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Antimicrobial Activities and Chemical Profiles of Fungal Species Isolated from Bovine Dung in the North West Region of Cameroon

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ABSTRACT: The global rise of drug-resistant pathogens has intensified the challenge of antimicrobial resistance, rendering many conventional antibiotics ineffective against infectious diseases. Coprophilous fungi are saprophytic fungi that live on animal dung and are recognized as promising sources of bioactive compounds, including antibiotics, enzymes, and biocontrol agents. This study aimed to document the diversity of fungi from cow dung samples collected in the Mezam Division, North West Region of Cameroon, to evaluate the antimicrobial activity of their crude extracts, and annotate compounds in the most active crude extracts. A total of 100 fungal isolates were recovered from 21 dung samples sourced from three cattle ranches using the moist chamber technique. Based on morphological characteristics, 12 representative strains were selected and identified through the internal transcribed spacer (ITS) gene region. Ethyl acetate extracts from solid-state cultures of these strains were screened for antimicrobial activity against available in-house strains, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, using the microdilution method. Metabolite annotation of these active extracts was performed using Ultra-High Performance Liquid Chromatography – Quadrupole Time-of-Flight – Electrospray Ionization – Tandem Mass Spectrometry (UHPLC-QTOF-ESI-MS/MS). The extracts displayed variable antimicrobial activity, with minimum inhibitory concentrations (MICs) ranging from 125 to 1000 µg/mL. Notably, crude extracts of *Scopulariopsis brevicaulis*, *Papulaspora gansuensis*, *Clonostachys epichloe*, and *C. rosea* demonstrated the most promising activity (MICs 125–500 µg/mL). Metabolite profiling revealed diverse chemical constituents, including uridine, spiroxamine, adipic acid, and sebacic acid, which have been previously reported for their antimicrobial properties. These findings highlight coprophilous fungi as a rich source of structurally

diverse metabolites with potential relevance for drug discovery against microbial pathogens.

1. INTRODUCTION

Coprophilous fungi encompass a diverse ecological group of fungi adapted to live in animal feces and play important roles in the decomposition and recycling of nutrients, the carbon flow and ecosystem energetics (Melo et al., 2016). These fungi have evolved features that enable adaptation to the dung environment (Misra et al., 2014) and withstand stress. Their spores depend on passage through the animals' digestive tracts to facilitate spore germination (Richardson, 2003). Previous studies on coprophilous fungi were limited to macroscopic fungi encountered during sampling. To obtain more comprehensive results, broader research involving the isolation of microscopic fungi from herbivorous animal waste is needed (Zuber et al., 2011). Species of Ascomycota identified using molecular methods, include: *Alternaria alternata*, *Paraconiothyrium fungicola*, *Chaetomium murorum*, *Fusarium solani*, *Cladosporium herbarum*, *Sarocladium strictum*, and *Epicoccum nigrum* as fungal mycobiota associated with the feces of birds (Torbat, 2016). Representatives from nearly all major fungal taxonomic groups have been identified in animal dung (Crous et al., 2009), and many appear to exhibit a degree of adaptability toward specific types of dung (Krug et al., 2004). Coprophilous fungi are represented across several groups, including Ascomycota, Basidiomycota, Mucoromycota, and Zoopagomycotina, with the latter being the most encountered group (Melo et al., 2020). According to Krug et al. (2004), most fungi inhabit the dung of herbivorous livestock, such as sheep and cattle, play a predominant role in the decomposition of organic matter. One of the several traits that facilitates their survival in this competitive environment is their ability to produce a vast array of biologically active secondary metabolites, a chemical arsenal that fosters successful colonization of the dung substrate (Charria-Girón et al., 2025). Researchers seeking new antibiotics have often turned to biologists specializing in chemically mediated microbial interactions, particularly in soils, marine invertebrates, and plant–microorganism symbioses. Despite few efforts to explore their chemical constituents, coprophilous fungi remain a valuable source of new antimicrobials (Bills et al., 2013; Charria-Girón et al., 2025). In our continuous efforts to search for new anti-infective agents (Kapche et al., 2025; Tchamgoue et al., 2026), we investigated the antimicrobial properties of twelve fungi isolated from cow dung samples collected in the Northwest Region of Cameroon, profiled their chemical constituents, annotated the detected compounds, and herein present the results.

2. MATERIALS AND METHODS

2.1 Collection of dung samples

Dry cow dung was collected from three different cattle ranches, namely, the Alabukam cattle Ranch (R1), the Alahki cattle Ranch (R2), and the Upsation cattle Ranch (R3) in the Mezam Division in the North West Region of Cameroon. Seven dung samples were collected from each of the three sites using forceps, gloves, and zip lock bags. A total of twenty-one samples were then transported in coolers to the Centre of Competence for the Study of Natural Antimicrobial Products from Fungi (CECANAPROF) at the Higher Teacher Training College, University of Yaounde 1, Cameroon, for fungi isolation.

2.2 Coprophilous fungal isolation

The fungal isolation was performed using the moist chamber method as described by Krug et al. (2004). Briefly, the collected dung samples were placed in Petri dishes containing filter paper pre-treated with a chloramphenicol solution (0.2 g in 500 mL of distilled water). After an incubation period of 10 to 30 days, the emerging fungal spores were transferred to Malt Extract Agar (MEA) for further purification. The resulting isolates were subcultured to obtain pure strains, and a voucher plate of each isolate was preserved at -80°C at the Centre of Competence for the Study of Natural Antimicrobial Products from Fungi (CECANAPROF).

2.3 Fungi identification

A total of one hundred isolates were recovered from the different cow dung from the three rearing sites and discriminated based on their morphological characteristics, including colony size, shape, and color, and microscopic features such as morphology of conidia (spores) and conidiophores (spore-producing cells). Based on this, 12 representative isolates were selected and identified using the ITS nrDNA gene region and sanger sequenced by Inqaba Biotec (<https://inqababiotec.co.za/>).

For identification, genomic DNA was extracted using the Quick-DNA™ Fungal/Bacterial kit (Zymo Research, Catalogue No. D6005) according to the manufacturer's instructions. The ITS target region was amplified with OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) using ITS-1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White et al., 1990). The PCR product was analysed by gel electrophoresis using the stain Ethidium Bromide and cleaned up enzymatically using the EXOSAP method (Bell, 2008), conditioned at an initial denaturation at 95°C for 10 minutes, followed by 30-40 cycles of denaturation (94-96 °C for 30 seconds), annealing (variable, 30-40 seconds), and extension (72°C, 30 seconds to 1 minute). The extracted fragments were sanger sequenced in both the forward and reverse directions (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and subsequently purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analysed on the ABI 3500xl Genetic Analyser (Applied Biosystems, ThermoFisher Scientific). The resulting sequence data (*.ab1 files) were processed using DNASTAR software and then subjected to a BLAST search in NCBI (Mountessou et al., 2025).

2.4 Crude extracts preparation

Crude extracts were prepared following the protocol described by (Kapche et al., 2025). Briefly, each fungal strain was cultivated in two 500 mL conical flasks, each containing 100 g of rice and 150 mL of distilled water. The contents were autoclaved and subsequently inoculated with actively growing fungal cultures obtained from Malt Extract Agar (MEA) plates. The flasks were incubated at 27 °C for 21 days. After incubation, the cultures were extracted with 300 mL of ethyl acetate, and the resulting filtrates were concentrated under reduced pressure to yield the crude extracts. Table 1 shows the percentage yield of crude extracts of isolated fungi calculated from the amount of solid media used (200 g of rice).

Table 1. Percentage yields of coprophilous fungi crude extracts

Code of strain	Mass of crude extracts (g)	Percentage yield
CECA 179	2.33	1.2 %
CECA 184	2.40	1.2 %
CECA 217	2.25	1.1 %
CECA 147	2.31	1.2 %
CECA 174	2.20	1.1 %
CECA 163	3.02	1.5 %
CECA 164	2.53	1.3 %
CECA 178	2.45	1.2 %
CECA 188	2.67	1.3 %
CECA 180	3.13	1.6 %
CECA 189	2.37	1.2 %
CECA 170	2.28	1.1 %

2.5 Antimicrobial assay

The *in vitro* antimicrobial activities of the extracts were evaluated against two bacterial strains, *Staphylococcus aureus* ATCC 43300 (SA ATCC 43300) and *Escherichia coli* ATCC 25922 (EC ATCC 25922), and one fungal strain, *Candida albicans* ATCC 14516 (CA ATCC 14516). All strains were obtained from the 'Centre Pasteur' of Cameroon (STM CPC) and stored at 4 °C in the Laboratory of Phytobiochemistry and Medicinal Plant Studies, Department of Biochemistry, University of Yaounde 1, Cameroon. Bacterial strains were maintained in tubes containing Mueller-Hinton Agar, while the yeast strain was preserved on Sabouraud Dextrose Agar.

Twelve crude extracts (CECA 179, CECA 184, CECA 217, CECA 147, CECA 174, CECA 163, CECA 164, CECA 178, CECA 188, CECA 180, CECA 189 and CECA 170) were prepared at 100 mg/mL in Dimethyl Sulfoxide (DMSO 100 %) and then stored at 4 °C. Ciprofloxacin and Amphotericin B, used as the reference antibiotics, were prepared under the same conditions at 100 µg/mL and 1 mg/mL, respectively in acidified water (with HCl 0.04N).

2.6.1 Antibacterial assay

The Minimum Inhibitory Concentrations (MIC) after the test on two bacterial strains *Staphylococcus aureus* ATCC 43300 (SA ATCC 43300) and *Escherichia coli* ATCC 25922 (EC ATCC 25922), were determined using the broth microdilution method

according to the M07-A10 protocol of the Clinical and Laboratory Standards Institute (CLSI, 2015) with slight modifications. The test was performed in triplicate in a sterile 96-well microplate. Indeed, 196 μL of Muller Hinton Broth (MHB) was introduced into the first wells and 100 μL into the remaining wells. Then, 4 μL of sterile extract solutions prepared at 100 mg/mL was introduced into the corresponding wells, followed by a two-fold serial dilution. After that, 100 μL of bacterial suspension (10^6 UFC/mL) was distributed into wells except those for sterility control. The concentrations of extracts and ciprofloxacin in the wells ranged from 1000 $\mu\text{g/mL}$ to 31.25 $\mu\text{g/mL}$ and from 0.25 $\mu\text{g/mL}$ to 0.0078 $\mu\text{g/mL}$ with a final volume of 200 μL . The negative control consisted of culture medium and bacterial suspension, and the positive control consisted of culture medium, bacterial suspension, and ciprofloxacin. In contrast, the control of sterility contained only the culture media. The plates were covered and incubated for 24 hrs at 37 °C. At the end of the incubation period, 20 μL of freshly prepared resazurin (0.15 mg/mL) was added to all wells, and the wells were re-incubated under the same conditions for 30 minutes. The lowest concentration at which there was no change in colouration from blue to pink, corresponding to a lack of visible growth of bacteria, was considered the Minimum Inhibitory Concentration (MIC) of the extract.

2.6.2 Antifungal assay

The antifungal activity of the crude extracts against the pathogenic fungus *Candida albicans* ATCC 14516 (CA ATCC 14516) (subculturing in a liquid medium) was evaluated according to CLSI M27 guidelines (CLSI, 2017). The tests were performed in duplicate in 96-well microplates. Indeed, 196 μL of culture medium (Sabouraud Dextrose Broth) was added to the cups of the first line and 100 μL into the remaining wells. Then, 4 μL of each extract and the reference antifungal (Amphotericin B) at initial concentrations of 100 mg/mL and 1 mg/mL, respectively, were added to the first wells, followed by a series of 5 two-fold dilutions. To 100 μL of a suspension of a single yeast strain prepared from 48-hour cultures (10^4 cells/mL) were seeded into the wells, except for the sterility control. The plates were sealed and incubated at 37 °C for 48 hours. The smallest concentration with no visible growth was considered as the MIC.

2.7 Chemical profiling

2.7.1 Sample preparation

1 mg of each crude extract was dissolved in LC-grade methanol to obtain a 1 mg/mL solution. For the analysis, a diluted solution was prepared by taking 20 μL of the prepared solution and adding 980 μL of methanol.

2.7.2 UHPLC-QTOF-MS/MS analysis and compound annotation

High resolution mass spectra were obtained with a QTOF Spectrometer (Bruker, Germany) equipped with an ESI source. The spectrometer was operated in negative ion mode (mass range: 100-1500, scan rate: 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements with a deviation of 0.40 ppm using Na Formate as the calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, and capillary temperature of 200 °C. Nitrogen was used as sheath gas (10 L/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) UHPLC system consisting of an LC-pump, a Diode Array Detector (DAD) (λ : 190-600 nm), an autosampler (injection volume 10 mL), and a column oven (35 °C). The separations were performed using a Synergi MAX-RP 100A (50 x 2 mm, 2.5 μ particle size) with H₂O (+0.1 % HCOOH) (A) /Acetonitrile (+0.1 % HCOOH) (B) gradient (flow rate 500 $\mu\text{L/min}$, injection volume 5 μL). Samples were analyzed using a gradient program as follows: 95 % A isocratic for 1.5 min, linear gradient to 100 % B over 6 min, after 100 % B isocratic for 2 min, the system returned to its initial condition (90 % A) within 1 min, and was equilibrated for 1 min.

Raw data files from the Bruker spectrometer MS were converted to a format compatible with our analysis software (.raw to .mzML). Spectral data (.mzML files) were imported and subjected to *ChromAnnot* (Bertrand et al., 2023; 2025) for annotation using a minimum cosine of 0.7, and an m/z tolerance of 10 ppm.

3. RESULTS AND DISCUSSION

3.1 Identification of Coprophilous fungi

Growth of coprophilous fungi on MEA was observed 10 to 30 days after incubation. Microscopic examination revealed distinct spore colonies, spore morphology, and septate and aseptate hyphae, as shown in Figure 1.

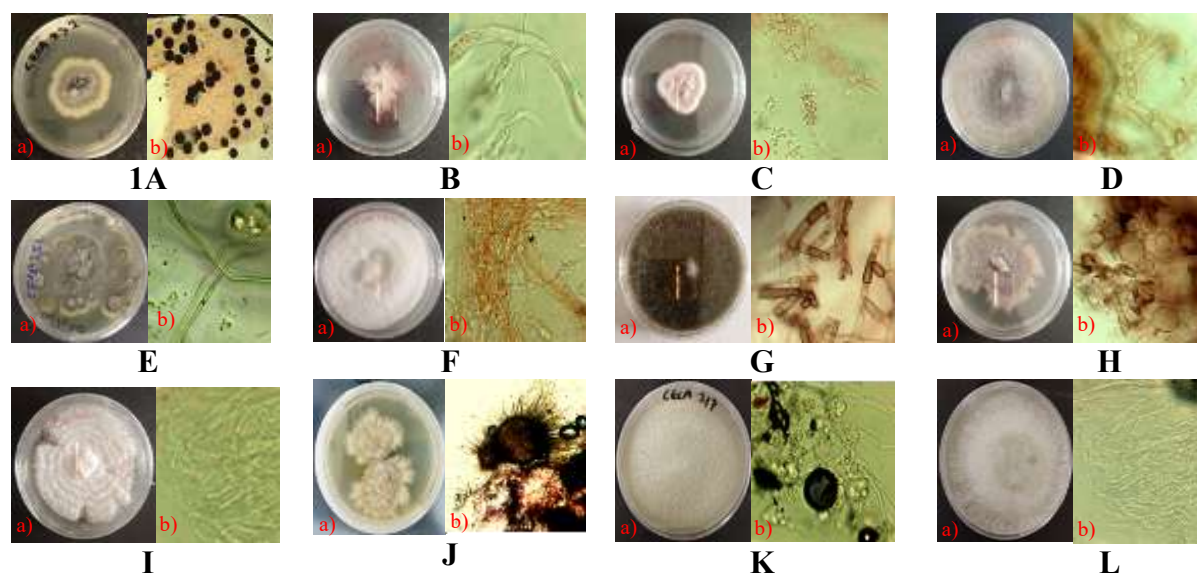


Figure 1. Morphological (a) and microscopic (b) observations of coprophilous fungi: A-*Scopulariopsis candida*; B-*Chaetomium madrasense*; C-*Purpureocillium lilacinum*; D-*Podospora bulbilosa*; E-*Aspergillus lanuginosus*; F-*Scopulariopsis brevicaulis*; G-*Papulaspora gansuensis*; H-*Cladorrhinum bulbilosa*; I-*Clonostachys epichloe*; J-*Chaetomium globosum*, K-*Gibberella fujikuroi*; L-*Clonostachys rosea*

In this study, all fungi isolated from cow dung were identified molecularly as members of the Ascomycota (Table 2), the phylum most frequently associated with animal dung (Richardson, 2003). This observation is consistent with previous reports, such as those of Lakshmi et al. (2015), who documented species of *Aspergillus*, *Fusarium*, and *Scopulariopsis* from cow dung in India. Similarly, the occurrence of *Chaetomium* and *Podospora* species in our samples aligns with the findings of Simões Calaça et al. (2020), who described *Chaetomium* species from dung substrates. Notably, *Chaetomium globosum* detected in our study has also been reported from soil and plant material (Sharma et al., 2025), highlighting its ecological versatility. Moreover, Wang (2000) reported 12 species of *Podospora* isolated from dung samples in Taiwan. The author also mentioned the work of several researchers related to the species of *Podospora* isolated in the dung samples of New Zealand.

To the best of our knowledge, our study reports the first isolation of *Purpureocillium lilacinum* from animal dung, whereas it has been described from soil and the rhizosphere of many crops (Prasad et al., 2015). Likewise, *Clonostachys epichloe* has been observed as a hyperparasite on the grass *Puccinellia distans* (Jacq.) (Górzyńska et al., 2023), and is also reported here for the first time as coprophilous fungi. In addition, *Clonostachys rosea*, an entomopathogenic fungus, and *Gibberella fujikuroi* are reported here for the first time from animal dung, although they had been isolated from the pupae of jujube fly (*Carpomya vesuviana*) (Mahmoudi et al., 2018) and from rice (*Oryza sativa*) (Karov et al., 2009), respectively.

Table 2. Molecular identification of fungal isolates

Code of strain	Species identified	Length	Identity	GenBank accession
CECA 179	<i>Scopulariopsis brevicaulis</i>	935	98.39 %	MT316372.1
CECA 184	<i>Papulaspora gansuensis</i>	540	100 %	MK252099.1
CECA 217	<i>Gibberella fujikuroi</i>	574	100 %	MW995793.1
CECA 147	<i>Scopulariopsis candida</i>	643	99.83 %	KP132441.1
CECA 174	<i>Cladorrhinum bulbiliosum</i>	535	99.22 %	KT321070.1
CECA 163	<i>Chaetomium madrasense</i>	939	93.74 %	OB0748941
CECA 164	<i>Clonostachys epichloe</i>	545	100 %	KJ780769.1
CECA 178	<i>Clonostachys rosea</i>	580	99.63 %	OR810514.1
CECA 188	<i>Purpureocillium lilacinum</i>	584	99.83 %	KJ207400.1
CECA 180	<i>Aspergillus lanuginosus</i>	604	92.76 %	NR184866.1
CECA 189	<i>Podospora bulbilosa</i>	1109	96.25 %	MK926861.1
CECA 170	<i>Chaetomium globosum</i>	948	100 %	OL741814.1

3.2 Antimicrobial activity and compound annotation

As summarized in Table 3, the tested extracts exhibited varying degrees of antimicrobial activity with MIC values ranging from 125 to 1000 µg/mL. The extracts of *Scopulariopsis brevicaulis*, *Papulaspora gansuensis*, *Podospora bulbillosa*, *Clonostachys epichloe*, *Clonostachys rosea*, and *Purpureocillium lilacinum* showed moderate antibacterial activities with MIC values of 125 µg/mL each. Previous investigations of species of these genera corroborate the present investigation. In fact, the study by Yu et al. (2008) identified two novel cyclodepsipeptides (scopularides A and B) from *Scopulariopsis brevicaulis*, which showed weak activity against Gram-positive bacteria. Likewise, Ramos et al. (2010) reported that the crude extract of *Papulaspora immersa* inhibited the growth of *S. aureus*, *K. rhizophila*, *P. aeruginosa*, and *E. coli*. The most relevant results were observed for the ethyl acetate extract of *P. immersa* against *P. aeruginosa* (90 µg/mL). Che et al. (2001) reported the antibacterial activity of two compounds, viz. anserinone A and B, derived from *Podospora anserina*. In addition, the extract of *P. lilacinum* has been reported to exhibit antibacterial activity against *B. cereus*, *E. coli*, and *P. stuartii*, with MICs below 62.6 µg/mL (Lenta et al., 2016). Previous studies have highlighted the antibacterial activity of compounds derived from *Clonostachys* species (Han et al., 2020), including the isolation of bisorbicillinoids from *Clonostachys rosea*, which exhibited potent antibacterial activity (Zhai et al., 2016). Similarly, several studies have reported the isolation of antibacterial constituents from *Purpureocillium* species (Chen and Hu, 2021), including an ergochrome from *P. lilacinum* exhibiting antibacterial activity against *E. coli* ATCC879 (Lenta et al., 2016). It is worth mentioning that the antibacterial activities of the extracts of *Scopulariopsis brevicaulis*, *Papulaspora gansuensis*, *Podospora bulbillosa*, and *Clonostachys epichloe* are reported herein for the first time.

Regarding antifungal activity, most fungal extracts were inactive against the pathogenic fungus *C. albicans*, whereas extracts of *P. gansuensis* and *C. epichloe* exhibited good activity, with MIC values of 125 and 250 µg/mL, respectively. Moreover, extracts of *S. brevicaulis* and *C. rosea* exhibited moderate activity, with a MIC of 500 µg/mL. *Clonostachys epichloe* has been reported for its antifungal activity while acting as a hyperparasite on the fungus *Epichloe typhina* (Górzyńska et al., 2023). *Papulaspora* sp. isolated from herbivores' dung was reported to inhibit the growth of *Fusarium oxysporum* (Moghalles et al., 2014). The antifungal activity of *C. rosea* against plant pathogens has been extensively documented (Jaffer et al., 2025). All these strains are reported herein for the first time with their activity against *C. albicans*.

Overall, the results of this study demonstrate that extracts from coprophilous fungi possess antimicrobial properties, thereby justifying further investigation into their chemical constituents.

Table 3. Antimicrobial activities of some coprophilous strains

S/N	Sample codes	Name of strains	Minimum Inhibitory Concentrations (MIC) (µg/mL) *		
			<i>Candida albicans</i> ATCC 14516	<i>Staphylococcus aureus</i> ATCC 43300	<i>Escherichia coli</i> ATCC 25922
1	CECA 179	<i>Scopulariopsis brevicaulis</i>	500	125	125
2	CECA 184	<i>Papulaspora gansuensis</i>	125	125	125
3	CECA 217	<i>Gibberella fukikuroi</i>	-	125	125
4	CECA 147	<i>Scopulariopsis candida</i>	-	1000	1000
5	CECA 174	<i>Cladorrhinum bulbilosum</i>	-	500	500
6	CECA 170	<i>Chaetomium globosum</i>	1000	125	500
7	CECA 163	<i>Chaetomium madrasense</i>	-	500	500
8	CECA 164	<i>Clonostachys epichloe</i>	250	125	125
9	CECA 178	<i>Clonostachys rosea</i>	500	250	500
10	CECA 188	<i>Purpureocillium lilacinum</i>	-	125	125
11	CECA 180	<i>Aspergillus lanuginosus</i>	-	250	125
12	CECA 189	<i>Podospora bulbillosa</i>	-	125	125
	Ciprofloxacin	Control sample 1	/	0.031	0.031
	Amphotericin B	Control sample 2	0.625	/	/

*: The values are means of duplicates; -: not determined, significantly active (100 ≤ MIC ≤ 512 µg/mL), moderately active (512 < MIC ≤ 2048 µg/mL), low activity (MIC > 2048 µg/mL), and not active (MIC > 10 mg/mL) Tamokou et al. (2017)

3.3 Qualitative chemical profiling and annotation

Fungal extracts with both antibacterial and antifungal properties were selected for chemical profiling and compound annotation. Thus, the extracts of *S. brevicaulis*, *P. gansuensis*, *C. epichloe*, and *C. rosea* were selected for chemical investigation. The UHPLC-MS/MS profiles of the extracts, recorded in negative ionization mode, are presented in Figure 2.

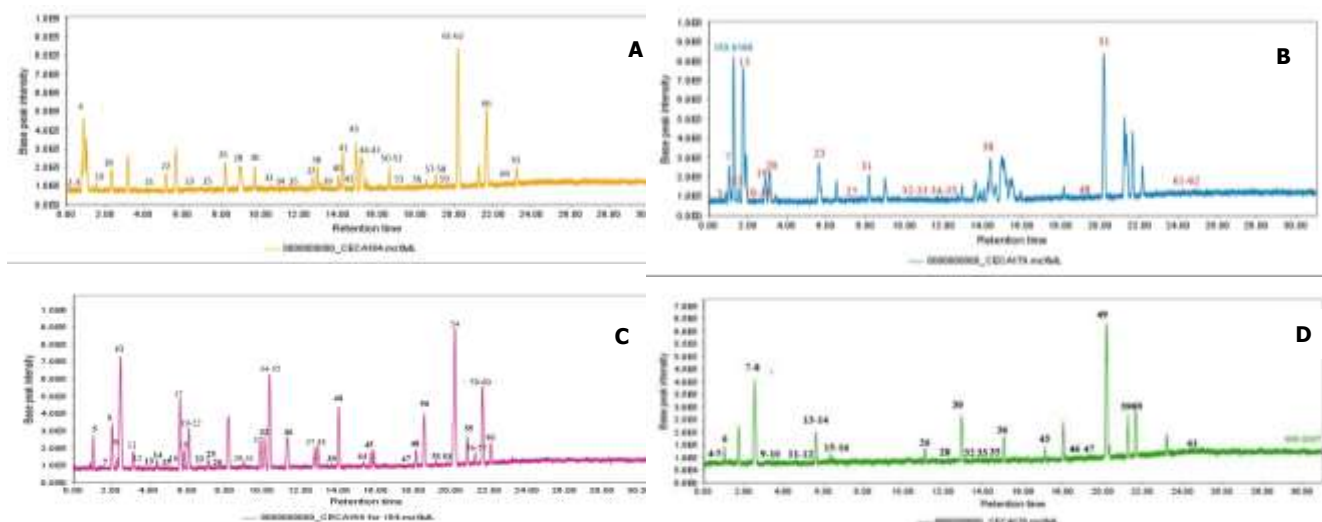


Figure 2. Total ion chromatograms (TICs) recorded in negative ionization mode for the crude extracts of: A. *Papulaspora gansuensis*, B. *Scopulariopsis brevicaulis*, C. *Clonostachys epichloe*, D. *Clonostachys rosea*

From the extract of *P. gansuensis*, a total of 28 compounds were annotated (Table S1), with alkaloids, terpenoids, and fatty acids representing the most abundant chemical classes. Global Natural Product Social-based molecular networking combined with cytoscape visualization enabled clustering and depiction of the relative distribution of these compounds (Figure 3). Notably, this is the first report of the chemical constituents of the genus *Papulaspora*, thereby expanding the chemical diversity known from coprophilous fungi.

Several of the annotated metabolites are structurally linked to previously reported antimicrobial compounds, which may explain the observed bioactivity of the extract. For instance, oct-2-ene-4,6-diynoic acid methyl ester (**18**), a polyacetylene derivative, belongs to a structural class widely documented in plants and fungi for broad-spectrum antimicrobial, antibiofilm, and quorum-sensing inhibitory activities (Samra and Abdel, 2026). Ricinoleic acid derivatives (**61**) have been reported to exhibit potent antimicrobial activity comparable to sorbic acid A (Novak et al., 1961), while spiroxamine (**52**) is patented as a fungicide.

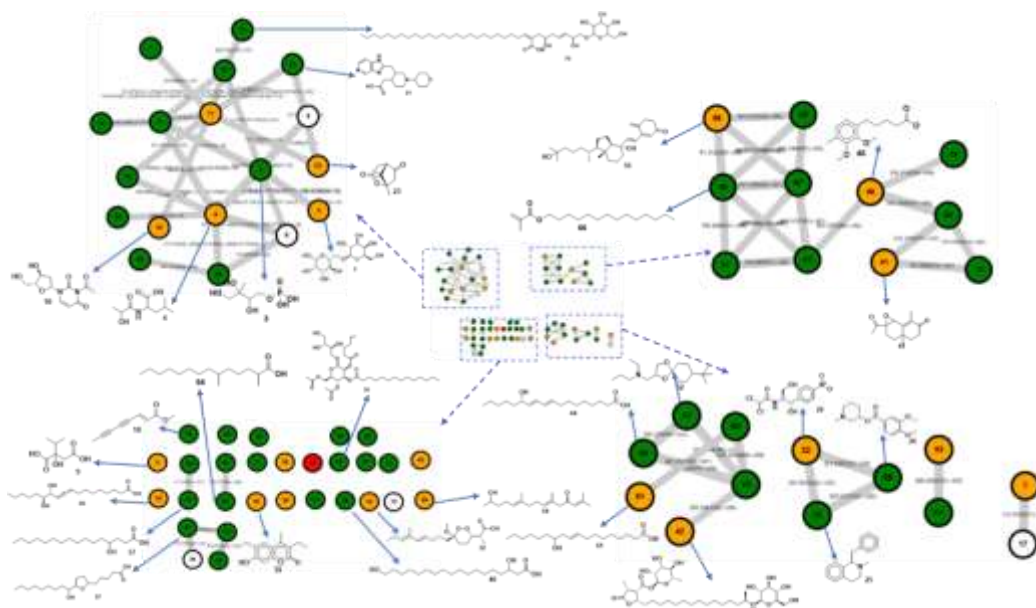


Figure 3. Molecular networking of *Papulaspora gansuensis* extract

Twenty-two compounds were annotated from the extract of *Scopulariopsis brevicaulis* (Table S2; Figure 4), with nucleosides, phenolic compounds, and fatty acids being the most abundant classes of compounds. This genus is known to produce a wide range of bioactive secondary metabolites, including cyclodepsipeptides, alkaloids, xanthenes, naphthoquinones, terpenoids, alkenoic acids, and diketopiperazines with antibacterial, antifungal, and antiviral effects (Tchokokam et al., 2026). In the present study, compounds with reported antimicrobial activities were identified, supporting the observed bioactivity. Specifically, uridine (**3**) and 5'-O-acetyluridine (**7**) have been reported for their antibacterial properties (Munia et al., 2023). Caffeic acid (**13**) is widely recognized as an alternative strategy to combat microbial pathogenesis and chronic infections caused by bacteria, fungi, and viruses (Khan et al., 2021). In addition, ricinoleic acid (**38**) was reported to exhibit potent antimicrobial activity (Jalageri et al., 2025).

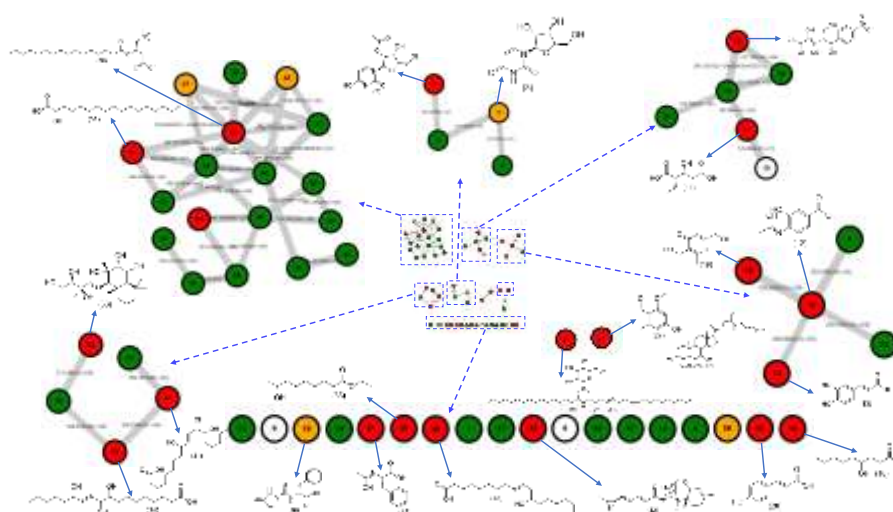


Figure 4. Molecular networking of *Scopulariopsis brevicaulis* extract

From the extracts of *C. epichloë* and *C. rosea*, 15 and 25 compounds were annotated, respectively (Table S3 and S4; Figures 5 and 6), with nitrogen-containing metabolites and fatty acids as the most abundant classes. Previous chemical investigations of *Clonostachys* species have revealed numerous secondary metabolites with antimicrobial and antiparasitic activities (Han et al., 2020; Kapche et al., 2025). In the current study, compounds with previously reported antimicrobial properties were annotated from both species, confirming the bioactivity of their extracts. In *C. epichloë*, metabolites such as 3,3-dimethylpentanedioic acid (7), azelaic acid (17), and nonactic acid (27) (Figure 5) are known for their antimicrobial activities (Devereux et al., 2003; Bojar et al., 1991; Kusche et al., 2009). Similarly, in *C. rosea*, derivatives of adipic acid (4) and sebacic acid (16) (Figure 6) have been reported for their antimicrobial effects (Irfan et al., 2022; Shen et al., 2021).



Figure 5. Molecular networking of the extract of *Clonostachys epichloë*

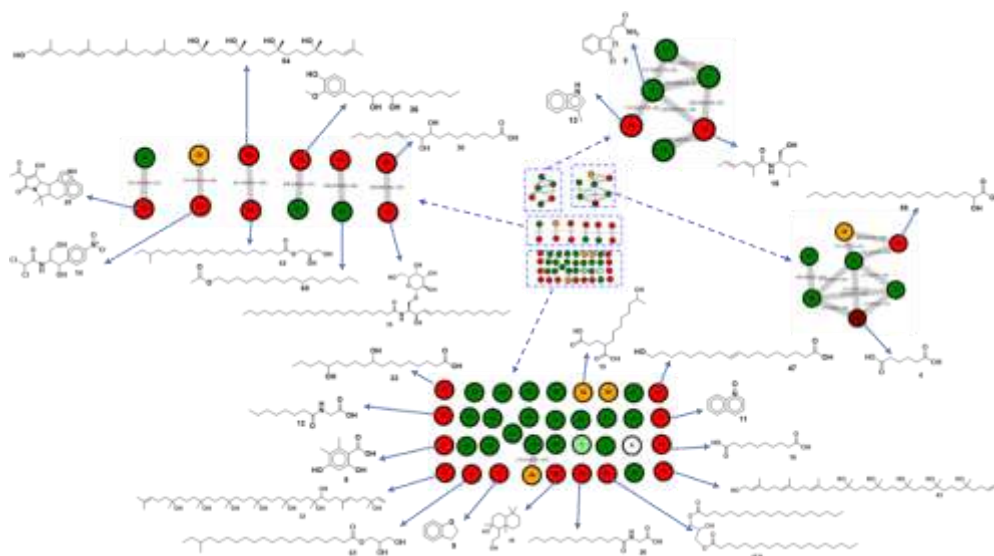


Figure 6. Molecular networking of the extract of *Clonostachys rosea*

4. CONCLUSION

This study investigated the antimicrobial activity of twelve coprophilous fungi isolated from cow dung samples collected in the Mezam Division of the North West Region of Cameroon. The extracts displayed varying degrees of antimicrobial potential, with *Papulaspora gansuensis* showing the most pronounced antibacterial and antifungal effects. Interestingly, the presence of *Purpureocillium lilacinum*, *Clonostachys epichloë*, *Clonostachys rosea*, and *Gibberella fujikuroi* in animal dung is reported here for the first time, thereby expanding the known ecological niche of these species. Chemical profiling of the most active extracts revealed a

remarkable chemical richness, with structurally diverse metabolites including compounds previously documented for the antimicrobial activity. These findings provide valuable insights into the diversity and bioactivity of coprophilous fungi in African ecosystems and highlight their potential as sources of novel antimicrobial agents.

Nevertheless, the use of crude extracts and a limited panel of in-house test strains represents an important constraint, as such preliminary screenings cannot reliably predict therapeutic relevance. The complexity of crude mixtures may mask synergistic or antagonistic interactions, and the restricted microbial spectrum may overlook strain-specific activity. Therefore, future work should prioritize purification and structural elucidation of the active constituents, followed by mechanistic investigations, toxicity assessment, and activity-guided isolation. Such comprehensive studies are essential they will more robustly position these fungi as promising contributors to the search for new antimicrobial agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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