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Research article

Metabolic profiling of antimicrobial secondary metabolites produced by *Penicillium bilaiae* EWB-3 isolated from electronic wastes in Algeria

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Abstract
Penicillium species research has progressed far beyond their ability to produce secondary metabolites with potential biological applications, particularly as antimicrobial agents. In this work, *Penicillium bilaiae* EWB-3 was isolated from electronic waste and identified using morphological and molecular (ITS and β-tubulin regions) methods. For 15 days, *Penicillium bilaiae* EWB-3 was grown into Czapek Yeast Broth using an orbital shaker. Finally, the secondary metabolites in this strain's filtrates were extracted using ethyl acetate. The agar well diffusion method tested this crude extract for antimicrobial activity. The *Penicillium bilaiae* EWB-3 extract exhibited strong antimicrobial potential against all tested microorganisms, including *Pseudomonas aeruginosa, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans* and *Aspergillus niger*. With diameters of 31, 26, and 25 mm, the largest inhibition zones were observed against *C. albicans, S. aureus,* and *E. faecalis,* respectively. The presence of 24 active compounds was revealed by gas chromatography-mass spectrometry (GC-MS) analysis of the crude extract of *Penicillium bilaiae* EWB-3. The findings suggest that the secondary metabolite extract of *Penicillium bilaiae* EWB-3 could be a promising active pharmaceutical component.

Keywords
Antimicrobial activity, electronic waste, GC-MS, *Penicillium bilaiae,* secondary metabolites.

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Introduction

Penicillium is one of the largest and most well-known fungal genera (Ali Shah et al., 2022). Secondary metabolites identified in this genus with various structural properties have piqued the interest of the pharmaceutical industry and mycologists, beginning with the discovery of penicillin and progressing to all of the different synthetically derived drugs currently in use (Omeike et al., 2019). Therefore, the role of *Penicillium* species as suppliers of bioactive compounds for medical use is crucial (Ashtekar et al., 2022). Studies have reported that industrial waste from current production processes can produce bioactive metabolites (Stierle and Stierle, 2014). It is possible to find novel antibiotics by exploring environments that have not yet been explored (Velasco-Rodríguez et al., 2021). The discovery of various commercially available drugs derived from extreme habitats such as salt mines, marine habitats and compost has elevated the importance of microbial research (Yadav et al., 2018; Kour et al., 2019; Abdel-Razek et al., 2020). Nature offers a broad spectrum of structurally distinct secondary metabolites. These structural differences reflect many biological activities (Yadav et al., 2020). Natural products can be arranged into multiple classes, terpenoids and steroids, fatty-acid-derived substances and polyketides, alkaloids, and nonribosomal polypeptides (Vemireddy et al., 2020). Developing microbial resistance against common antibacterial, antifungal, and antiviral medications is a major threat to human health (Hashem et al., 2021). Therefore**,** research of new bioactive compounds with antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral, cytotoxic, antitumoral and immunomodulatory activity through several pathogen-killing ways is necessary (Toghueo and Boyom, 2020). The screening procedure for producing secondary metabolites from filamentous fungi, which have already shown interesting biological activity, has been the subject of a few studies in Algeria. *Penicillium bilaiae* Chalab. is frequently used as a seed inoculant to improve phosphorus efficiency in various crops (Raymond et al., 2018). Thus, discovering new natural compounds from this filamentous fungus piqued our interest as a research topic. This study aims to isolate *Penicillium* spp., and in particular *P. bilaiae*, from new resources as electronic wastes that can produce the bioactive secondary metabolites and identify them by gas chromatography-mass spectrophotometry (GC-MS), evaluating their antimicrobial activity against human pathogens.

Materials and Methods

Sampling

In 2021, electronic samples were aseptically collected from a mixture of batteries and printed circuit boards waste at a depth of 10 cm below the surface of waste storage tanks in the Bordj-Bou Arreridj region (Algeria). These samples were immediately transferred to the laboratory for analysis.

Isolation of fungal strain

The fungal strain was isolated from samples electronic waste under aseptic conditions on Potato Dextrose Agar (PDA) plates using a standard serial dilution plating technique. Ten g of a mixture of batteries and printed circuit boards waste were washed using sterile distilled water, then stirred in 90 ml of sterile physiological water for 45 min. Serial dilutions from the supernatant were prepared until 10^3. One hundred µl were put on Petri plates containing PDA. In 2021, electronic samples were aseptically collected from a mixture of batteries and printed circuit boards waste at a depth of 10 cm

below the surface of waste storage tanks in the Bordj-Bou Arreridj region (Algeria). These samples were immediately transferred to the laboratory for analysis.

The plates were incubated at 28 °C for 7 days during repeated sub-culturing, and distinct isolates were purified. The isolated strains were then transferred in plates filled with five different culture media: Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA), Czapek Yeast Agar (CYA), and Oxytetracyclin-Glucose-Agar (OGA). After three days, the morphological characteristics of filamentous fungal colonies, including spore morphology, colony colors, and hyphal patterns, were observed. Based on their characteristics, macroscopic and microscopic slides were used to identify and select *Penicillium* isolates. Pure fungal isolates were stored at 4 °C for further examination (Yadav et al., 2018).

Molecular analyses

DNA extraction, PCR amplification and sequencing

Genomic DNA extraction from a selected fungal culture growing on PDA was performed using a NucleoSpin Plant II DNA isolation kit (Macherey-Nagel, Germany), following the manufacturer's instructions. The PCR amplification of Internal Transcribed Spacer (ITS) region of the fungal genome and 5.8S ribosomal genes was performed using the universal primers ITS1F (5' CTTGGTCATTTA GAGGAAGTAA 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') and the primers BT2A (5'GGTAACCAAATCGGTGCTGCTTTC 3'), BT2B (5'ACCCTCAGTGTAGTGACCCTTGGC 3') for the β-tubulin gene. In total, 25 µl of the reaction mixture was used to amplify all fragments, including 5 µl of Taq Buffer (Solis Biodyne). One and a half µl of 25 mM $MgCl_2$, 0.2 µl of 25 mM dNTP, 1 µl of each primer (ITS1F and ITS4), 2 µl of genomic DNA, 0.2 µl of 5 U μl^{-1} Taq polymerase (Solis Biodyne) and ultra-pure water (Kumar et al., 2016).

The thermal cycle program starts at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and a final extension at 72 °C for 7 min. The PCR conditions for the β-tubulin region are 5 min at 95 °C for pre-denaturation, followed by 35 cycles at 95 °C for 30 s, 30 s at 60 °C, 72 °C for 45 s and a final extension at 72 °C for 7 min (Guo et al., 2020). The amplified PCR products were separated by electrophoresis and then purified by NucleoSpin® Gel and PCR Clean-up from Macherey-Nagel (Germany) Mini kit. The Sanger technique sequenced the purified PCR products (Sanger et al., 1977) using the BigDye v3.1 kit from Applied Biosystems and the PCR primers used to amplify fragments of interest. The obtained sequences were analyzed and cleaned using the CHROMAS PRO software. Then the final sequences were compared with those of the GenBank database by BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgiBlast) for the identification of studied isolates based on the percentage homology with reference strains.

Phylogenetic analysis

The ITS and β-tubulin sequences of EWB-3 isolate and those of phylogenetically related species obtained from NCBI were aligned using a muscle algorithm in MEGA7 software to generate the phylogenetic trees. The obtained individual sequences, including ITS and β-tubulin, and the concatenated sequences were subjected to maximum likelihood analyses. The analyses were conducted in MEGA7 software with 1,000 replicates of the Tamura-Nei model. The trees were rooted with *Penicillium levitum* CBS: 345.48.

Fermentation and extraction of secondary metabolites

The cultures in shake flasks were performed in 500 ml Erlenmeyer flasks containing 200 ml Czapek's liquid medium by inoculating agar blocs of actively growing pure culture (6 mm in diameter) of the selected fungal strain. The cultures were incubated at 28 °C at 150 rpm on a rotary shaker for 15 days for the highest production. The Czapek medium composed of (g L^{-1}): $NaNO_3$, 0.3 g; K_2HPO_4, 0.1 g; KCl, 0.05 g; $MgSO_4$, $7H_2O$, 0.05 g; $FeSO_4$ $7H_2O$, 0.001 g; sucrose, 3.0 g; Yeast Extract, 5.0 g (Pitt, 1979). After incubation, the cultures were filtered through Whatman paper N°1 to separate the culture filtrate and mycelial material. The filtrate was extracted three times with an equal volume of ethyl acetate (EtOAc) using a separating funnel. Combined ethyl acetate extract evaporated at 40 °C by rotary evaporator (BUCHI R-100). The crude extracts were then dissolved in Dimethyl Sulfoxide (DMSO) and stored at at 4 °C for further analysis (Lykholat et al., 2021; Nischitha and Shivanna, 2021).

In vitro antimicrobial activity

Agar well diffusion method

Antimicrobial activity of crude ethyl acetate extract was performed by agar well diffusion method described by Fatima et al. (2016) against ten pathogenic bacterial and fungal strains *Klebsiella pneumoniae* ATCC 70063, *Enterococcus faecalis* ATCC 29212, Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Escherichia coli* ATCC 29522, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 43306, *Bacillus cereus* ATCC, *Aspergillus niger* CTM 10099 and *Candida albicans* ATCC 10231. Bacterial and fungal suspensions were adjusted to a final concentration of 10^8 CFU ml^{-1} and 10^6 CFU ml^{-1}, respectively. One hundred µl of each bacterial and fungal suspension were spread on Mueller-Hinton agar and Sabouraud chloramphenicol agar plates, respectively. Then, wells with a diameter of 6 mm were created, and 20 µl of the metabolic extract was added. Dimethyl sulfoxide (DMSO) was used as a negative control. Subsequently, the plates were incubated for 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi. All the assays were conducted in triplicate. The antibacterial and antifungal activities were evaluated by measuring the diameter of inhibition zones (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC)

The minimal inhibitory concentration was established using the 96-well broth microdilution technique (Pierce et al., 2008). The final concentrations of tested ethyl acetate extract varied between (90 - 45 - 22.5 - 11.2 - 5.6 - 2.8 - 1.4 - 0.7 - 0.3 µg ml^{-1}), and the bacterial and fungal suspension was adjusted to 0.5 McFarland. The Gentamycin (40 µg ml^{-1}) and Fluconazole (50 µg ml^{-1}) antimicrobial agents were used as controls, and the indicator strains were used for antimicrobial assays. Each well was inoculated with 90 µl of different ethyl acetate crude extract concentrations and 10 µl of bacterial and fungal suspensions. After incubation at 37 °C for 24 h for bacterial strains and 48 h for fungal strains, the presence or absence of growth was observed with the resazurin fluorometric/colorimetric assay by addition of 20 µl of resazurin to each well (Eloff, 2019). The MIC was defined as the lowest concentration of ethyl acetate crude extract without any microbial growth.

Characterization of compounds

Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive metabolites

The protocol described by Lykholat et al. (2021), with some modifications, was used to search for volatile metabolites. GC-MS (Thermo Fisher Scientific) was used to analyze the biologically active fungal crude ethyl acetate extract, and the sample was derivatized to impart volatility to the compounds for maximum detection. The derivatization procedure was realized by adding 50 µl of a sample extract (20% in ethyl acetate) was placed into a 2 ml glass vial and treated sequentially with 100 µl of acetonitrile and 100 µl of NO-Bis (trimethylsilyl)trifluoroacetamide (BSTFA). The sample was shaken at room temperature for 1 min then the vial was heated at 60 °C for 30 min. The resulting solution is analyzed directly by capillary gas chromatography connected to a mass-spectrometry assay. GC-MS analysis of the extracts was performed using a Thermo Fisher ZB-5 MS column (length 30 m × 0.25 mm, film thickness 0.25 µm) containing 5% diphenyl/ 95% dimethyl polysiloxane as a fixed liquid phase. The column temperature was maintained at 50 °C for 5 min. After that, the temperature gradient was programmed to rise by 10 °C each minute, achieving 300 °C and maintaining there for 10 min. The carrier gas helium passed at a 20 ml/min flow rate. Injection volume 1 µl was employed (split ratio 20%); the injector temperature was 280 °C.

Identification of compounds

The National Institute of Standards and Technology (NIST) database was used to interpret the GC-MS mass spectrum. The obtained spectrum was compared to the spectrum of the database's standard component. Each compound's name, retention time, and chemical structure were also determined.

Statistical analysis

The data were analyzed using Microsoft Excel 2013 and were expressed as mean ± SD value.

Results

Isolation and identification of the fungal strain

Based on morphological characteristics, one of the isolated fungal colonies was identified as *Penicillium* sp. from preliminary screening of electronic waste. After 10 days at 28 °C, this isolate grew on various growth media, including PDA, OGA, CYA, MEA, and SDA, as shown in Figure 1. On most culture media, this isolate grew at a similar rate, with colony diameters ranging from 22 to 30 mm. However, its colonial morphology was significantly different, with colonies on PDA appearing green with a white outline, mycelium on CYA, OGA, and MEA becoming green with yellow centers, covered with yellow exudates for MEA with age, and mycelium on SDA appearing green with a white center. A diffusion of yellow pigment around colonies was observed in all culture media.

After sequencing and correction, the blast of the obtained sequences from ITS and β-tubulin regions against the previously archived sequences in the NCBI databases revealed that both sequences had a similarity percentage of 98% with those of the *P. bilaiae* strain NRRL 3391. A total of 34 ITS and β-tubulin sequences of the closely related species to the sequences of EWB-3 isolate downloaded from NCBI, one sequence from this study and another sequence as an outgroup, were used for the paleogenetic analyses and to construct the trees. The Maximum likelihood phylogenetic analysis results indicate that fungal isolate EWB-3 is classified within the *Penicillium* genus, specifically in

section *Sclerotiorum*, series *Adametziorum*. Phylogenetic trees created using ITS sequences, β-tubulin, and concatenated data generally exhibit identical topologies. The EWB-3 isolate is positioned among the other species in the same section and has the closest phylogenetic affinity to *P. bilaiae*, supported by very high bootstrap values of 100% (ITS tree), 99% (β-tubulin tree) and 98% (concatenated tree) (Figs. 2-4). The internal transcribed spacer (ITS) and β-tubulin sequences of the isolate EWB-3, was deposited to the GenBank with accessions numbers: ITS: OR225221, β-tubulin: OR243415.

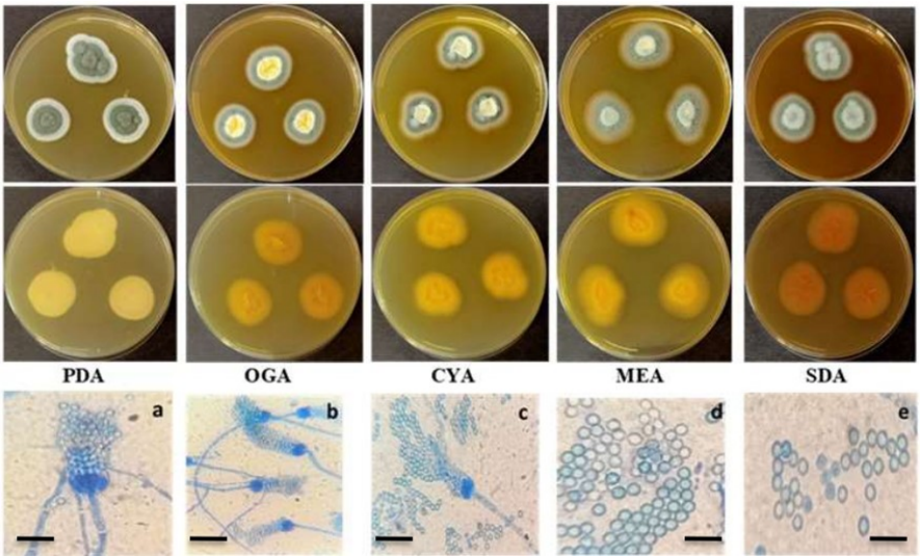


Fig. 1 – Morphological characteristics of EWB-3 isolate on different growth media after 10 days at 28°C: conidiophores (**a-c**), conidia (**d-e**). Scale bars: a, c, = 250 µm; b = 500 µm; d, e = 10 µm.

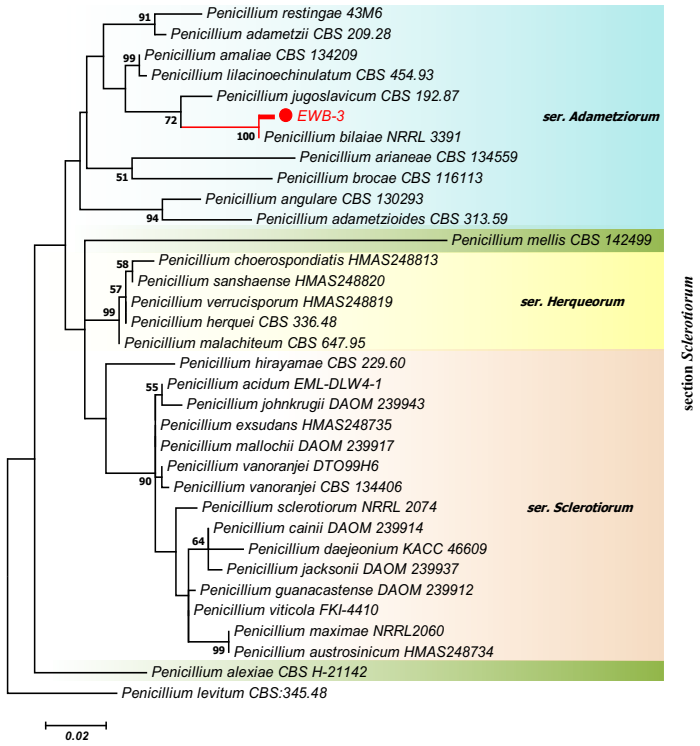


Fig. 2 – Maximum-likelihood phylogenetic tree inferred from the data set of ITS locus for *Penicillium* section *Sclerotiora*. The Bootstrap values are greater than 50%. The phylogram is rooted with *Penicillium levitum* CBS: 345.48.

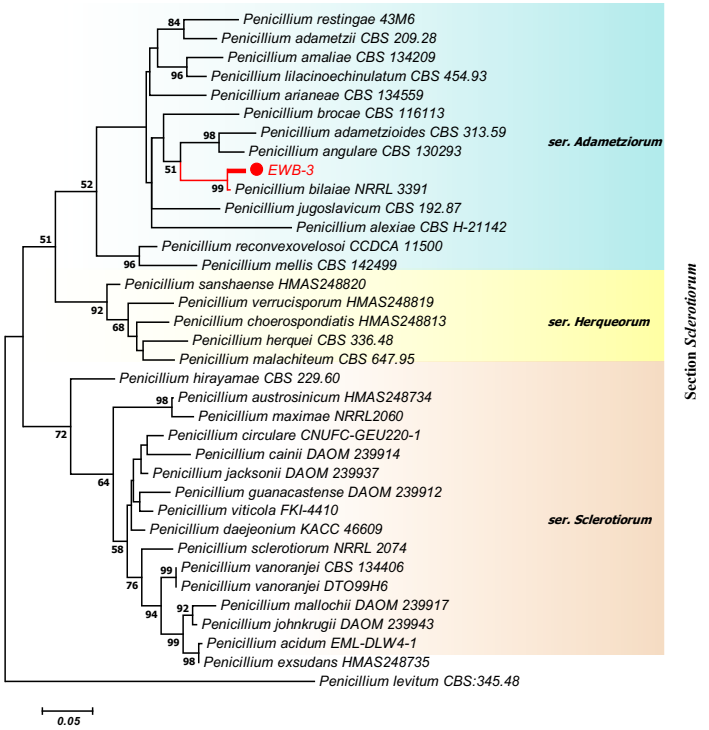


Fig. 3 – Maximum-likelihood phylogenetic tree inferred from the data set of BenA locus for *Penicillium* section Sclerotiora. The Bootstrap values are greater than 50%. The phylogram is rooted with *Penicillium levitum* CBS:345.48.

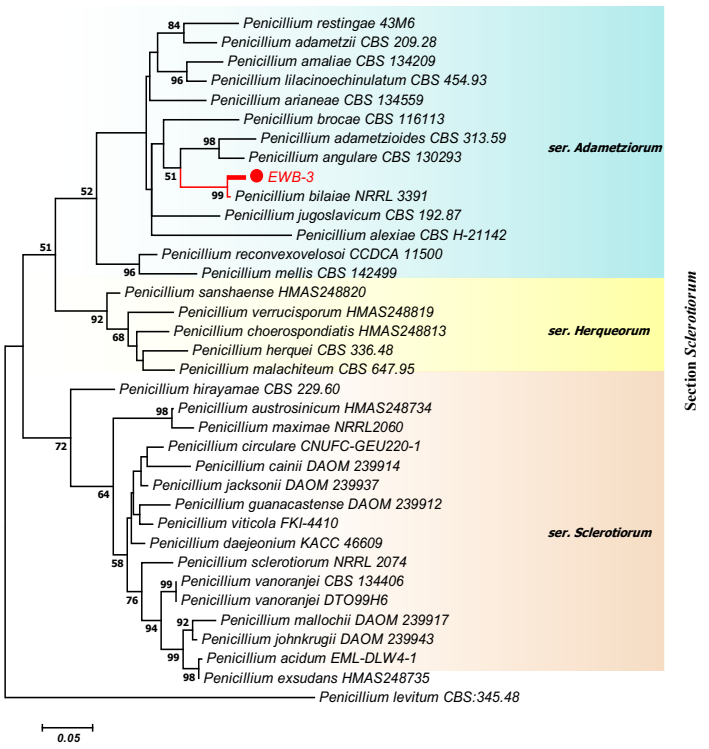


Fig. 4 – Maximum-likelihood phylogenetic tree inferred from the ITS and BenA loci combined data set for *Penicillium* section *Sclerotiora*. The Bootstrap values are greater than 50%. The phylogram is rooted with *Penicillium levitum* CBS:345.48.

In vitro antimicrobial activity (well diffusion method)

The crude ethyl acetate extract exhibited high antibacterial and antifungal activities by inhibiting many pathogens (Table 1). A strong antimicrobial activity against *B. cereus* ATCC 14579, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 43306, *C. albicans* ATCC 10231 can be noted, as well as a strong activity against *E. coli* ATCC 29522, *K. pneumoniae* ATCC 70063, MRSA ATCC 43300 and *A. niger* CTM 10099. On the other hand, the fungal extract displayed a weak zone for *S. typhimurium* ATCC 13311of only 11 mm. The inhibition zones varied from 11 to 31 mm in diameter, as shown in Figure 5. The largest inhibition zone was observed against *C. albicans*, *B.cereus* and *S. aureus*, with 31, 26, and 25 mm, respectively.

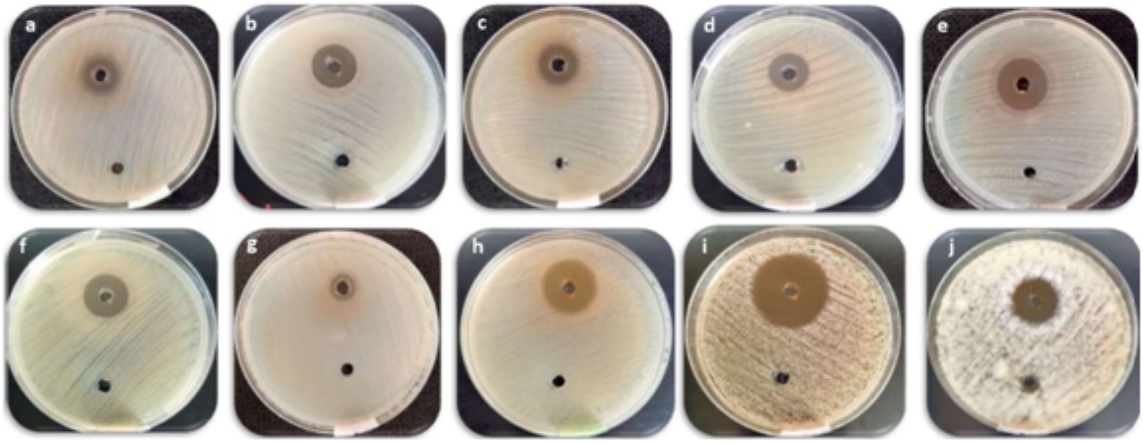


Fig. 5 – Antimicrobial activity of ethyl acetate extract against *Escherichia coli* (a), *Pseudomonas aeruginosa* (b), *Klebsiella pneumoniae* (c), *Enterococcus faecalis* (d), *Staphylococcus aureus* (e), Methicillin-Resistant *Staphylococcus aureus* (f), *Salmonella typhimurium* (g), *Bacillus cereus (h), Candida albicans* (i), *Aspergillus niger* (j).

Table 1 - Inhibition diameters of antimicrobial activity of ethyl acetate extract against the tested microorganisms.

Microbial strains	Zones of inhibition (mm) ± SD	Negative control DMSO
Escherichia coli ATCC 29522	17 ± 2.52	00 ± 00
Pseudomonas aeruginosa ATCC 27853	23 ± 2.08	00 ± 00
Klebsiella pneumoniae ATCC 70063	18 ± 2.08	00 ± 00
Salmonella typhimurium ATCC 13311	11 ± 1.15	00 ± 00
Enterococcus faecalis ATCC 29212	24 ± 5.13	00 ± 00
Bacillus cereus ATCC 14579	26 ± 1.11	00 ± 00
Staphylococcus aureus ATCC 43306	25 ± 3.46	00 ± 00
Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300	19 ± 1.15	00 ± 00
Candida albicans ATCC 10231	31 ± 3.00	00 ± 00
Aspergillus niger CTM 10099	13 ± 1.53	00 ± 00

Table 2 - Minimum Inhibitory Concentration (µg ml^{-1}) of ethyl acetate crude extract.

	Human bacterial pathogens							
Crude ethyl acetate extract	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*S. typhimurium*	*E. faecalis*	*B. cereus*	*S. aureus*	*MRSA*
	5.62	90	22.5	90	45	1.40	1.40	0.35

MRSA: Methicillin-Resistant *Staphylococcus aureus.*

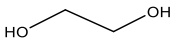
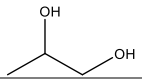
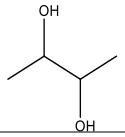
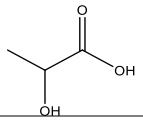
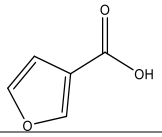
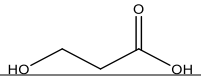
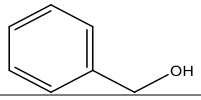
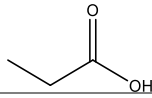
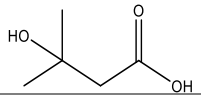
Table 3 - Minimum Fungicide Concentration (MFC µg/ml) of ethyl acetate crude extract.

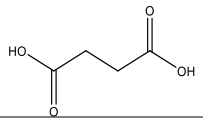
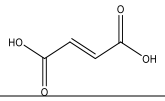
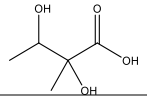
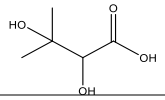
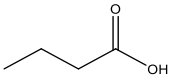
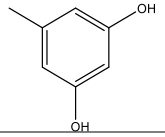
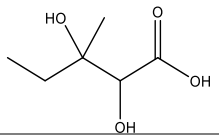
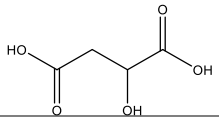
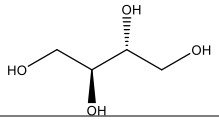
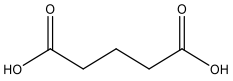
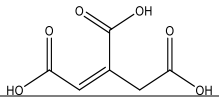
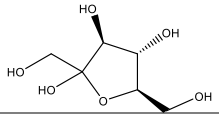
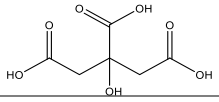
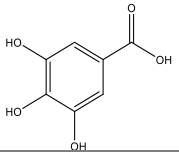
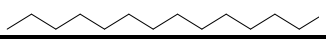
	Human Fungal pathogens	
Crude ethyl acetate extract	*C. albicans*	*A. niger*
	11.25	5.62

Characterization using Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis provides a representative spectrum of each metabolite in the tested sample. The GC-MS chromatograms (Supplementary Fig. S1) show that the fungal isolate's crude ethyl acetate extract contains the compounds listed in Table 4, their name, retention time (RT), probability %, chemical structure and formula. In total, 24 different metabolites were identified.

Table 4 - The detected compounds through GC-MS in a crude extract of *Penicillium bilaiae* EWB-3.

No	Compound name	Retention Time (min)	Probability %	Chemical structure	Formula
1	Ethylene glycol, 2TMS derivative	9.29	25.39		$C_8H_{22}O_2Si_2$
2	Propylene glycol, 2TMS derivative	9.58	53.88		$C_9H_{24}O_2Si_2$
3	2.3-Butanediol, 2TMS derivative	10.40	51.51		$C_{10}H_{26}O_2Si_2$
4	Lactic acid, 2TMS derivative	10.78	87.10		$C_9H_{22}O_3Si_2$
5	3-Furoic acid, TMS derivative	12.13	20.71		$C_8H_{12}O_3Si$
6	Hydracrylic acid, 2TMS derivative	12.28	89.33		$C_9H_{22}O_3Si_2$
7	Benzyl alcohol, TMS derivative	12.45	22.00		$C_{10}H_{16}OSi$
8	Propanoic acid, 2TMS derivative	12.54	92.38		$C_{10}H_{24}O_3Si_2$
9	3-Hydroxyisovaleric acid, 2TMS derivative	13.32	80.67		$C_{11}H_{26}O_3Si_2$

10	Butanedioic acid, 2TMS derivative	14.90	88.93		$C_{10}H_{22}O_4Si_2$
11	2-Butanedoic acid, (E)-, 2TMS derivative	15.40	88.49		$C_{10}H_{20}O_4Si_2$
12	2.3-Dihydroxy-2-methylbutanoic acid, 3TMS derivative	15.71	97.70		$C_{14}H_{34}O_4Si_3$
13	®-2.3-Dihydroxy-3-methylbutyric acid, 3TMS derivative	15.94	65.12		$C_{14}H_{34}O_4Si_3$
14	Butanoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	16.20	44.43		$C_{13}H_{32}O_4Si_3$
15	Orcinol, 2TMS derivative	16.68	77.25		$C_{13}H_{24}O_2Si_2$
16	2.3-Dihydroxy-3-methylpentanoic acid, 3TMS derivative	16.95	83.24		$C_{15}H_{36}O_4Si_3$
17	Malic acid, 3TMS derivative	17.19	96.91		$C_{13}H_{30}O_5Si_3$
18	Erythritol, 4TMS derivative	17.40	45.18		$C_{16}H_{42}O_4Si_4$
19	Pentanedioic acid, 2-[(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	18.27	93.34		$C_{14}H_{32}O_5Si_3$
20	Aconitic acid, 3TMS derivative	20.28	54.10		$C_{15}H_{30}O_6Si_3$
21	D-(-)-Fructofuranose, pentakis, 5TMS derivative	20.92	18.92		$C_{21}H_{52}O_6Si_5$
22	Citric acid, 4TMS derivative	21.00	36.02		$C_{18}H_{40}O_7Si_5$
23	Gallic acid, 4TMS derivative	22.43	90.50		$C_{19}H_{38}O_5Si_4$
24	Palmitic acid, TMS derivative	23.37	64.62		$C_{19}H_{40}O_2Si$

Discussion

Penicillium bilaiae EWB-3 was isolated from electronic waste for the first time in Algeria. The ability of this EWB-3 isolate to produce bioactive compounds was evaluated. Natural antimicrobial compounds derived from extremophile fungi are safer for human health and food preservation than synthetic antimicrobials (Akbar et al., 2019). *Penicillium bilaiae* EWB-3 showed a very important antimicrobial activity against all tested pathogens. The findings from previous studies support the genus *Penicillium*'s ability to produce a wide variety of secondary metabolites (Toghueo and Boyom, 2020). Earlier studies have employed *P. bilaiae* for the bioremediation of metal-contaminated soil (Arwidsson et al., 2010). Moreover, the phosphorus-solubilizing fungus has been used as an inoculant to promote plant growth (Hansen et al., 2020). Meng et al. (2014) investigated some metabolic compounds of *P. bilaiae* MA-267 isolated from the rhizospheric soil of a mangrove plant with high activity against the plant pathogenic fungus *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. The current study is considered a first report where *P. bilaiae* EWB-3 was isolated from an unusual source, electronic waste, and its characterized metabolites exhibited a high activity against all tested microorganisms known as human pathogens.

The media composition influences secondary metabolite profiles. The yeast extract containing czapek yeast medium is regarded as a secondary metabolite-producing medium (Frisvad, 2012). The most common organic solvent employed to extract fungal secondary metabolites is ethyl acetate (EtOAc) with medium polarity extraction (Nawaz et al., 2020). Data from the literature confirm that the antibacterial effects of fungal extracts are related to the extraction solvent (Ben Mefteh et al., 2018). Further, Al-Saleem et al. (2022) reported an antimicrobial activity of ethyl acetate extract from *P. chrysogenum* against *S. aureus* with a MIC value of 250 µg ml^{-1}. The authors also observed fungicidal activity against *C. albicans* and *Cryptococcus neoformans*. According to Kumari et al. (2021), the antibacterial activity of *Penicillium citrinum* Thom of ethyl acetate extract displayed a strong activity against human pathogenic bacteria, including *S. aureus, Bacillus subtilis, E. coli, Shigella boydii, Vibrio cholera.*

Some other works affirmed the antibacterial activity of the crude extract of *Penicillium griseofulvum* Dierckx (Zerroug et al., 2018) and *Penicillium brevicompactum* Dierckx ANT13 (Sadrati et al., 2023) against pathogenic bacteria with the maximal activity against *E. coli* (45.5 mm) and *S. aureus* ATCC 25923 (45.5 mm) respectively. Omeike et al. (2019) also reported a potent activity of *P. citrinum* ethyl acetate extract with 16 mm of inhibition zone against *Klebsiella peneumoniae*. Nischitha and Shivanna (2021) studied the antimicrobial activity of the ethyl acetate extract of *Penicillium pinophilum* Hedgc. The researchers obtained good activity for all tested bacteria except *S. aureus* and *P. aeruginosa*. Pan et al. (2017) indicated the potent antibacterial activity of *Penicillium* sp. ethyl acetate extract against *S. aureus* and *B. subtilis*. Comparatively with our work, the results revealed that ethyl acetate extract of *P. bilaiae* EWB-3 inhibited both Gram-positive and Gram-negative bacteria tested, such as *S. aureus, B. cereus* and *P. aeruginosa* with an important inhibition zone 25 mm and 23 mm, respectively. The antimicrobial activity of these *Penicillium* spp. may be explained by the presence of chemical compounds that can inhibit microbial growth.

Penicillium bilaiae EWB-3 produces a wide range of chemical compounds belonging to different classes, organic acids, esters, fatty acids and alcohols that appeared during GC-MS analysis. The most abundant compounds in the fungal extract are 2,3-butanidiol, lactic acid, 3-furoic acid, propanoic acid, 2-butanedioic acid, malic acid, pentanedioic acid, D-(-)-fructofuranose, 3-

hydroxyphenylacetic acid, gallic acid, palmitic acid. These compounds have different biological properties, such as antifungal and antibacterial activities. This study is the first in Algeria to target the biomolecules produced by this isolate. Research carried out about *P. bilaiae* has reported the crucial role of this fungus in dissolving insoluble forms of inorganic phosphorus (Hansen et al., 2020; Zhao et al., 2021) and highlighted the utility of using *P. bilaiae* as an inoculant to promote plant growth or phosphorus uptake in various crops. This process may release organic acids for phosphorus mobilization and promote plant growth.

Arwidsson et al. (2010) reported that *P. bilaiae* could produce organic acids such as citric acid, oxalic acid, and acetic and formic acid for bioremediation in metal-contaminated soils. In this study, we characterized unusual and interesting organics compounds isolated for the first time from this fungus and having potent activity against all tested pathogenic human microorganisms, such as 3-hydroxyisovaleric acid, pinosylvin, 3-furoic acid, palmitic acid and 2,3-dihydroxy-2-methylbutanoic acid. On the other hand, work on *P. bilaiae* in co-culture with *P. chermesinum* carried out by Meng et al. (2020) detected new bioactive meroterpenoids in chermebilaenes A and B. The obtained compounds were tested against the human and aqua-pathogenic bacteria. Only 9, 12-octadecadienoic acid showed moderate activity against the tested pathogens. Nakahara et al. (2004) isolated a new acetylenic with nematicidal activity from *P. bilaiae* against *Pratylenchus penetrans*. Our results are according to Lykholat et al. (2021), who analyzed different compounds from ethyl acetate extract from *Penicillium* sp., the same compounds using GC-MS analysis were noticed, propanoic acid, 2-butanoic acid, and pentanedioic acid also known as glutaric acid. In addition, different studies confirm what we found about the antimicrobial activity of malic acid (Agoramoorthy et al., 2007), 3-furoic acid (Liu et al., 2012), ethylene glycol (Gurtler and Mai, 2014) and palmitic acid (Zhang et al., 2020).

Furthermore, 3-hydroxyisolvaleric acid, present in our work, was also identified by Menezes et al. (2022) as a bioactive secondary metabolite exhibiting antimicrobial and antiparasitic activity. Song et al. (2020) reported propanoic acid as the antibacterial metabolite against *Acinetobacter baumannii, P. aeruginosa, E.coli* and *S. aureus.* Gong et al. (2014) found that the *P. oxalicum* synthesis pathway for generating acids is directly influenced by the presence of a nitrogen source. This strain largely secreted malic acid, acetic acid, propionic acid and citric acid in this condition. These findings suggest that fatty acids could enhance the penetration into bacterial cell walls, plasma membranes, protein synthesis, and nucleic acid metabolism, subsequently increasing the antimicrobial activity against tested pathogenic microorganisms (Li et al., 2015; Watanabe et al., 2019). Therefore, it can be noted that *P. bilaiae* EWB-3 can produce a potential number of secondary metabolites with therapeutic benefits. These include phenolic acids, esters, and fatty acids.

Conclusion

Penicillium bilaiae EWB-3 was identified morphologically and genetically from electronic waste in this study. This is the first report of this strain being isolated in Algeria. This filamentous fungus is a rich source of biologically active natural compounds. *Penicillium bilaiae* EWB-3 crude extract was tested for antimicrobial activity against Gram-positive and Gram-negative human pathogenic bacteria and fungi. As a result, the high potential of this fungal strain suggests that it could be a promising source of bioactive substances with potential applications in the pharmaceutical industry.

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