

Antioxidant and anti-inflammatory activities of endophytic *Fusarium* sp. isolate NN-Fus-1

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Abstract

Endophytic fungi are a valuable source of unprecedented bioactive metabolites of medical and pharmaceutical potential. In the current study, the fungal isolate NN-Fus-1 was selected following a screening process for bioactive crude extracts. The isolate was cultivated on a solid rice medium, and the culture was extracted with ethyl acetate. The fungal isolate was identified morphologically and via partial sequencing of the 18S rDNA. The isolate was identified as *Fusarium* sp. isolate NN-Fus-1F, and its sequence was deposited in GenBank. Its crude extract revealed antioxidant activity by scavenging DPPH radicals with an IC_{50} of 109.17 ± 1.30 μ g/ml (6.41 ± 0.08 μ g Trolox/mg crude extract) but not nitric oxide radicals. Although it did not stabilize the membrane of heat-induced RBCs, it showed anti-inflammatory potential by inhibiting heat-induced albumin denaturation ($IC_{50} = 469.05 \pm 3.72$ μ g/ml) and reducing lipooxygenase 5-LOX activity ($IC_{50} = 83.51 \pm 1.36$ μ g/ml) *in vitro*, as well as decreasing rat paw edema ($IC_{50} = 159.76 \pm 3.6$ μ g/g body weight) compared to untreated controls *in vivo*. The extract was ineffective against tested bacterial strains in the agar diffusion assay. *Fusarium* sp. isolate NN-Fus-1F crude extract comprised of alkaloids, phenolic derivatives, flavonoids, and organic acids as main constituents. The presence of these classes of compounds in the crude extract reflects its potential antioxidant and anti-inflammatory activities detected herein. *Fusarium* sp. isolate NN-Fus-1 is considered a candidate for secondary metabolites with medical and pharmaceutical applications.

Keywords: Anti-inflammatory, Antioxidant, Bioactive metabolites, Endophytes, *Fusarium* sp.

1. Introduction

Fungi have drawn the attention of several natural product scientists since the discovery of penicillin by Fleming in 1928 (Saxena *et al.*, 2024). Part of these microorganisms colonize the plant host's internal tissues or intracellular spaces without causing adverse effects (Al-Qaralleh *et al.*, 2021). The *Fusarium* genus is placed in the third level of plant-associated fungi, after the *Penicillium* and *Aspergillus* genera. It is a cosmopolitan filamentous fungus in the class Sordariomycetes (order: Hypocreales, family: Nectriaceae) with more than 70 species (Ahmed *et al.*, 2023). *Fusarium* species are widely distributed in various habitats, not only as phytopathogens but also found in soil, plants, insects, and animal substrates in terrestrial and marine environments (Amuzu *et al.*, 2024). They are reservoirs of novel compounds, including peptides, polyketides, quinones, steroids, terpenoids, alkaloids, flavonoids, and phenolic acids (Wei and Wu, 2020), metabolites that revealed unprecedented activities in different biological assays. Therefore, they are exploited as a rich source of novel bioactive metabolites, including anticancer, antioxidant, antibacterial, antiviral, antidiabetic, anti-inflammatory, and immunomodulatory agents (Singh *et al.*, 2021).

Three hundred ninety compounds from various *Fusarium* species were reported from 1999 to 2022; more

than 50% were isolated throughout 2017-2022, and 92 compounds (23%) were novel (Ahmed *et al.*, 2023), highlighting the importance of this genus as a source for natural bioactive metabolites. Among the reported bioactive metabolites derived from endophytic *Fusarium* species were the cytotoxic azaanthraquinone derivatives and the anti-inflammatory fusopolide A metabolite from *F. solani*, the antimicrobial aminobenzamide derivative fusarithioamide B from *F. chlamydosporum* (Wei and Wu, 2020), and Rohitukine alkaloid from *F. proliferatum* as an anti-cancer, anti-inflammatory, and immunomodulatory agent (Ahmed *et al.*, 2023).

Notably, some endophytes synthesized secondary metabolites previously known from their hosts (Yan *et al.*, 2018; Archana and Nagadesi, 2022), raising questions about the exact biosynthetic origin of these metabolites. For example, the anticancer drug Taxol, which was primarily isolated from the *Taxus brevifolia* plant (Pacific yew), was synthesized by the associated *Taxomyces andreanae* fungus (Stierle *et al.*, 1995). Similarly, the vinca alkaloids vinblastine and vincristine, identified in the Madagascar periwinkle plant *Catharanthus roseus*, are synthesized by some endophytic fungi, including *Fusarium oxysporum*, *Alternaria* sp., and *Nigrospora sphaerica*, which are fungal species associated with the same plant (Ayob *et al.*, 2017). These findings highlighted the potential of endophytes as prolific producers of

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pharmacologically active compounds, often in greater quantities than their host plants.

Inflammation is a protective response triggered by microbial infections, oxidative stress, or tissue injury (Mang *et al.*, 2018). It involves changes in vascular permeability, pain induction via inflammatory mediators (such as prostaglandins, kinins, and histamine), and tissue damage due to the release of lysosomal enzymes and protein denaturation (DiStasi and Ley, 2009). However, prolonged or excessive inflammation can lead to chronic disorders. Managing inflammation is achieved using non-steroidal anti-inflammatory drugs (NSAIDs); however, their side effects limit their therapeutic utility (Panchal and Sabina, 2023). This underscores the need for safer, naturally derived anti-inflammatory and antioxidant agents (Al-Zereini *et al.*, 2023). Accordingly, this study aims to screen endophytic fungi for crude extracts with potential antioxidant and anti-inflammatory properties.

2. Materials and Methods

2.1. Plant sample collection and isolation of the endophytic fungus NN-Fus-1

A healthy *Marrubium vulgare* (white horehound, family Lamiaceae) specimen was collected from the Al-Jami'ah sub-district in Western Amman, Jordan (GPS 32.0015346, 35.8449004). The plant was taxonomically authenticated by Dr. Feryal Kherissat in the Department of Biological Sciences at Mutah University-Jordan, where a voucher specimen was deposited for future reference. Isolation of the endophytic fungus NN-Fus-1 from the plant stem was performed as described in Hazalin *et al.* (2009). Briefly, the plant aerial parts were aseptically surface sterilized by submersion in 70% ethanol for 2 min, then in 3% sodium hypochlorite solution for 1 min, followed by submersion in 70% ethanol for 30 seconds. This was followed by rinsing in sterilized distilled water for 5 min. The sterilized parts were cut aseptically into segments of 1 cm long and placed on potato dextrose agar plates (Oxoid, UK) supplemented with 100 µg/mL of streptomycin and penicillin G (Bio Basic Inc, Canada). Aliquots of the sterilized rinsing water were plated on a PDA plate to ensure the sterilization effectiveness and the absence of epiphytic microorganisms. The agar plates were incubated at 27°C for 2-4 weeks to observe mycelial growth. The grown mycelia with different morphological appearances were picked and transferred to malt extract agar (Oxoid, UK). Pure cultures of NN-Fus-1, cultivated on malt extract agar slants, were maintained at 4°C for long-term storage.

2.2. Cultivation of NN-Fus-1 and preparation of crude extracts:

The fungal isolate NN-Fus-1 was inoculated in a 500 ml Erlenmeyer flask containing sterilized rice medium (50 g of rice submerged in 75 ml of distilled water, autoclaved at 121°C for 20 min). Five agar blocks (1 x 1 cm) from a well-grown fungal culture on potato dextrose agar (PDA) were transferred aseptically to the rice flask. The flask was incubated statically at 27°C for 30 days, allowing complete colonization of the medium by fungal mycelia (Chen *et al.*, 2020). Post-incubation, the biomass and rice matrix were homogenized and exhaustively extracted with ethyl acetate (2 x 200 ml, 24h each). The pooled organic phases were

filtered through Whatman No. 1 paper containing anhydrous sodium sulfate for drying. The water-free organic solvent was concentrated *in vacuo* at 50°C using a rotary evaporator. The resulting crude extract was dissolved in methanol to prepare a 100 mg/ml stock solution, which was stored at 4°C till bioassays (Al-Qaralleh *et al.*, 2021).

2.3. Morphological and molecular identification of the NN-Fus-1

The fungal isolate NN-Fus-1 was characterized morphologically as mentioned in Watanabe (2010). The fungal isolate was cultured on PDA plates at 27°C for 14 days. Colony features (e.g., pigmentation, texture, margin) were documented using a stereomicroscope at 40x, while microscopic structures (hyphae, conidiophores, conidia) were examined under a compound microscope at 400x magnification. For molecular identification, genomic DNA was extracted using the fungal DNA Mini Kit (Omega BioTek, USA), and the internal transcribed spacer (ITS1-5.8S-ITS2) was amplified via PCR with the universal primers ITS1 and ITS4. The PCR reaction constituents and conditions were previously described (Al-Qaralleh *et al.*, 2021). The amplified product was sequenced and compared to the NCBI GenBank database via BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic analysis was conducted in MEGA X software using the Maximum Likelihood Algorithm to confirm taxonomic affiliation (Kumar *et al.*, 2018).

2.4. Evaluation of antioxidant and anti-inflammatory activities

2.4.1. Determination of antioxidant activity

The radical scavenging potential of the NN-Fus-1 isolate's crude extract was assessed against nitric oxide (NO) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals, as outlined by Al-Qaralleh *et al.* (2023). For the DPPH assay, extract concentrations (50-500 µg/ml) were mixed with 0.1 mM methanolic DPPH solution at 37°C for 30 min in the dark. The reduction in the color of the DPPH radical solution was measured at 517 nm using a UV/Vis spectrophotometer (Novaspec Model 80-2088-64, Pharmacia Biotech, UK). In the NO scavenging assay, the same concentrations of the crude extract were reacted with 10 mM sodium nitroprusside in 0.2 M phosphate-buffered saline (pH 7.4) for 3 h at 25°C. An equal volume of Griess reagent (Sigma-Aldrich, Germany) was added, and the absorbance of the resulting chromophore was read at 540 nm. Trolox (Sigma-Aldrich, Germany) was employed as a positive control. All experiments were conducted in triplicate, and the scavenging activity (%) was calculated compared to untreated controls.

2.4.2. Assessment of anti-inflammatory activity

2.4.2.1. Albumin denaturation test

The inhibition of heat-induced bovine serum albumin denaturation (BSA) was tested following the method of Sakat *et al.* (2010), with slight modifications. Ten microliters of the crude extract were added to 2 ml of 1% aqueous BSA (pH 5.3±0.2) at final concentrations (50-1000 µg/ml) with incubation at 37°C for 20 min. The preparation was heated at 56°C for 20 min. After cooling, the inhibition of protein agglutination was measured at 660 nm using a spectrophotometer, relative to untreated control

samples. Indomethacin (5-25 µg/ml) in 1% BSA was used as a reference standard. All assays were conducted in triplicate. The percentage of inhibition in albumin denaturation was calculated using the following equation: Albumin denaturation inhibition (%) = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$ [1]

Where A_{control} : absorbance of denatured albumin without treatment, and A_{sample} : absorbance of denatured albumin including the test sample.

2.4.2.2. Red blood cells (RBCs) membrane stability test

Venous blood samples (~ 5-10 ml each) in anticoagulant-containing blood tubes were generously obtained from the clinical center at Mutah University/Karak-Jordan; samples were collected from healthy individuals attending the center for routine blood count checks. The blood tubes were centrifuged at 3,500 rpm for 10 min (Combi-514R, Hanil, South Korea), and the resulting cell pellets were washed three times and suspended in normal saline at a concentration of 10% (v/v). Following the method of Tantary *et al.* (2017), the erythrocyte membrane stabilization assay involved incubating 10% human RBC suspensions with 50-500 µg/ml of the test crude extract at 56°C for 30 min, followed by cooling under running tap water and centrifuging at 2500 rpm for 5 minutes. The decrease in the released hemoglobin was quantified at 560 nm. As a negative control, normal saline was added instead of the RBC suspension. The assay was carried out in triplicate.

2.4.2.3. Lipoxigenase inhibitor assay

The ability of the fungal crude extract to inhibit the lipoxigenase enzyme (LOX) activity was conducted as described by Gopi and Jisho (2018). The tested extract (50-500 µg/ml) was reacted for 5 min with ice-cold LOX enzyme (2000 U/ml in 0.2 M borate buffer). After that, 1 ml of ice-cold 0.6 M linoleic acid substrate, prepared in the same buffer, was added to the reaction mixture. The decrease in absorbance of the formed hydroperoxy-linoleic acid product was checked for 2 min at 234 nm to determine the % LOX activity inhibition. Nordihydroguaiaretic Acid (NDGA) was used as a reference standard, and the test was repeated three times.

2.4.2.4. Carrageenan-induced paw edema inflammation in rats

In vivo carrageenan-induced paw edema model was employed using 25 albino rats (aged 7-8 weeks, 180-200 g body weight). The rats were kept in cages in a 12 h light/dark cycle, with food and water, in a temperature-controlled room. The rats were divided into four groups, each of five rats. They were lightly anesthetized with ether before treatment with test samples. Rats were pretreated with the extract or indomethacin for 1 h before injecting 100 µl of 1% λ-carrageenan into the right hind paw. Group 1 was injected with 100 µl buffer saline solution containing 100 µg/g b.w crude extract, Group 2 received the same volume with 200 µg/g b.w extract, Groups 3 and 4 were treated with 10 µg/g b.w and 25 µg/g b.w of indomethacin, respectively, while Group 5 was injected with 100 µl of buffer saline solution into the right paw as an untreated control. The left paw in all rats received 100 µl of buffer saline as a negative control. Paw swelling in the right paw was measured at 0, 1, and 4 h post-injection using a digital caliper and compared to the left paw measurement for each rat in each group. The anti-

inflammatory potency of the tested samples was evident from the difference in paw thickness between the right and the left limb due to treatments with either fungal crude extract or the positive control (indomethacin) relative to that of the untreated Group 5.

2.5. Chemical analysis

2.5.1. Thin-layer chromatography (TLC)

Preliminary chemical screening of the main classes of compounds present in the crude extract was performed on silica gel 60 TLC plate (20 x 20 cm, Alugram® SIL G/UV254, Macherey & Nagel, Düren) following Jork *et al.* (1990). The TLC plate was developed in toluene: acetone: formic acid (70:30:1, v/v/v). The developed bands were visualized under UV light (254 and 366 nm) and were stained with specific spray reagents: 1% ethanolic solution of aluminum chloride for detecting flavonoids; a mixture of 0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml 97% sulfuric acid for the detection of phenols; 37% formaldehyde in concentrated sulfuric acid at a ratio (1:10) for the detection of alkaloids and aromatic hydrocarbons, terpenes, sugars, and steroids; mixture of 0.1g Bromocresol green in 500 ml ethanol and 5 ml of 0.1 M NaOH for the detection of organic acids; a solution of 1g sodium nitrite in 100 ml 1M HCl for the detection of thiazoles and indoles.

2.5.2. High-performance liquid chromatography (HPLC)

Chemical constituents of the ethyl acetate crude extract of *Fusarium* sp. NN-Fus-1 was analyzed using an HPLC instrument (Shimadzu Prominence-i LC-2030, Japan). The instrument is equipped with a Shim-pack GISS C18 column with dimensions of I.D. 4 x 125 mm and 5 µm particle size (Shimadzu, Japan) and a photodiode array detector (PDA). Analysis was performed using a mobile phase mixture of solvent A (0.1% formic acid solution) and solvent B (Acetonitrile). The run program was a linear gradient elution as follows: 0-1 min, 0-1% B; 1-11 min, 1-100% B; 5 min, 100% B; decreasing in solvent B from 100% to 1% within 2 min with a flow rate of 1 ml/min. The injection volume was 20 µl of the sample, the PDA detection wavelength was set to 190-600 nm, and the column temperature was 40°C. The sample was filtered pre-analysis through a 0.45 µm disposable PTFE filter.

2.6. Statistical analysis

Data were expressed as means ± standard deviations (SD). Statistical significance ($P < 0.05$) was determined using one-way ANOVA followed by Turkey's post-hoc test in SPSS ver. 26.0 (SPSS Inc., Chicago, IL, USA) software.

3. Results

During screening for endophytic fungi with potent anti-inflammatory activity, fungal isolate NN-Fus-1 was selected for further investigations. The crude extract of this isolate did not exhibit antibacterial activity nor stabilize the membrane of heat-induced RBC. However, it demonstrated pronounced DPPH radical scavenging and lipoxigenase activities starting at 100 µg/ml, along with 80-90% inhibition in the albumin denaturation test at 1 mg/ml, indicating promising anti-inflammatory properties. The fungal isolate NN-Fus-1 forms colonies with fluffy,

cottony aerial mycelia, displaying whitish pigmentation and yellow-colored edges after 3-4 weeks of cultivation on PDA medium. Microscopic examinations revealed lateral hyaline conidiophores with flexible, irregular secondary conidiophore branches. The macroconidia were hyaline, 1-2 celled, ovoid to pyriform shaped (13.6-30.9 μm long and 5.7-8.4 μm wide), and arose from slender phialides. The chlamydospores were yellowish brown, measuring 32-60 μm wide (Fig. 1).

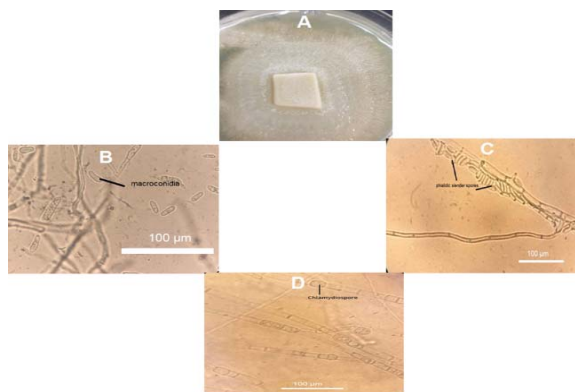


Figure 1. Microphotographs of *Fusarium* sp. isolate NN-Fus-1. A) fungal block grown on PDA medium at 100X magnification, B) macroconidia, C) an aggregate of spores forming the phialide, and D) chlamydospores.

Amplification of the partial 18S rRNA gene partial/sequence yielded a ~515 bp amplicon, which showed 98% similarity to *Fusarium redolens* isolate-FWN2 (Accession no. MN537039.1) upon alignment with sequences in the GeneBank (Fig. 2). The partial 18S rDNA gene sequence of the isolate was deposited in GenBank as *Fusarium* sp. isolate NN-Fus-1 (Accession number MW047084.1) (www.ncbi.nlm.nih.gov/nuccore/MW047084.1).

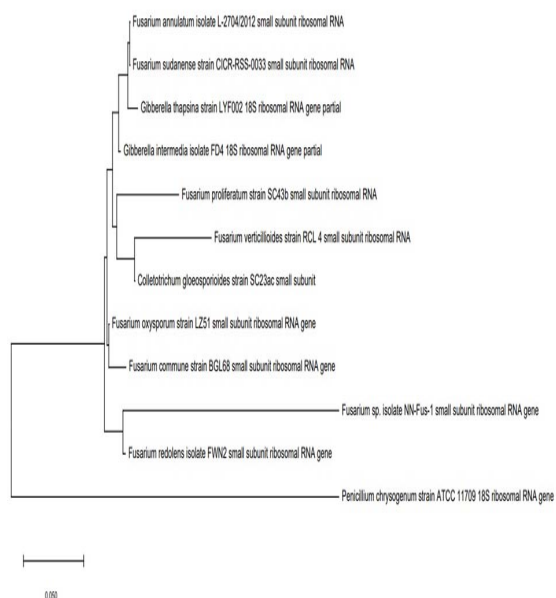


Figure 2. Phylogenetic tree of fungal isolate NN-Fus-1 constructed using the Maximum Likelihood Algorithm in MEGA7 software (Kumar *et al.*, 2018).

3.1. Biological activity

3.1.1. Antioxidant activity

Fusarium sp. isolate NN-Fus-1 crude extract exhibited significant antioxidant activity, neutralizing DPPH radicals with an IC_{50} value of 109.17 ± 1.30 $\mu\text{g/ml}$ (6.41 ± 0.08 μg Trolox/mg crude extract) (Table 1). However, the extract did not show scavenging activity against the nitric oxide radical at concentrations up to 500 $\mu\text{g/ml}$, suggesting differences in the type of metabolites active in the DPPH assay compared to NO radical scavenging.

3.1.2. Anti-inflammatory activity assay

3.1.2.1. Albumin denaturation inhibition

Fusarium sp. isolate NN-Fus-1 crude extract inhibited heat-induced albumin denaturation with an IC_{50} of 469.05 ± 3.72 $\mu\text{g/ml}$, indicating its ability to abate inflammatory reactions caused by the denaturation of cellular proteins. In comparison, indomethacin showed inhibition with an IC_{50} of 18.35 ± 1.99 $\mu\text{g/ml}$ (Table 2).

Table 1. DPPH radical scavenging activity of *Fusarium* sp. isolate NN-Fus-1 crude extract.

Sample	Concentration ($\mu\text{g/ml}$)	% Scavenging activity $\pm$ SD	IC_{50}	TEAC (μg Trolox/mg crude extract)
crude extract	50	33.73 ± 0.09^a	109.17 ± 1.30	6.41 ± 0.08
	100	57.20 ± 1.85^b		
	300	$76.41 \pm 1.68^{b,c}$		
	500	87.11 ± 1.59^c		

Post hoc Tukey HSD test analysis of scavenging activities, results with similar letters are not significantly different from each other.

Table 2. Inhibiting heat-induced albumin denaturation by *Fusarium* sp. isolate NN-Fus-1 crude extract.

Sample	Concentration ($\mu\text{g/ml}$)	% Inhibition $\pm$ SD	IC_{50} ($\mu\text{g/ml}$)
Crude extract	50	21.302 ± 1.454^a	$469.05 \pm 3.72^*$
	100	23.872 ± 3.15^a	
	300	38.435 ± 3.55^a	
	500	$53.741 \pm 5.98^{a,b}$	
	1000	85.551 ± 4.44^b	
Indomethacin	5	7.91 ± 0.65^c	18.35 ± 1.99
	10	14.22 ± 3.05^c	
	25	58.36 ± 3.98^d	
	50	76 ± 7.63^d	

Post hoc Tukey HSD test analysis of inhibition percentages; results with similar letters are not significantly different from each other. Asterisk indicated a statistically significant difference in IC_{50} value of the tested sample to the positive control ($p < 0.05$).

3.1.2.2. Lipoxigenase inhibition assay

Lipoxigenase (LOX) converts fatty acids into peroxide derivatives, including arachidonic acid to leukotrienes, which are inflammatory mediators. Using linoleic acid as a substrate, the crude extract of *Fusarium* sp. NN-Fus-1 inhibited LOX activity in a concentration-dependent manner, with an IC_{50} value of 83.51 ± 1.36 $\mu\text{g/ml}$. In

contrast, NDGA revealed LOX inhibitory activity at an IC_{50} of 0.11 ± 0.004 $\mu\text{g/ml}$ (Table 3).

Table 3. Inhibition of the lipoxygenase enzyme by the *Fusarium* sp. isolate NN-Fus-1 crude extract.

Sample	Concentration ($\mu\text{g/ml}$)	% Inhibition $\pm$ SD	IC_{50} ($\mu\text{g/ml}$)
Crude extract	50	38.77 ± 0.78^a	$83.51 \pm 1.36^*$
	100	56.38 ± 1.46^a	
	300	80.4 ± 1.41^b	
	500	87.77 ± 1.22^b	
NDGA	0.05	31.4 ± 0.32^c	0.11 ± 0.004
	0.1	47.31 ± 1.54^d	
	0.2	58.29 ± 3.95^d	
	0.3	83.9 ± 1.75^e	

Post hoc Tukey HSD test analysis of inhibition percentages; results with similar letters are not significantly different from each other. Asterisk indicated a statistically significant difference in IC_{50} value of the tested sample to positive control ($p < 0.05$).

3.1.2.3. Carrageenan-induced paw edema inflammation in rats

Treatment with the fungal crude extract (100 and 200 $\mu\text{g/g}$ b.w) ameliorates the thickness of the edema due to the carrageenan inflammatory effect, with a maximum

inhibition of 55.5% at 200 $\mu\text{g/g}$ b.w and an IC_{50} of 159.76 ± 3.6 $\mu\text{g/g}$ b.w. Indomethacin (25 $\mu\text{g/g}$ b.w) reduced paw thickness by 28%, with an IC_{50} of 58.05 ± 2.85 $\mu\text{g/g}$ b.w (Table 4).

Table 4. Inhibition of carrageenan-induced paw edema in a rat model ($n=5$).

Sample	Concentration ($\mu\text{g/g}$ b.w)	Inhibition (%) $\pm$ SD	IC_{50} ($\mu\text{g/g}$ b.w)
Crude extract	100	41.887 ± 4.0	$159.76 \pm 3.6^*$
	200	55.453 ± 3.2	
Indomethacin	10	18.176 ± 2.1	58.05 ± 2.85
	25	28.111 ± 3.6	

*Statistically significant difference in IC_{50} value of the tested sample to the positive control ($p < 0.05$).

3.2. Chemical analysis

Chemical analysis of the ethyl acetate crude extract of *Fusarium* sp. NN-Fus-1 revealed the presence of alkaloids, phenolic derivatives, flavonoids, and organic acids as the major detected constituents. The presence of phenolic compounds and flavonoids was highly detected at 280 nm (Fig. 3). In comparison, alkaloids were detected at 230 nm (Fig. 4). These wavelengths were chosen because the principal peaks exhibited maximum absorption, and the sample matrix effect is minimal. The eluted constituents were within the retention times 7.5-13.7 min for flavonoids as well as phenolic compounds, and 6.8-11.5 min for alkaloids.

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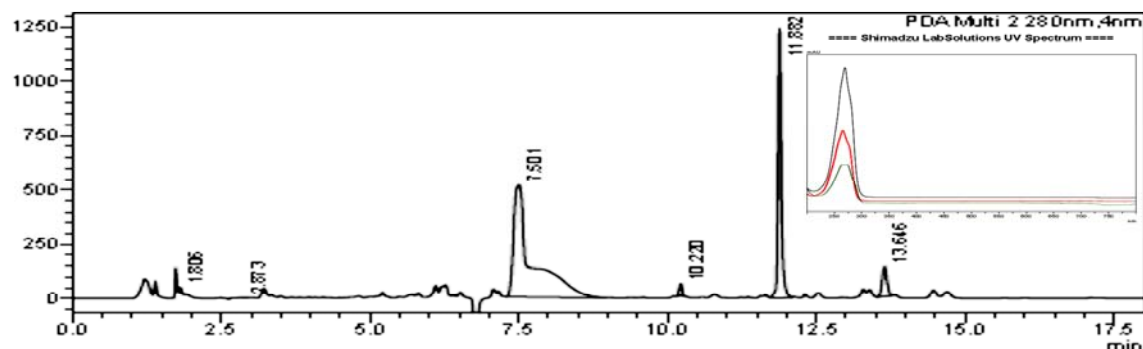


Figure 3. HPLC chromatogram of *Fusarium* sp. NN-Fus-1 ethyl acetate crude extract using an RP-C₁₈ column at 280 nm wavelength. Inset is the UV spectra of the major peaks.

<Chromatogram> mAU

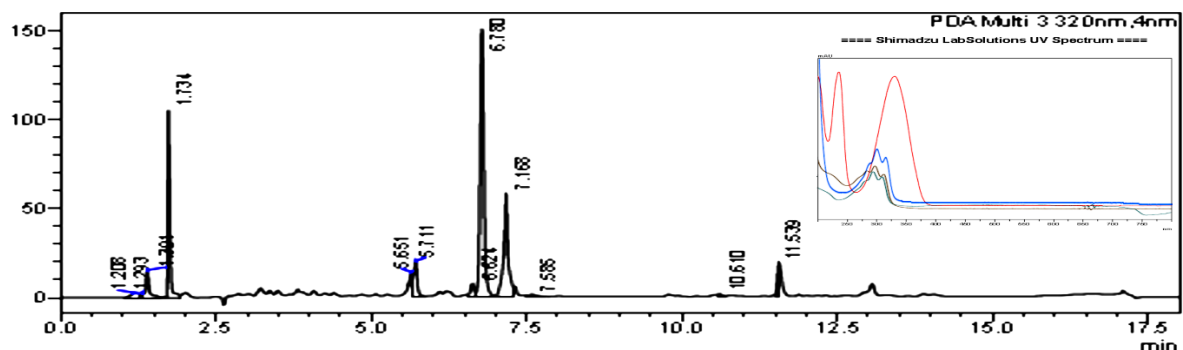


Figure 4. HPLC chromatogram of *Fusarium* sp. NN-Fus-1 ethyl acetate crude extract using an RP-C₁₈ column at 320 nm wavelength. Inset is the UV spectra of the major peaks.

4. Discussion

Human health is endangered by various infections and inflammatory disorders, often intensified by uncontrolled inflammatory responses to various noxious stimuli. Given the adverse effects associated with the consumption of synthetic anti-inflammatory drugs, the search for novel bioactive metabolites from unconventional sources, including microbial compounds, has become a vital issue. Intriguingly, over the years, microbial-derived therapeutic agents have improved human health (Dias *et al.*, 2019), with *Fusarium* species as prolific producers of drug precursors with potential pharmaceutical applications (Kyekyeku *et al.*, 2017).

The host cellular response to many inflammatory reactions triggers the production of pro-inflammatory cytokines such as interleukin-1 beta (IL-1 β), IL-6, tumor necrosis factor-alpha (TNF- α), interferons (INF- α and - β), nitric oxide (NO), prostaglandins, arachidonic acid metabolites, and proteolytic enzymes (Henrotin *et al.*, 2005). These mediators trigger increased vascular permeability, leucocyte infiltration, protein denaturation, membrane destabilization, and edema formation, as key hallmarks of inflammation (Bouriche *et al.*, 2016). Furthermore, oxidative stress resulting from an imbalance in free radical regulation contributes to chronic inflammatory disorders such as rheumatoid arthritis, asthma, allergy, and autoimmune diseases (Al-Qaralleh *et al.*, 2021). Therefore, the potential anti-inflammatory and antioxidant activities of secondary metabolites produced by the *Fusarium* sp. isolate NN-Fus-1 were evaluated herein.

Chemical analysis of the crude extract of *Fusarium* sp. isolate NN-Fus-1F revealed the presence of phenolic compounds, flavonoids, and alkaloids. The eluted constituents were within the retention times 7.5-13.7 min for flavonoids as well as phenolic compounds, and 6.8-

11.5 min for alkaloids, implying their nonpolar to moderate polar nature. Phenolic and flavonoids are successfully visualized in the chromatogram when analyzed at 280 nm (Al-Rimawi, *et al.*, 2024); an absorption band caused by electron transition of the benzoyl group in these groups of compounds (between 240-280 nm), in addition to absorption band corresponding to the cinnamoyl group (300-400 nm) (Feng *et al.*, 2017; Nistor and Butnariu, 2023), depicted in Fig.4 (red spectrum). However, most alkaloids revealed maximum UV absorptions at 210-230 nm, 240-290 nm, and 300-350 nm (Kabulov *et al.*, 1992; Hisiger and Jolicœur, 2007; Aigotti *et al.*, 2022).

The crude extract was identified as a potent source of antioxidant compounds in the DPPH radical scavenging assay (IC_{50} = 109.16 μ g/ml), comparable to 6.41 $\pm$ 0.08 μ g Trolox equivalents per mg crude extract. However, it was not effective in scavenging NO radicals at concentrations up to 500 μ g/ml. This inconsistency suggests that the bioactive constituents preferentially neutralize hydrophobic radicals (DPPH being prepared in an organic solvent) rather than NO radicals derived from sodium nitroprusside in an aqueous solution, highlighting the hydrophobic nature of the metabolites (Baliga *et al.*, 2003).

The antioxidant potential of *Fusarium* sp. isolate NN-Fus-1F extract renders it a promising source for anti-inflammatory compounds. The extract's anti-inflammatory mechanism appears to involve direct protein stabilization at the site of inflammation, rather than reducing the release of lysosomal constituents from neutrophils that cause inflammation. The extract caused inhibition in heat albumin denaturation (IC_{50} of 469.05 $\pm$ 3.72 μ g/ml), a process implicated in inflammatory diseases such as rheumatoid arthritis (Moharram *et al.*, 2017), but failed to stabilize RBCs membrane, where erythrocyte membranes model lysosomal membranes (Parvin *et al.*, 2015; Santos *et al.*, 2020). Moreover, the 5-LOX enzyme catalyzes the oxidation of polyunsaturated fatty acids into pro-inflammatory leukotrienes, exacerbating conditions such as rheumatoid arthritis, ulcerative colitis, asthma, and atherosclerosis (Gopi and Jisho, 2018). Intriguingly, the extract inhibited 5-LOX activity with an IC_{50} of 83.51 $\pm$ 1.36 μ g/ml, likely by scavenging lipid-peroxy-radicals and disrupting the production of lipid hydroperoxides and leukotrienes (Ruma *et al.*, 2013). The dual antioxidant and LOX-inhibitory activity of the crude extract is correlated with *in vivo* anti-inflammatory effects, as demonstrated by a 55.4 % reduction in carrageenan-induced paw edema at 200 μ g/g b.w (IC_{50} of 159.76 $\pm$ 3.6 μ g/g b.w) compared to indomethacin (28.1% inhibition at 25 μ g/g b.w). This suggests suppression of vascular permeability and leukocyte infiltration, key drivers of edema (Fatima *et al.*, 2021).

Coinciding with our findings, the ethyl acetate extract of the endophytic *Fusarium* sp. and *Aspergillus fumigatus* revealed the presence of phenolic compounds, which endow them with antioxidant properties and a capacity to neutralize DPPH radicals with IC_{50} values of 20 μ g/ml and 42.25 μ g/ml, respectively. These metabolites also revealed anti-inflammatory potential by inhibiting LOX in a dose-dependent manner with IC_{50} values of 62 μ g/ml and 63 μ g/ml, respectively (Ruma *et al.*, 2013). Several *Fusarium* species, including *F. redolens*, were isolated from the bulbs of *Fritillaria unibracteata* var. *wabuensis*. They revealed antioxidant activities in various scavenging assays, especially their ethyl acetate and ethanolic extracts, due to their content of phenolic and flavonoid compounds (Pan *et al.*, 2017). Meanwhile, the high flavonoid content in *Xylaria* isolated from the medicinal plant *Conyza blinii* was the primary cause of the radical scavenging activity of their crude extract in DPPH and ABTS assays, with IC_{50} values of 110 μ g/ml and 200 μ g/ml, respectively (Tang *et al.*, 2020). Interestingly, the phenolic derivative fusagunolics B identified from the endophytic *F. guttiforme* revealed moderate anti-inflammatory activity by inhibiting NO production with an IC_{50} value of 28.6 μ M (Yue *et al.*, 2024). In addition, the glycosylated flavonoid vitexin from *F. solani* caused a reduction in the pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, and IL-33) and enhanced the anti-inflammatory cytokine (IL-10) as a promising anti-inflammatory drug (Ahmed *et al.*, 2023).

However, rohitukine, an alkaloid produced by the endophytic *F. proliferatum*, displayed anti-inflammatory and immunomodulatory properties (Kumara *et al.*, 2012). Moreover, Azaphilone and Pseurotin A, alkaloids from *Penicillium sclerotiorum* cib-411 and *Aspergillus fumigatus*, exert their anti-inflammatory activity by

inhibiting NO production in LPS-induced RAW264.7 macrophages (Shi *et al.*, 2015; Tang *et al.*, 2019). Similarly, Neoechinulin A and Aspersiamides, marine-derived alkaloids, were isolated from *Eurotium* sp. SF-5989 and *A. versicolor*, respectively, inhibited the production of pro-inflammatory mediators (prostaglandins E-2, IL-1 β , NO, and TNF- α) and suppressed the expression of COX-2 and iNOS in lipopolysaccharide (LPS)-stimulated murine RAW264.7 cells (Souza *et al.*, 2020; Bai *et al.*, 2021). Furthermore, the marine isolate *Aspergillus* sp. SCSIO 41024 synthesized cyclopiazonic acid indole alkaloid derivatives, causing inhibition in the DPPH scavenging assay with IC₅₀ values ranging from 31.9–228.4 μ g/ml (Chen *et al.*, 2020).

In line with our results and as documented in all aforementioned studies, crude extracts encompassing alkaloid, phenolic, or flavonoid compounds are considered potential lead antioxidant agents to treat oxidative stress-associated inflammatory disorders, such as cardiovascular, cancer, and neurodegenerative diseases. In general, flavonoid synthesis involves both the acetate pathway to generate ring A and the shikimate pathway to generate the B ring; the two rings condense to form chalcone (a precursor of flavonoids) (Hu *et al.*, 2025), which is linked to the pyran C-ring. The hydrophilicity of glycosylated flavonoids decreases their passage through biological membranes, reducing their bioactivity, but improves their stability in cells. Meanwhile, the aglycone exhibits high bioactivity with poor bioavailability (Dias *et al.*, 2021). Therefore, glycosylated flavonoids undergo enzymatic hydrolysis in biological cells to aglycone congeners, which are degraded enzymatically, producing phenolic compounds of low molecular weight. The impact of flavonoid metabolism exhibited bioactivity rather than the parental flavonoids (Carregosa *et al.*, 2022).

In fact, flavonoids exert their antioxidant activity through radical stabilization, where the hydroxyl group donates electrons and hydrogen through resonance and generates stable phenoxy-radical flavonoids and phenolic acids. They can also activate electrophile-responsive elements to regulate enzymes involved in the generation of free radicals, including NAD(P)H oxidoreductase, mitochondrial succinoxidase, xanthine oxidase, and glutathione S-transferase, thereby resisting oxidative stress (Andrés *et al.*, 2023). The oxo-group of flavonoids participates in the conjugated system to enhance electron delocalization (Hu *et al.*, 2025). The number and position of the hydroxyl groups in the catechol B-ring and their position on the pyran C-ring influence the free radical scavenging ability (Dias *et al.*, 2021). Moreover, they inhibit ROS generation by chelating trace elements such as iron, leading to a reduction in the Fenton reaction kinetics (Lahey-Beitia *et al.*, 2021).

Notably, phenolic compounds and flavonoids can suppress mitogen-activated protein kinase (MAPK), NF- κ B, cyclooxygenase (COX), LOX, and inducible nitric oxide synthase (iNOS), as well as downregulate TNF- α , IL-1 β , and IL-6 (Al-Khayri *et al.*, 2022; Ysrafil *et al.*, 2023); thereby mediating their anti-inflammatory activity. The hydroxylation number on “B” and “C” rings, the presence of a double bond in C2–C3 and/or a ketone in C4 in the “C” ring are structural features that influence their anti-inflammatory potential (Silva *et al.*, 2021). The ability of these metabolites to decrease the production of

arachidonic acid and inhibit the crucial pro-inflammatory enzymes leads to a significant decrease in the production of prostaglandins, leukotrienes, and NO, the key inflammatory substances (Stachelska *et al.*, 2025).

On the other hand, synthesis of most alkaloids starts with amino acid precursors; however, terpenoid and steroidal alkaloids are biosynthesized from isoprenoid and steroidal backbones (Letchuman *et al.*, 2025). They undergo decarboxylation, cyclization, methylation, and oxidation reactions to form the characteristic heterocyclic cores of alkaloids. The nitrogen atoms of alkaloids can engage in hydrogen bonding and ionic interactions that influence their biological activities. They can interact with various biological systems, affecting enzyme activity and cellular signaling pathways (Leclerc and Pierre Fournier, 2024). Alkaloids stabilize the free radicals by donating the hydrogen of their OH and NH functional groups (Dalimunthe *et al.*, 2018) and exert their anti-inflammatory activity by reducing the production of iNOS and COX-2 (Souza *et al.*, 2020; Bai *et al.*, 2021).

5. Conclusion

Fusarium sp. isolate NN-Fus-1 crude extract demonstrates significant antioxidant and anti-inflammatory properties, likely mediated by its content of alkaloids, phenolics, and flavonoids. These metabolites can modulate oxidative stress, inhibiting LOX, reducing protein denaturation, and ameliorating the effect of carrageenan on the induction of paw edema in the mouse model. *Fusarium* sp. isolate NN-Fus-1 is a potential candidate for further pharmaceutical investigations. Future work exploring the mechanistic pathways of its activity and isolation of the lead bioactive compounds is required.

Conflict of interest

The authors declare no conflicts of interest.

Ethical Consent

The study was approved by the Scientific Ethics Committee in the Department of Biology/Mutah University. All animal experiments complied with the ARRIVE guidelines and were performed per the ethical and welfare standards in European Union legislation (EU Directive 2010/63/EU).

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