus* [24]. Our study is considered the first research to obtain spiculisporic acid from endophytic fungi *A. cejpuii* with very promising antimicrobial activities.

Spiculisporic acid isolated from *A. cejpuii* exhibited significant promising broad-spectrum antimicrobial activities, with MICs much lower than those obtained by the total extract. The isolated compound achieved significant high activity against drug-resistant Gram-positive bacteria, MRSA, and *Ps. aeruginosa* (PS 16) with MIC value of 31.25 µg/mL which is considered a very promising result compared to tested positive standards, kanamycin which showed no activity against the tested resistant strains and tetracycline with MIC value of 125 µg/mL. Our results came in agreement with a previous study that reported a high antibacterial activity for endophytic *A. cejpuii* from *Nelumbo nucifera* against MRSA [43]. In addition, spiculisporic acid exhibited significant high activity against *A. baumannii* (ACT 322), where it showed similar antimicrobial activity to that of kanamycin with MIC value of 7.8 µg/mL. Similar results were observed in a previous study where spiculisporic acid derivatives viz., spiculisporic acids B–D isolated from the culture of *Aspergillus* sp. showed promising antibacterial activities against *S. aureus* ATCC 51650 with inhibition zones of 9.6, 11.6, and 11.5 mm at 20 mg/mL [41].

On the other hand, another group of researchers isolated spiculisporic acid E compound from the culture of the marine-sponge associated fungus *Talaromyces*

trachyspermus and evaluated its antimicrobial activity against Gram-positive (*S. aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and Gram-negative (*E. coli* ATCC 25922 and *Ps. aeruginosa* ATCC 27853) bacteria, *Candida albicans*, and MDR isolates from the environment; however, it showed no activity at the highest concentration tested (256 µg/mL). In another study, two antibiotic spiculisporic acid analogues, identified as spiculisporic acid F and G, which were isolated through bioactivity-guided fractionation from the fermentation broth of the sea urchin-derived *Aspergillus candidus*, also displayed antibacterial activity against Gram-negative *Ps. solanacearum* and Gram-positive *S. aureus* [42].

GC-MS analysis of the crude ethyl acetate extract of *A. cejpuii* revealed the presence of numerous compounds of various classes with hydrocarbons being the predominant class followed by fatty acids and their derivatives. Identified compounds were reported to possess numerous biological activities including antimicrobial activities. *n*-Pentatriacontane, the major identified hydrocarbon (8.93%), was reported to display antimicrobial potential against fungal pathogens *Mycogone pernicioso* and *Rhizoctonia solani*, as well as against the bacteria *Bacillus megaterium*, *E. coli*, and *Ps. fluorescens* [44]. *n*-Tetatriacontane, the second most abundant hydrocarbon (6.39%), was previously identified from the methanolic extract of a medicinal plant *Plantago lanceolata* showing excellent antibacterial activities against *E. coli* and *Bacillus cereus* [45]. Tetracosane was also reported to possess antibacterial activity against *S. aureus* and *S. epidermidis* as well as antifungal activity against *Aspergillus niger* and *Bacillus cinerea* [46]. *n*-Hexadecanoic acid (4.67%) and its ethyl ester in addition to spiculisporic acid (0.69%) were found as major components in the extract. Fatty acids play an important role in the formation of lipids in plants, animals, and microorganisms. There have long been reports on the antibacterial activity of fatty acids against both Gram-positive and Gram-negative pathogens. According to previous studies, *n*-hexadecanoic acid has been shown to possess strong antifungal activity towards several human and phytopathogens through inhibition of spore germination or mycelial growth by disruption of plasma membrane [30]. In a research analyzing the essential oil of the leaves *Solanum spirale* Roxb., *n*-hexadecanoic acid was the major component in the essential oils, and it was found to have significant antibacterial activity against both Gram-negative *E. coli* and Gram-positive *S. aureus* [47]. Additionally, derivatives of spiculisporic acid including spiculisporic acids B–D, F, and G isolated from sea urchin-derived fungus *Aspergillus* sp. and *Aspergillus candidus* strain exhibited significant inhibitory activity against *P. solanacearum* and *S. aureus* which was higher than that achieved by the tested positive controls (streptomycin sulfate and kanamycin) [42]. Thus, GC-MS analysis confirms the presence of a number of antimicrobial chemical compounds in the ethyl acetate crude fungal extract, the synergetic effect of which can be credited for the observed promising antimicrobial activity.

Conclusion

Endophytes are considered a hidden gem that is required to be explored to find new and promising secondary metabolites with diverse biological activities. Our study focused on isolating different fungi from the various organs of the well-known medicinal plant *H. helix* L. The endophytic fungus *A. cejpaii* was isolated from the roots of *H. helix*. The fungal extract and spiculisporic acid isolated compound exhibited a broad-spectrum antimicrobial activity against several Gram-positive and Gram-negative strains as well as showing promising activity against the resistant ones. Spiculisporic acid may be used alone or in combination with currently available antibiotics to augment their effects, leading to the improvement of the efficacy against resistant microorganisms. Additional studies are recommended to reveal other biological activities of endophytic fungi hidden in the various organs of the plant as well as further profiling of their secondary metabolites and their mechanism of action.

The outcomes of this study are considered very promising for the discovery of new secondary metabolites with broad-spectrum antimicrobial activity as these findings indicated that the fungus isolated from natural plants could be used as natural agents for pathogenic bacteria bio-control which could offer a natural solution in the pharmaceutical field to fight this bacterial infection.

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Author contribution Sarah Osama: investigation, data curation, writing—original draft and editing. Marwa M. Raafat: investigation, writing—review and editing, supervision, conceptualization, methodology. Moshera El Sherei: supervision, conceptualization, methodology, writing—review and editing. Dalia A. Al-Mahdy: investigation, writing—review and editing, supervision, conceptualization, methodology. Osama Salama and Mokhtar Bishr: supervision, conceptualization, methodology.

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Declarations

Conflict of interest The authors declare no competing interests.

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References

- Demirci B, Goppel M, Demirci F, Franz G (2004) HPLC profiling and quantification of active principles in leaves of *Hedera helix* L. *Pharmazie* 59:770–774
- Bezruk I, Georgiyants V, Ivanauskas L (2020) Comparison of components profile in herbal raw material, extract and pharmaceuticals of *Hedera helix*. *ScienceRise: Pharmaceutical. ScienceRise: Pharm Sci* 4:36–39. <https://doi.org/10.15587/2519-4852.2020.210768>
- Bedir E, Kirmizipekmez H, Sticher O, Caliş I (2000) Triterpene saponins from the fruits of *Hedera helix*. *Phytochemistry* 53:905–909. [https://doi.org/10.1016/s0031-9422\(99\)00503-8](https://doi.org/10.1016/s0031-9422(99)00503-8)
- Hostettmann K (1980) Saponins with molluscicidal activity from *Hedera helix* L. *Helv Chim Acta* 63:606–609. <https://doi.org/10.1002/hlca.19800630307>
- Elias R, Lanza AMD, Vidal-Ollivier E, Balansard G, Faure R, Babadjamian A (1991) Triterpenoid saponins from the leaves of *Hedera helix*. *J Nat Prod* 54:98–103. <https://doi.org/10.1021/np50073a006>
- Tucker AO, Maciarello MJ (1994) Essential oil of English Ivy, *Hedera helix* L. ‘Hibernica’. *J Essent* 6:187–188. <https://doi.org/10.1080/10412905.1994.9698352>
- Osama S, El Sherei M, Al-Mahdy DA, Refaat MM, Bishr M, Salama O (2023) Genus *Hedera*: a comprehensive review of its phytoconstituents, diverse pharmacological activities and medicinal properties. *Egypt J Chem.* <https://doi.org/10.21608/ejchem.2023.176914.7236>
- Al-Snafi AE (2018) Pharmacological and therapeutic activities of *Hedera helix*. A review. *J Pharm Pharmacol* 8:41–53
- Uddin G, Rauf A, Qaisar M, Latif A, Ali M (2011) Preliminary phytochemical screening and antimicrobial activity of *Hedera helix* L. *Middle-East J Sci Res* 8:198–202
- Shawky E, El Sohafy SM (2020) Untargeted and targeted chemical profiling for efficacy-directed discrimination of *Hedera helix* L. subspecies using HPTLC-image analysis and HPTLC/MS. *Ind Crops Prod* 145:111980. <https://doi.org/10.1016/j.indcrop.2019.111980>
- Zazharskyi VV, Davydenko PO, Kulishenko OM, Borovik IV, Brygadyrenko VV (2020) Antibacterial and fungicidal activities of ethanol extracts from *Cotinus coggygria*, *Rhus typhina*, *R. trilobata*, *Toxicodendron orientale*, *Hedera helix*, *Aralia elata*, *Leptopus chinensis* and *Mahonia aquifolium*. *Regul Mech Biosyst* 11:305–309
- Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol Res* 106:996–1004. <https://doi.org/10.1017/S0953756202006342>
- Ajilore BS, Oluwadairo TO, Olorunnisola OS, Fadahunsi OS, Adegbola PI (2021) GC–MS analysis, toxicological and oral glucose tolerance assessments of methanolic leaf extract of *Eucalyptus globulus*. *Future J Pharm Sci* 7:162. <https://doi.org/10.1186/s43094-021-00312-5>
- Guo B, Wang Y, Sun X, Tang K (2008) Bioactive natural products from endophytes: a review. *Appl Biochem* 44:136–142. <https://doi.org/10.1134/s0003683808020026>
- Besbes Hlila M, Mosbah H, Majouli K, Ben Nejma A, Ben Jannet H, Mastouri M, Aouni M, Selmi B (2016) Antimicrobial activity of *Scabiosa arenaria* Forssk. Extracts and pure compounds using bioguided fractionation. *Chem Biodivers* 13:1262–1272. <https://doi.org/10.1002/cbdv.201600028>
- Mawabo IK, Nkenfou C, Notedji A, Jouda JB, Lunga PK, Eke P, Fokou VT, Kuate JR (2019) Antimicrobial activities of two secondary metabolites isolated from *Aspergillus niger*, endophytic fungus harbouring stems of *Acanthus montanus*. *Bio Sci Pharma Res* 7:7–15. <https://doi.org/10.15739/ibspr.19.002>

17. Hazalin NAMN, Ramasamy K, Lim SSM, Wahab IA, Cole ALJ, Abdul Majeed AB (2009) Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complement Altern Med* 9:46. <https://doi.org/10.1186/1472-6882-9-46>
18. Hubka V, Kolařík M, Kubátová A, Peterson SW (2013) Taxonomic revision of *Eurotium* and transfer of species to *Aspergillus*. *Mycologia* 105:912–937. <https://doi.org/10.3852/12-151>
19. Turan C, Nanni IM, Brunelli A, Collina M (2015) New rapid DNA extraction method with Chelex from *Venturia inaequalis* spores. *J Microbiol* 115:139–143. <https://doi.org/10.1016/j.mimet.2015.06.005>
20. Morgan MC, Boyette M, Goforth C, Sperry KV, Greene SR (2009) Comparison of the Biolog OmniLog Identification System and 16S ribosomal RNA gene sequencing for accuracy in identification of atypical bacteria of clinical origin. *J Microbiol* 79:336–343. <https://doi.org/10.1016/j.mimet.2009.10.005>
21. Simpson AJG, Reinach FC, Arruda P, Abreu FA, Acencio M, Alvarenga R, Alves LMC, Araya JE, Baia GS, Baptista CS, Barros MH, Bonaccorsi ED, Bordin S, Bové JM, Briones MRS, Bueno MRP, Camargo AA, Camargo LEA, Carraro DM et al (2000) The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature* 406:151–157. <https://doi.org/10.1038/35018003>
22. Elissawy AM, Ebada SS, Ashour ML, El-Neketi M, Ebrahim W, Singab ANB (2019) New secondary metabolites from the mangrove-derived fungus *Aspergillus* sp. AV-2. *Phytochem* 29:1–5. <https://doi.org/10.1016/j.phytol.2018.10.014>
23. Alan M, Sugar MD, Xiuping L (1995) Comparison of three methods of antifungal susceptibility testing with the proposed NCCLS standard broth macrodilution assay: lack of effect of phenol red. *Diagn Microbiol Infect Dis* 21:129–133. [https://doi.org/10.1016/0732-8893\(95\)00067-K](https://doi.org/10.1016/0732-8893(95)00067-K)
24. Moriwaki-Takano M, Asada C, Nakamura Y (2021) Production of spiculisporic acid by *Talaromyces trachyspermus* in fed-batch bioreactor culture. *Bioresour Bioprocess* 8:1–11. <https://doi.org/10.1186/s40643-021-00414-1>
25. Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. *FEMS Immunol Med Microbiol* 48:367–372. <https://doi.org/10.1111/j.1574-695X.2006.00155.x>
26. Stinson M, Ezra D, Hess WM, Sears J, Strobel G (2003) An endophytic *Gliocladium* sp. of *Eucryphia cordifolia* producing selective volatile antimicrobial compounds. *Plant Sci* 165:913–922. [https://doi.org/10.1016/S0168-9452\(03\)00299-1](https://doi.org/10.1016/S0168-9452(03)00299-1)
27. Sanghee K, Dong-Sun S, Taeho L, Ki-Bong O (2004) Periconicins, two new fusicoccane diterpenes produced by an endophytic fungus *Periconia* sp. with antibacterial activity. *J Nat Prod* 67:448–450. <https://doi.org/10.1021/np030384h>
28. Atmosukarto I, Castillo U, Hess WM, Sears J, Strobel G (2005) Isolation and characterization of *Muscodora albus* I-41.3s, a volatile antibiotic producing fungus. *Plant Sci* 169:854–861. <https://doi.org/10.1016/j.plantsci.2005.06.002>
29. Nair DN, Padmavathy S (2014) Impact of endophytic microorganisms on plants, environment and humans. *Sci World J* 2014:250693. <https://doi.org/10.1155/2014/250693>
30. Singh A, Kumar J, Sharma VK, Singh DK, Kumari P, Nishad JH, Gautam VS, Kharwar RN (2021) Phytochemical analysis and antimicrobial activity of an endophytic *Fusarium proliferatum* (ACQR8), isolated from a folk medicinal plant *Cissampelos quadrangularis* L. *S Afr J Bot* 140:87–94. <https://doi.org/10.1016/j.sajb.2021.03.004>
31. Farhat H, Urooj F, Sohail N, Hameedi SF, Ali MS, Ehteshamul-Haque S (2022) Evaluation of antibacterial potential of endophytic fungi and GC-MS profiling of metabolites from *Talaromyces trachyspermus*. *S Afr J Bot* 150:240–247. <https://doi.org/10.1016/j.sajb.2022.07.004>
32. Youssef FS, Alshammari E, Ashour ML (2021) Bioactive alkaloids from genus *Aspergillus*: mechanistic interpretation of their antimicrobial and potential SARS-CoV-2 inhibitory activity using molecular modelling. *Int J Mol Sci* 22:1866
33. VanderMolen KM, Raja HA, El-Elimat T, Oberlies NH (2013) Evaluation of culture media for the production of secondary metabolites in a natural products screening program. *AMB Express* 3:71. <https://doi.org/10.1186/2191-0855-3-71>
34. Sadrati N, Zerroug A, Demirel R, Bakli S, Harzallah D (2020) Antimicrobial activity of secondary metabolites produced by *Aspergillus neobridgeri* isolated from *Pistacia lentiscus* against multi-drug resistant bacteria. *Bangladesh J Pharmacol* 15:82–95
35. Bezerra JDP, Nascimento CCF, Barbosa RDN, Da Silva DCV, Svedese VM, Silva-Nogueira EB, Gomes BS, Paiva LM, Souza-Motta CM (2015) Endophytic fungi from medicinal plant *Bauhinia forficata*: diversity and biotechnological potential. *Braz J Microbiol* 46:49–57
36. Elkady WM, Raafat MM, Abdel-Aziz MM, Al-Huqail AA, Ashour ML, Fathallah N (2022) Endophytic fungus from *Opuntia ficus-indica*: a source of potential bioactive antimicrobial compounds against multidrug-resistant bacteria. *Plants* 11:1070
37. Poole K (2011) *Pseudomonas aeruginosa*: resistance to the max. *Front Microbiol* 2. <https://doi.org/10.3389/fmicb.2011.00065>
38. Kerr KG, Snelling AM (2009) *Pseudomonas aeruginosa*: a formidable and ever-present adversary. *J Hosp Infect* 73:338–344. <https://doi.org/10.1016/j.jhin.2009.04.020>
39. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK (2015) Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol* 29:996–1011. <https://doi.org/10.1086/591861>
40. Ohneck EJ, Arivett BA, Fiester SE, Wood CR, Metz ML, Simeone GM, Actis LA (2018) Mucin acts as a nutrient source and a signal for the differential expression of genes coding for cellular processes and virulence factors in *Acinetobacter baumannii*. *PLoS One* 13:e0190599
41. Wang R, Liu T, Shen M, Yang M, Feng Q, Tang X, Li X (2012) Spiculisporic acids B–D, three new γ -butenolide derivatives from a sea urchin-derived fungus *Aspergillus* sp. HDF2. *Molecules* 17:13175–13182. <https://doi.org/10.3390/molecules171113175>
42. Wang R, Guo Z, Li X, Chen F, Zhan X, Shen M (2015) Spiculisporic acid analogues of the marine-derived fungus, *Aspergillus candidus* strain HDF2, and their antibacterial activity. *Antonie Van Leeuwenhoek* 108:215–219. <https://doi.org/10.1007/s10482-015-0462-y>
43. Techaoui S, Jirayuthcharoenkul C, Jarmkom K, Dumrongphutti-decha T, Khobjai W (2020) Chemical evaluation and antibacterial activity of novel bioactive compounds from endophytic fungi in *Nelumbo nucifera*. *Saudi J Biol Sci* 27:2883–2889. <https://doi.org/10.1016/j.sjbs.2020.08.037>
44. Kaushik RD, Kumar A, Arora C (2003) Fractionation, characterization and evaluation of biocidal potential of active principles of leaves of *Vitex negundo* Linn. *Asian J Chem* 15:1659–1664
45. Khan I, Javaid A (2021) Identification of biologically important compounds in neem leaves through GC-MS analysis. *Jordan J Pharm Sci* 14:359–366
46. Dosoky NS, Moriarity DM, Setzer WN (2016) Phytochemical and biological investigations of *Conradina canescens*. *Nat Prod Commun* 11:8–25. <https://doi.org/10.1177/1934578X1601100109>
47. Keawsa-Ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Pyne SG (2012) Chemical constituents and antioxidant and biological activities of the essential oil from leaves of *Solanum spirale*. *Nat Prod Commun* 7:955–958. <https://doi.org/10.1177/1934578X1200700740>

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