

Antimicrobial and Antidermatophytic Dihydroisocoumarins and Picolinic Acids Produced by Endophytic Fungus *Fusarium napiforme* of *Faurea saligna* Harv.

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Abstract

Fungal metabolites are reservoirs of bioactive molecules that impart therapeutic potential to medicinal plants. The fungus *Fusarium napiforme* was isolated from the internal tissues of the leaves of *Faurea saligna*. In this study, four dihydroisocoumarin: 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-9,11-dienylisochroman-1-one (**1**), 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (**2**), 7-butyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (**3**), 7-butyl-6,8-dihydroxy-3(R)-pentylisochroman-1-one (**4**) and two picolinic acid derivatives: dehydrofusaric acid (**5**) and fusaric acid (**6**) were isolated from endophytic fungus *F. napiforme*. The elucidation of the chemical structures of the isolated compounds was performed by analysis based on spectroscopic analysis and comparison with reported literature. Compounds **1-6** were tested for antimicrobial and antidermatophytic activity against selected bacteria and fungi. All the compounds exhibited selective antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with a minimum inhibitory concentration (MIC) range of 62 – 175 µg/mL. Compounds **5** and **6** further exhibited antibacterial activity against *Klebsiella pneumoniae* with MIC value of 150 µg/mL. None of the compounds showed activity against *Pseudomonas aeruginosa*. Furthermore, all the compounds exhibited antifungal activity against *Candida albicans* with MIC values ranging from 48 µg/mL to 150 µg/mL. Compounds **1-4** further exhibited antidermatophytic activity against *Trichophyton rubrum*, *Trichophyton benhamiae* and *Trichophyton interdigitale*, with minimum inhibitory concentration (MIC) values ranging from 33.3 µg/mL to 66.6 µg/mL. Compounds **5** and **6** did not show any antidermatophytic activity against the highest concentration of 66.6 µg/mL used. Although, these compounds have been previously identified, this is the first instance of their reporting in endophytic fungi *F. napiforme* isolated from *F. saligna*. These results provide promising baseline information for the potential use of the compounds of this endophytic fungi as an antimicrobial and antidermatophytic for the control of diseases.

Keywords: Antimicrobial; antidermatophytic; Dihydroisocoumarins; Picolinic acids; Endophytic fungi; *Fusarium napiforme*; *Faurea saligna* Harv.

INTRODUCTION

Fungi have been proven to be a prolific pool of bioactive and structurally unique secondary metabolites with promising pharmacological properties (Ibrahim et al., 2023; Kellogg & Raja, 2017; Mohamed et al., 2020). Endophytic fungi are kind of microorganisms, which colonize within a living plant without any obvious negative effects or external symptoms (Clay & Schardl, 2002; Li et al., 2021). They grow within their plant hosts and in this environment, the fungus and hosts engage in constant metabolic interaction (Rodrigo et al., 2021; Schulz et al., 2002). They have also been identified as a promising sources of new and secondary metabolites with very interesting properties in a broad range of

applications (Schulz et al., 2002). They are also a source of a diverse array of multidimensional bioactive secondary metabolites such as alkaloids, terpenoids, steroids, quinones, iso-coumarins, lignans, phenylpropanoids, phenols and lactones (Ahmed et al., 2023; Mao et al., 2021).

Faurea saligna Harv. is a small to medium-sized medicinal plant that occurs naturally in bushland and woodland in tropical Africa (Maroyi, 2023). Different parts of the plant have been used in the ethno medicinal preparation for the treatment of different human diseases (Amuka et al., 2015) such as bilharziasis and helminthiasis (Chimponda & Mukanganyama, 2010), diarrhea (Hamill et al., 2003; Mahavy et al., 2020; Masevhe et al., 2015; Oosthuizen et al., 2019), epilepsy

(Masevhe et al., 2015; Stafford et al., 2008; Tshikalange et al., 2016), venereal diseases (Lawal et al., 2019) and pneumonia (Nyagumbo et al., 2022).

Fusarium is a widely distributed group of filamentous ascomycetes (Summerell, 2019), which are widely abundant in soil, subterranean and aerial plant parts, plant debris and other organic substrates (Nucci & Anaissie, 2007). They are considered to be a rich source of bioactive compounds such as butenolides, alkaloids, terpenoids, cytochalasins, phenalenones, xanthenes, sterols, and diphenyl ether and anthraquinones derivatives, with multidimensional bioactivities such as antimicrobial, antiviral, anticancer, antioxidant, antiparasitic and immunomodulating activity (Ahmed et al., 2023; Singh et al., 2021). *Fusarium napiforme* has been firstly isolated from millet and sorghum from southern Africa, and soil debris from one grassland in Australia (Supratman et al., 2021). In the study presented here, the isolation of internal tissues of the leaves of *F. saligna* led to the isolation of endophytic fungi *F. napiforme* and the chemical investigation of an EtOAc extract of *F. napiforme* culture led to the isolation of four dihydrocoumarins and two picolinic acids.

Dihydroisocoumarins (1*H*-2-benzopyran-1-ones or isocoumarin derivatives) are isomers of coumarins that have a reversed α -pyrone ring moiety in their structures that might possess the 6,8-dioxygenated pattern, 3-(un)substituted phenyl ring or 3-alkyl chain (C1-C17) (Pal et al., 2011). They represent a large class of secondary metabolites, which can be found in bacteria, fungi, lichens, marine sponges and to a lesser extent in higher plants (Chen et al., 2019; Sachs et al., 2019; Saeed, 2016). These natural products are well known for their biological activities including cytotoxins, anti-virus and antimicrobial activities (Saeed, 2016; Xu et al., 2021). 5-alkylpicolinic acids are a major group of secondary metabolites biosynthesized by fungi in the genus *Fusarium*. Numerous studies have shown that picolinic acids possess many important biological functions including antifungal (Huang et al., 2020; Li-jian et al., 2010), antibacterial (Thanh et al., 2020; Zhang et al., 2021), antimycobacterial (Pan et al., 2011), antiviral and cytotoxic (Li et al., 2013; Mamur et al., 2020; Seçme et al., 2023) activities. In addition, these natural products serve as exceptional frameworks for the development of novel and potent pharmaceuticals (Newman & Cragg, 2020).

This paper therefore presents the isolation and structural elucidation of four dihydroisocoumarins and two picolinic acids from fungus *F. napiforme* isolated from the leaves of *F. saligna* Harv. Antibacterial and antifungal assays were performed on gram positive bacterium *Staphylococcus aureus* (ATCC 6538), Gram negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumoniae* (ATCC 10031), and yeast *Candida albicans* (ATCC 10231). Antidermatophytic assays were

performed on *Trichophyton rubrum* (CCF 4933), *Trichophyton benhaniae* (CCF 6474) and *Trichophyton interdigitale* (CCF 6575). This is the first report of isolation of compounds **1** – **6** from endophytic fungus *Fusarium napiforme* of *F. saligna* Harv. and the first isolation of endophytic fungi *F. napiforme* from the leaves of *F. saligna*. However, it should be noted that compounds **5** (Dehydrofusaric acid) and **6** (fusaric acid) have been isolated before from other species of *Fusarium*.

MATERIALS AND METHODS

General experimental procedures.

NMR spectra were recorded on a Bruker Avance II 400 (resonance frequencies 400 MHz for ^1H and 100 MHz for ^{13}C) equipped with a 5 mm N₂-cooled cryo probe head (prodigy) with z-gradients at room temperature with standard Bruker pulse programs. Chemical shifts are given in parts per million, referenced to residual solvent signals (3.31 ppm for ^1H and 49.0 for ^{13}C) and coupling constants calculated in Hertz (Hz). ^{13}C -Jmod spectra with WALTZ16 ^1H decoupling was acquired using 64k data points.

HPLC analysis were done on Shimadzu preparative HPLC on a reversed phase column Gemini NX C-18 (21.20 $\times$ 150 mm, 5 μm , 110Å). The eluents were solvent A: deionized water and solvent B: Methanol. LC-HRMS/MS were recorded on a Vanquish LC system coupled to a QExactive HF, Thermo Fischer scientific via C-18-RP using a Gemini[®] NX-C18 column (5 μm , 110Å, 150 $\times$ 2 mm, Phenomenex, Torrance, CA, USA). The UHPLC-system was coupled to a QExactive HF Orbitrap mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) via a heated ESI interface operating in fast-polarity switching mode (positive/negative ionization). For Column chromatography, silica gel 60 (0.063-0.2 mm), Macherey-Nagel were used.

Fungal material

Fresh leaves of *F. saligna* were collected from Mt. Elgon Forest (1.1493° N, 34.5418° E) in Kenya. The leaves were washed in running water and surface sterilized by successive soaking in 70% EtOAc for 1 min and sodium hypochlorite solution for 5 min and then rinsed twice with water. Aseptically, the leaves were cut into approximately 2 $\times$ 2 mm² pieces and placed on agar in petri dishes. After incubation at 25 °C, individual hyphal tips of the emerging fungi were removed and placed on potato dextrose agar (PDA). During the first two weeks of incubation, the cultures were periodically checked for purity and successively subcultured by hyphal tipping method until pure cultures were obtained. The isolated strain was classified as *Fusarium napiforme* based on the ITS rDNA sequence (Genbank accession no. MH 862670) and morphology using the procedures described in Kolařík et al. (2023). The assembling was done by

genious program and the BLAST search was done in the NCBI website. The strain is deposited in the Bioactive Microbial Metabolites (BiMM) Fungal Collection, UFT-Tulln in Austria.

Fermentation, Extraction and isolation

The fungal strain was cultured on potato dextrose agar plates at 25 °C for 10 days. Solid fermentation was done by weighing 90g of rice and added into 500 mL Erlenmeyer flasks containing 90 mL of distilled water and autoclaved twice at 121 °C for 20 mins and allowed to cool. On sterile conditions, a cork-borer of 7 mm was used to cut the fungal culture into several pieces and six plugs were inoculated into each flask containing sterile rice and incubated at 21 °C for 28 days. The fungal cultures in the flasks were then crushed into small pieces and soaked in methanol for 24 hours. After 24 hours it was ultrasonicated for about 2 hours and the methanol extract filtered and evaporated under reduced pressure using a rotary evaporator to obtain the methanol crude extract. The crude extract was then suspended in distilled water followed by sequential partitioning between hexane and ethyl acetate solvents. The obtained extracts were evaporated to obtain hexane and ethyl acetate crude extracts respectively. The ethyl acetate crude extract was then subjected to column chromatography (CC) over silica gel 60 and eluted with hexane/EtOAc and then EtOAc/MeOH mixtures of increasing polarities to give seven fractions (A-G). Fraction D (EtOAc 100%) and fraction G (EtOAc/MeOH) (20:80) was further purified using reversed-phase preparative HPLC using deionized H₂O as solvent A and Methanol as solvent B at a flow rate of 5 mL/min on a gradient method; 20 % - 55 % B in 15 minutes increasing to 85 % B in 10 minutes, then maintained at 85 % B for 10 minutes and the system returned to initial conditions of 20% in 10 minutes to afford six fractions (D1-D6) from fraction D and four fractions (G1-G4) from fraction G. gradient method yielded semi pure fractions, which were further purified on the same column on isocratic method. Compound **1** (2.1 mg) was obtained from fraction D2 with an isocratic elution of 40% solvent B for 20 minutes while compound **2** (2.9 mg), **3** (1.5 mg) and **4** (2.0 mg) was obtained from fraction D4 with an isocratic gradient of 50% solvent B for 20 minutes. Finally, compound **5** (1.7 mg) and compound **6** (2.5 mg) was obtained from G3 using isocratic gradient of 65% solvent B for 15 minutes.

Antimicrobial Assay

The antibacterial and antifungal activities (minimum inhibition concentration, MIC₅₀) were determined using serial dilution technique in 96-well microplate and measured with a synergy HI microplate reader at 600nm. The MIC values were defined as the lowest concentration of compounds exhibiting 50% inhibition compared to the negative growth control. Briefly, compounds were then dissolved and diluted in ethanol and in Muller Hinton media respectively. 200 µl of the stock solutions were

pipetted into the first row of a 96-well microtiter plate with 100 µl of Muller Hinton added to the remaining rows. Then 100 µl of Muller Hinton media aliquoted to all remaining rows. Next, 100 µl of the first row was pipetted to the second row, mixed by repeated pipetting and 100 µl of this mixture was transferred to row three. A serial dilution (in ration 1:1) was performed in the following rows with 100 µl casted off after the final row. The bacteria and fungi were grown overnight within Muller Hinton media, and the 600nm optical density was determined. For the MIC₅₀ determination, the cultures were freshly diluted to an OD₆₀₀ of 0.02 in Muller Hinton media. 100 µl of these inoculi were pipetted onto the media containing the compounds. After inoculation, the 'breathe – easy' membrane was used to seal the plates and placed into a synergy HI plate reader (Souza et al., 2018). The plates took 24 hours in incubation at 37°C, with measurements of OD₆₀₀ taken every hour. The antimicrobial activity was expressed as the sample concentration capable of reducing micro-organisms growth by 50% (MIC₅₀). Nystatin was used as positive control while oxytetracycline was used as positive control.

Antidermatophytic activity

The method was adopted from Kolarczykova et al. (2024) and Štěpánek et al. (2022). Three dermatophyte strains were used (*T. benhamiae* CCF6474, *T. interdigitale* CCF6575, *T. rubrum* CCF4933). Strains were cultivated on SDA (Sabouraud Dextrose agar, Trios, Prague, Czech Republic) for 1-2 weeks at 25°C. The inoculum suspensions were prepared in sterile distilled water supplemented with 0.1% Tween 20. To adjust the density of the suspension to 0.5 McFarland (concentration of $2-5 \times 10^6$ conidia mL⁻¹), distilled water was added and the resulting density was checked using the densitometer DEN-1B (BIOSAN, Riga, Latvia). The suspension was then diluted 1:10 with liquid YM6 medium to obtain a final working inoculum of $2-5 \times 10^5$ CFU mL⁻¹. All compounds were dissolved in methanol (98%) and tested in the concentration range 66.6-0.52 µg. Terbinafine was used as a standard. The microplates with YM6 medium were incubated at 25°C in ambient air for 5 days. The endpoints (MICs) were read visually as 50% inhibition. The toxicity of the methanol was considered in the experiments.

RESULTS AND DISCUSSION

Elucidation of the compounds

Six compounds were isolated from the fungus *Fusarium napiforme* and characterized based on their spectral data and literature comparison. Among the isolated compounds, compounds **1** to **4** were identified as dihydroisocoumarins and compounds **5** and **6** as picolinic acids derivatives (see Fig. 1). These compounds were confirmed as: 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-

9,11-dienylisochroman-1-one (**1**) (Tabopda et al., 2009), 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (**2**) (Kim et al., 2018; Kongsaree et al., 2003), 7-butyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (**3**) (Hoque et al., 2022; Kim et al., 2018; Kongsaree et al., 2003; Tabopda et al., 2009), 7-butyl-6,8-dihydroxy-3(R)-pentylisochroman-1-one (**4**) (Kim et al., 2018; Kongsaree et al., 2003), dehydrofusaric acid (**5**) (Boonman et al., 2012; Chen et

al., 2026) and fusaric acid (**6**) (Boonman et al., 2012; Chen et al., 2026).

These compounds have been extracted from diverse endophytic fungi isolated from diverse medicinal plants. However, it is important to highlight that, to the best of our knowledge, this represents the first documentation of the isolation of fungus *Fusarium napiforme* from the leaves of *F. saligna* and the first isolation of compounds **1-6** from fungus *F. napiforme*.

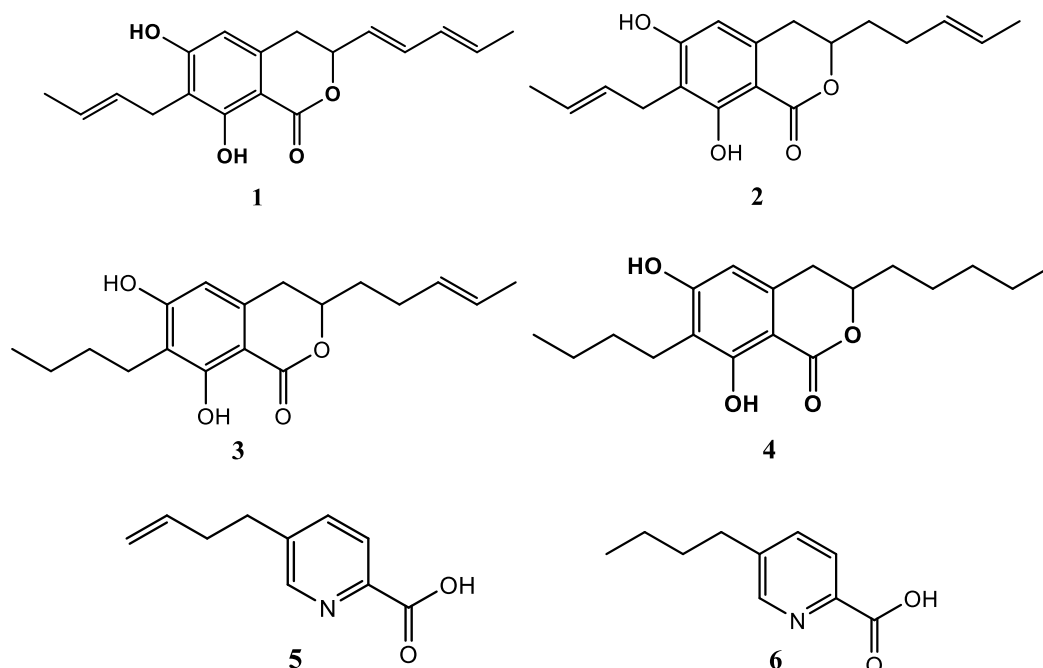


Figure 1. Dihydroisocoumarins (**1- 4**) and picolinic acids (**5 and 6**) derivatives isolated from fungus *F. Napiforme*.

Physicochemical and spectrometric data of the isolated compounds

Compound 1: This compound was isolated as a white amorphous powder (MeOH); UV (MeOH), λ_{max} 220, 276, 315 nm; HRESIMS m/z 301.14277 $[M + H]^+$, corresponding to the molecular formula $C_{18}H_{20}O_4$ (calculated for 301.14344); ^1H NMR (CD_3OD , 400 MHz) (supplementary figure S1) δ_H : 6.39 (dd, $J = 10.4$, 15.3 Hz, H-10), 6.21 (s, H-5), 6.09 (m, H-11), 5.83 (m, H-12), 5.69 (dd, $J = 6.8$, 15.0 Hz, H-9), 5.51 (m, H-15), 5.44 (m, H-16), 5.02 (dd, $J = 7.1$, 14.1 Hz, H-3), 3.24 (d, $J = 6.0$ Hz, H-14), 2.90 (d, $J = 6.9$ Hz, H-4), 1.76 (dd, $J = 1.4$, 6.7 Hz, H-13), 1.59 (dd, $J = 1.4$, 6.1 Hz, H-17); ^{13}C NMR (CD_3OD , 100 MHz) (supplementary figure S2) δ_C : 171.9 (C-1), 166.1 (C-8), 163.1 (C-6), 139.7 (C-4a), 135.2 (C-10), 132.9 (C-12), 131.7 (C-11), 129.9 (C-15), 127.9 (C-9), 125.5 (C-16), 114.5 (C-7), 108.2 (C-5), 100.5 (C-8a), 80.5 (C-3), 34.3 (C-4), 26.4 (C-14), 18.3 (C-13), 18.0 (C-17). After comparison with literature compound **1** was determined as 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-9,11-dienylisochroman-1-one (Tabopda et al., 2009).

Compound 2: This compound was isolated as a white amorphous powder (MeOH); UV (MeOH), λ_{max} 223, 273, 307 nm; HRESIMS m/z 303.15884 $[M+H]^+$, corresponding to molecular formula $C_{18}H_{22}O_4$ (calculated for 303.15909); ^1H NMR (CD_3OD , 400 MHz) (supplementary figure S5): δ 6.21 (s, H-5), 5.49 (m, H-15), 5.47 (m, H-12), 5.46 (m, H-11), 5.43 (m, H-16), 4.45 (m, H-3), 3.23 (d, $J = 6.0$ Hz), 2.79 (m, H-4), 2.18 (m, H-10), 1.82 (m, H-9a), 1.71 (m, H-9b), 1.62 (dd, $J = 1.0$, 5.9 Hz), 1.57 (dd, $J = 1.3$, 6.0 Hz); ^{13}C NMR (CD_3OD , 100 MHz) (supplementary Figure S6): δ 172.2 (C-1), 164.4 (C-6), 163.1 (C-8), 140.2 (C-4a), 131.1 (C-11), 129.7 (C-15), 126.9 (C-12), 125.7 (C-16), 114.3 (C-7), 107.4 (C-5), 100.8 (C-8a), 80.2 (C-3), 35.7 (C-9), 33.7 (C-4), 28.9 (C-10), 26.4 (C-14), 18.1 (C-13), 18.0 (C-17). After comparison with literature, compound **2** was determined as 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (Kim et al., 2018; Kongsaree et al., 2003).

Compound 3: this compound was isolated as white amorphous powder (MeOH); UV (MeOH), λ_{max} 220, 274 nm; HRESIMS m/z 305.17420 $[M+H]^+$,

corresponding to molecular formula $C_{18}H_{24}O_4$ (calculated for 305.17474); ^1H NMR (CD_3OD , 400 MHz) (supplementary figure S9): 6.21 (s, H-5), 5.48 (m, H-12), 5.47 (m, H-11), 4.47 (m, H-3), 2.82 (m, H-4), 2.59 (t, $J = 7.4$ Hz, H-14), 2.19 (m, H-10), 1.84 (m, H-9a), 1.73 (m, H-9b), 1.64 (dd, $J = 1.0, 5.9$ Hz, H-13), 1.48 (m, H-15), 1.35 (m, H-16), 0.91 (t, $J = 7.3$ Hz, H-17); ^{13}C NMR (CD_3OD , 100 MHz) (supplementary figure S10): 172.2 (C-1), 163.8 (C-6), 163.2 (C-8), 139.9 (C-4a), 131.1 (C-11), 126.9 (C-12), 116.0 (C-7), 106.9 (C-5), 101.2 (C-8a), 80.2 (C-3), 33.7 (C-4), 35.7 (C-9), 32.0 (C-15), 28.9 (C-10), 23.8 (C-16), 23.1 (C-14), 18.1 (C-13), 14.2 (C-17). After comparison with literature compound **3** was determined as 7-butyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (Hoque et al., 2022; Kim et al., 2018; Kongsaree et al., 2003; Tabopda et al., 2009).

Compound 4: This compound was isolated as white amorphous powder (MeOH); UV (MeOH), λ_{max} 217, 269 nm: HRESIMS m/z 307.19009 $[M+H]^+$, corresponding to molecular formula $C_{18}H_{26}O_4$ (calculated for 307.19039); ^1H NMR (CD_3OD , 400 MHz) (supplementary figure S13): 6.18 (s, H-5), 4.47 (m, H-3), 2.80 (m, H-4), 2.57 (t, $J = 7.4$ Hz, H-14), 1.79 (m, H-9a), 1.69 (m, H-9b), 1.55 (m, H-11), 1.47 (m, H-15), 1.35 (m, H-16), 1.34 (m, H-10, H-12), 0.93 (m, H-17), 0.91 (t, $J = 7.3$ Hz, H-13); ^{13}C NMR (CD_3OD , 100 MHz) (supplementary figure S14): 172.4 (C-1), 165.8 (C-6), 163.3 (C-8), 139.9 (C-4a), 116.2 (C-7), 107.9 (C-5), 100.3 (C-8a), 80.2 (C-3), 35.9 (C-9), 33.9 (C-4), 32.7 (C-10), 32.1 (C-15), 25.7 (C-11), 23.8 (C-12), 23.6 (C-16), 23.2 (C-14), 14.4 (C-17), 14.3 (C-13). After comparison with literature compound **4** was determined as 7-butyl-6,8-dihydroxy-3(R)-pentylisochroman-1-one (Kim et al., 2018; Kongsaree et al., 2003).

Compound 5: this compound was isolated as a yellow amorphous solid (MeOH); UV (MeOH), λ_{max} 202, 225, 273 nm: HRESIMS m/z 178.0790 $[M+H]^+$, corresponding to molecular formula $C_{10}H_{11}NO_2$; ^1H NMR (CD_3OD , 400 MHz) (supplementary figure S17): 8.52 (s, H-6), 8.08 (d, $J = 8.2$ Hz, H-3), 7.87 (dd, $J = 8.0, 2.1$ Hz, H-4), 5.86 (ddt, $J = 17.0, 10.2, 6.7$ Hz, H-9), 5.00 (m, H-10), 2.85 (t, $J = 7.6$ Hz, H-7), 2.43 (q, $J = 7.1$ Hz, H-8); ^{13}C NMR (CD_3OD , 100 MHz) (supplementary figure S18): 168.1 (C-11), 149.7 (C-6), 147.9 (C-2), 142.9 (C-5), 139.7 (C-4), 138.1 (C-9), 125.9 (C-3), 116.4 (C-10), 35.9 (C-8), 33.2 (C-7). After comparison with literature compound **5** was determined as dehydrofusaric

acid (Boonman et al., 2012; Capasso et al., 1996; Chen et al., 2026; Liu et al., 2026)

Compound 6: This compound was isolated as a yellow amorphous solid (MeOH); UV (MeOH), λ_{max} 202, 225, 270 nm: HRESIMS m/z 180.10147 $[M+H]^+$, corresponding to molecular formula $C_{10}H_{13}NO_2$; ^1H NMR (CD_3OD , 400 MHz) (supplementary figure S20): 8.50 (s, H-6), 8.12 (d, $J = 8.0$ Hz, H-3), 7.95 (dd, $J = 8.0, 2.1$ Hz, H-4), 2.77 (t, $J = 7.7$ Hz, H-7), 1.67 (m, H-8), 1.40 (m, H-9), 0.97 (t, $J = 7.3$ Hz, H-10); ^{13}C NMR (CD_3OD , 100 MHz) (supplementary figure S21): 167.0 (C-11), 149.1 (C-6), 146.6 (C-2), 144.6 (C-5), 140.2 (C-4), 126.2 (C-3), 34.1 (C-8), 33.5 (C-7), 23.3 (C-9), 14.2 (C-10). After comparison with literature compound **6** was determined as fusaric acid (Boonman et al., 2012; Capasso et al., 1996; Chen et al., 2026).

Antibacterial and antifungal activity

Compounds **1-6** (table 1) were tested for their antibacterial and antifungal activity against four pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungus *Candida albicans*. All of the isolated isocoumarins compounds (**1-4**) showed selective activity against gram-positive bacterium *S. aureus*, gram-negative bacteria *E. coli* and fungus *C. albicans* with minimum inhibitory concentration (MIC) range of 48 $\mu g/mL$ to 135 $\mu g/mL$. *P. aeruginosa* and *K. pneumoniae* demonstrated resistance to all isocoumarins. Among the four isocoumarins compounds, compound **4** demonstrated the highest inhibitory activity, with a minimum inhibitory concentration (MIC) range of 48 – 95 $\mu g/mL$. Among the test strains, *C. albicans* exhibited greater susceptibility to the isocoumarins whereas *E. coli* demonstrated lower susceptibility to the isocoumarin compounds.

Picolinic compounds **5** and **6** also showed weak activity against *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans* with MIC range of 125 – 175 $\mu g/mL$. As compared to isocoumarins compounds which did not show any antibacterial activity against *K. pneumoniae*, picolinic acids **5** and **6** exhibited antibacterial activity against *K. pneumoniae* with MIC value of 150 $\mu g/mL$. No activity was seen against gram-negative bacteria *P. aeruginosa*. Compound **4** showed the most potent antimicrobial activity with MIC value of 95, 62 and 48 $\mu g/mL$ for *E. coli*, *S. aureus* and *C. albicans*, respectively.

Table 1. Antibacterial and antifungal activity of compounds **1-6** against selected clinically important pathogens.

Compounds	MIC concentrations ($\mu\text{g/mL}$)/ test organism				
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumonia</i>	<i>C. albicans</i>
1	120	85	n.i	n.i	95
2	135	95	n.i	n.i	85
3	125	110	n.i	n.i	60
4	95	62	n.i	n.i	48
5	130	125	n.i	n.i	150
6	150	175	n.i	n.i	125
Controls	4.0(T)	4.0(T)	4.0(T)	4.0(T)	3.1(N)

*n.i: No inhibition, T:tetracycline, N:nystatin; *The values are expressed as mean of three consistent replicates.

The antibacterial activity of dihydroisocoumarins compounds **1-4** has been assessed, demonstrating variability which could be influenced by their structural characteristics and mechanisms of action. Studies showed that the antibacterial activity of isocoumarins can be modulated by the presence of hydroxyl groups at position 6 and 8 together with alkyl substitutions at position 3 and 7 of the dihydroisocoumarins (Saeed, 2016). Moreover, the presence of OH groups in the molecules seems to enhance the inhibition of biofilm formation as well as the disruption of preformed biofilms (Alejo-Armijo et al., 2025). The presence of this hydroxyl groups on the benzene ring also enhances the lipophilicity of compounds and ability to disrupt bacterial membranes and proteins which may be associated with easier penetration into gram-positive bacteria (Devienne et al., 2005). As seen in this study, all the dihydroisocoumarins **1-4** had hydroxyl groups at position 6 and 8 which could have contributed to the activity shown against the gram-positive bacteria *S. aureus*. Other studies also showed that several dihydroisocoumarins have antimicrobial activity that in many cases is also due to the presence of a phenolic group in their structure (Braca et al., 2012). It has also been documented that coumarins proved to be slightly less active against Gram-negative bacteria, due to possibly particular physical properties ((Devienne et al., 2005; Kayser & Kolodziej, 1999).

Compound **5** and **6** isolated as picolinic acid also showed varying activity against selected bacteria and fungus. These compounds showed moderate to weak antibiotic effects, in accordance with other studies (Shi et al., 2018; Sondergaard et al., 2016; Thanh et al., 2020). They exerts antibacterial activity primarily through metal chelation and the disruption of bacterial cellular processes (Paterson et al., 2025). Research indicates that

the n-butyl side chain is responsible for increasing the lipophilicity and enables it to penetrate cell membranes or potentially insert into bacterial membranes and influence cell permeability (Arumugam et al., 2021; Paterson et al., 2025). Previous studies have also linked fusaric acid to fungal membrane disruption (Macías-Rubalcava et al., 2025). Other studies have shown that the structure-activity relationship analysis showed that a carboxyl on C-2 and an n-butyl on C-5 were essential for fusaric acid and dehydrofusaric acid to elicit antibacterial activity (Tung et al., 2017). It is worth noting that the fusaric acid and dehydrofusaric acid have been isolated from various endophytic fusarium species (Li et al., 2020; Liu et al., 2016; Zhou et al., 2019). In this study, fusaric acid and dehydrofusaric acid showed antifungal activity against *C. albicans* with MIC values of 150 and 125 $\mu\text{g/mL}$. However, the antifungal mode of action of these picolinic acid derivatives are not yet fully understood (Macías-Rubalcava et al., 2025).

Antidermatophytic assay

The results obtained from the dihydroisocoumarins compounds **1-4** (table 2) exhibited a varying range of activity with minimum inhibitory concentration (MIC) values ranging from 33.3 $\mu\text{g/mL}$ to 66.6 $\mu\text{g/mL}$ against strains *T. rubrum*, *T. interdigitale* and *T. benhamiae*. Among the test strains, *T. interdigitale* demonstrated the greatest susceptibility specifically to compounds **1** and **3**, with a minimum inhibitory concentration (MIC) of 33.3 $\mu\text{g/mL}$. Among the four isocoumarins compounds, compound **2** demonstrated resistances to *T. benhamiae* at the highest concentration of 66 $\mu\text{g/mL}$. The picolinic acid (compound **5** and **6**) showed no inhibitory activity with all the three strains (*T. rubrum*, *T. interdigitale*, *T. benhamia*) used under the maximum concentration of 66 $\mu\text{g/mL}$ used (Table 2).

Table 2. Antidermatophytic activity of compounds **1-6** isolated from fungus *F. napiforme*.

Compounds	MIC Concentration (µg/mL)/ Test Organisms		
	<i>T. rubrum</i>	<i>T. benhamiae</i>	<i>T. interdigitale</i>
1	66.6	66.6	33.3
2	66.6	>66.6	66.6
3	66.6	66.6	33.3
4	66.6	66.6	66.6
5	>66.6	>66.6	>66.6
6	>66.6	>66.6	>66.6
Terbinafine	0.52	0.52	0.52

*The values are expressed as mean of three consistent replicates

Many isocoumarin derivatives are proven to possess a broad range of biological activities (Hussain & Green, 2017; Kumar et al., 2020; Xiao et al., 2016). The coumarins' antifungal potency varies depending on coumarin core substituents, and those with phenolic, hydroxyl, and the carboxylic group has been proven as strong antifungal agents (Lončar et al., 2023). The results obtained from this study indicate that isocoumarins contains hydroxyl groups at position 6 and 8 and showed antidermatophytic efficacy against the fungal pathogens *T. rubrum*, *T. benhamiae* and *T. interdigitale*. Moreover, several microorganisms, such as fungi and bacteria, have been shown to produce coumarin compounds through specific metabolic routes and their biological activity is associated with their ability to form noncovalent interactions with different active places in living organisms (Ji et al., 2016).

CONCLUSIONS

In summary, the present study resulted in the extraction of four dihydroisocoumarins **1-4** and two picolinic acids **5** and **6** from the fungus *F. napiforme* isolated from the leaves of *F. saligna*. The compounds demonstrated a diverse range of antibacterial, antifungal and antidermatophytic properties. 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-9,11-dienylisochroman-1-one (**1**), 7-butyl-15-enyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (**2**), 7-butyl-6,8-dihydroxy-3(R)-pent-11-enylisochroman-1-one (**3**) and 7-butyl-6,8-dihydroxy-3(R)-pentyloisochroman-1-one (**4**) have been reported for the first time in *F. napiforme*. Dehydrofusaric acid (**5**) and fusaric acid (**6**) have been isolated from various endophytic *Fusarium* species. Further studies including isolation of other associated endophytic fungi of *F. saligna* may result in some unknown metabolites of this type.

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