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Original Research Article

Naphthoquinones Isolated from *Fusarium solani,* an Endophytic Fungi of *Cola nitida,* with Potentials for Pharmaceutical and Industrial Applications

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ABSTRACT

Endophytic fungi isolated from Nigeria Rainforest medicinal plants have been shown to possess potentials as sources of novel therapeutic agents. In our effort to further explore the rainforest medicinal plants in Nigeria for endophytic fungi populations, we investigated *Cola nitida* for the isolation of novel fungal endophytes that can synthesize unique compounds of pharmaceutical and industrial importance. The endophytic fungus was isolated from the leaves of *Cola nitida* using standard protocols. The identification was carried out by DNA amplification and sequencing of the internal transcribed spacer (ITS) region. The pure fungus was grown using solid fermentation on a rice medium and the metabolites were extracted using ethyl acetate. The crude extract was subjected to several chromatographic techniques to isolate compounds **1**-**4**. The structures of these compounds were elucidated using a combination of 1-and 2-D NMR and Mass Spectrometry. The fungus was identified as *Fusarium solani* and the isolated compounds were elucidated as Javanicin (**1**), Solaniol (**2**), 3-*O*-methyl fusarubin (**3**) and anhydrojavanicin (**4**). This study further contributes to the plethora of endophytic fungi isolated from the leaves of *Cola nitida.* The isolated naphthoquinones have potential for development into novel therapeutic agents as well as applications as pigments in food, cosmetics, pharmaceutical, and textile industries.

Keywords: Fungal endophytes, *Fusarium solani*, Naphthoquinones, *Cola nitida*, Pigments.

Introduction

In the past few decades, drug discovery from natural products has consistently drifted from the investigation of whole medicinal plants or parts to the microorganisms, known as endophytes, which reside within the organs and tissues of these plants. [1] This new paradigm has obvious advantages ranging from enhancing the chances of generating new and novel chemical entities to the preservation of plant species from over-exploitation, which could impart negatively on the ecosystem. [1,2] Besides there is the possibility of performing genetic modifications of these organisms, especially the unculturable ones, modulating their culture conditions and co-cultivating with other organisms so as to generate novel compounds and improve the yield of their secondary metabolites. [2,3] So far, a large number of fungal endophytes have been isolated and their secondary metabolites, which elicit a wide range of biologica activities, fully characterized.[4,5,6,7] It is now a common knowledge that every plant has at least one fungal endophyte occupying its organs/tissues,[8] which could easily be identified and quantified with the invention of high-throughput sequencing (HTS) techniques.[9]

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Fusarium species were previously seen only as plant disease-causing agents; however, their endophytic nature is now explored.[10] As fungal endophytes, *Fusarium* species are present in a wide range of plant species found in varying habitats, and they produce a large spectrum of bioactive secondary metabolites and pigments that exhibit antimicrobial, antioxidant, antiparasitic, immunosuppressing and immunomodulating, antiviral and anticancer activities.[11,12,13] Species of this genus are also quite consistent with their production of naphthoquinone derivatives,[14] a class of natural pigments that exhibit good inhibitory properties against bacterial, cancer cells, and malarial parasites. The unique biological activities stem from the ability of these naphthoquinones to form reactive oxygen species that target cellular molecules which regulate various signalling pathways, thus leading to the death of targeted cells.[15] Naphthoquinones have also been isolated from other fungal endophytes and their pharmacological properties equally reported.[16] In our continuous quest to explore Nigerian medicinal plants for novel endophytic fungi, *Fusarium solani*, was isolated from the healthy leaves of *Cola nitida,* and assessed for its secondary metabolites. *Cola nitida* is an evergreen plant found in tropical regions, whose seeds (kola nut) have traditional uses in many Nigerian cultures. Extracts and compounds with antidiabetic, antioxidant, anti-inflammatory, antimicrobial and antidiuretic activities[17,18] have been identified in different parts of the plant. Apart from its medicinal uses, the pigmentation property of *C. nitida* has also been reported,[18] and it is also a host to different fungal endophytes. Three fungal endophytes belonging to *Acremonium*, *Aspergillus* and *Trichophyton* genera were previously isolated from healthy leaves of *C. nitida*, and their extracts were shown to exhibit good activity against *Pseudomonas aeruginosa*.[19] *Trichoderma species* was also previously isolated from the leaves of *C. nitida* and was shown to produce two secondary metabolites with poor cytotoxic effect.[20] Apart from their potential therapeutic applications, naphthoquinones have been widely used in the food, cosmetics and pharmaceutical industries as colorants due to the increasing acceptance of natural pigments over those of synthetic origins. This current trend has stimulated our interest to

further explore the potentials of endophytic fungi of medicinal plant origin as sources of novel naphthoquinone pigments. In the present study, we isolated and characterized four naphthoquinone pigments from *F. solani*, an endophyte associated with *Cola nitida* leaves. These natural pigments have potential applications in the food, cosmetics, pharmaceutical, and textile industries.

Materials and Methods

General Experimental Procedures

H-NMR spectra (1-and 2-D) were recorded with AVANCE DMX 300 or 600 NMR spectrometers (Bruker, Rheinstetten, Germany). MS (ESI) data were obtained with Finnigan LCQ Deca mass spectrometers (Thermoquest, Bremen, Germany). Analytical HPLC was carried out with a Dionex P580HPLC system (Dionex Softron, Germering, Germany) coupled to a photodiode array detector (UVD340S). Detection was at 235, 254, 280 and 354 nm. Semipreparative HPLC was performed with Merck Hitachi L-7100 (Merck/Hitachi, Germany) coupled to a UV detector (L- 7400). A linear gradient of HPLC grade methanol and nano-pure water was used in each case of separation. TLC was performed on silica gel F_{254} coated plates (Merck) using the following solvent systems: Hexane/Ethyl acetate (5:5). Vacuum Liquid Chromatography (VLC) was carried out on silica gel (200–400 mesh, Merck).

Plant Material

Fresh, healthy leaves of *Cola nitida* were collected from Awo-Idemili, Orsu Local Government Area, Imo State, Nigeria in June, 2023. The leaves were placed in a sealed cellophane bag and transported the same day to the Pharmaceutical Microbiology laboratory of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria for use in the isolation of the endophytic fungi. The plant material was authenticated by a taxonomist, Mrs. Anthonia Emezie of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria and a voucher specimen (PCG/474/A/050) was deposited at the herbarium of Department of Pharmacognosy and Traditional Medicine of the same University.

Fungal Isolation, Purification and Identification

For fungal isolation, purification, and identification, a previously validated protocol by our research group was adopted.[4,5] The fresh, healthy leaves of *Cola nitida* were washed carefully under running water. They were subsequently surface-sterilized by soaking in 70% ethanol, 2% NaClO and 70% ethanol for 1, 2 and 2 min, respectively. Subsequently, the disinfected leaves were cut into small sizes, selected and placed on petri dishes of malt extract agar (MEA) made up of 15 g/L malt extract, 15 g/L agar and supplemented with chloramphenicol (200 mg/L) to prevent the growth of bacteria. The plates were incubated at room temperature for the emergence of hyphal tips. The emerged hyphal tips from the leaf segments were transferred to new plates of MEA, and this process was repeated until pure colonies of fungi were obtained. Fungal strain identification was done through DNA amplification by Polymerase Chain Reaction (PCR) (Hot StarTag Master Mix Taq polymerase and primer pairs ITS1 and ITS4), sequencing, and subsequent BLAST searches of the NCBI GenBank data.

Fermentation, Extraction, Isolation and Characterization of Metabolites

Solid state fermentation of the pure fungus was carried out in 1 L Erlenmeyer flasks containing Rice Medium (100 g of rice in 100 mL of distilled water, autoclaved at 121ºC at 15 psi for 1 h and allowed to cool). The flasks were inoculated with 3 mm diameter agar blocks of the pure fungus and incubated at 28ºC for 21 days. After the fermentation period, fungal secondary metabolites were exhaustively extracted with EtOAc, pooled and concentrated using a rotary evaporator.[21]

The extract was subjected to Vacuum Liquid Chromatography (VLC) on silica gel (200–400 mesh) using the gradient of hexane/EtOAc (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70 20:80, 0:100). About 100 g of the fraction eluted with hexane/EtOAc 70:30 was separated with semipreparative HPLC to obtain compounds **1** (2 mg), **2** (2.1 mg) and **3** (2.4 mg). Similarly, about 30 mg of the fraction eluted with hexane/EtOAc 60:40 was separated with semipreparative HPLC to obtain compound **4** (1.5 mg). The purity of the isolated compounds was assessed using analytical HPLC (Figures S1 to S4 in supplementary data). The chemical structures of the compounds were elucidated by a combination of 1-and 2-D NMR and mass spectrometry.

Results and Discussion

The endophytic fungus isolated from healthy leaves of *Cola nitida* was identified as *Fusarium solani*. This identification was carried out at the Institute of Pharmaceutical Biology, Universität Dusseldorf, Dusseldorf, Germany and the sequence has been deposited in the GenBank with accession number MG772815. Chromatographic purification of the ethyl acetate extract of the fermentation products of *Fusarium solani*, gave rise to four existing naphthoquinones viz. javanicin (**1**), solaniol (**2**), 3-*O*-methyl fusarubin (**3**) and anhydrojavanicin (**4**).

Javanicin (**1**). Dark orange crystal (2 mg); HPLC retention time: 38.23 min; UV λmax (PDA): 225.7, 304 and 502 nm; ESI-MS: 91.1 [M+1]; 313 [M+23]; 602.9 [2M+23]; 289.3 [M-1]$^-$; NMR (300MHz, MeOH-d4) δ_H: 6.37 (1H, s H-3), 4.04 (2H, s, H_2-10), 3.96 (3H, s, 2-MeO), 2.31 (3H, s, H_3-9), 2.23 (3H, s, H_3-12).

Solaniol (**2**). Dark orange crystal (2.1 mg); HPLC retention time: 36.57 min; UV λmax (PDA): 226.2; 304 and 503.5; NMR (300MHz, MeOH-d4) δ_H: 6.34 (1H, s, H-3), 4.08 (1H, s, H-11), 3.94 (3H, s, 2-MeO), 2.93 (2H, d, *J*= 7.1, H_2-10), 2.34 (3H, s, H_3-9), 1.24 (3H, d, *J*= 6.2, H_3-12).

3-O-methyl fusarubin (**3**). Wine-red coloured crystals (2.4 mg); HPLC retention time: 43.01 min; UV λmax (PDA): 224.4; 301.5 and 595.3 nm; NMR (600MHz, MeOH-d4) δ_H: 12.92 (1H, s, 10-OH); 12.64 (1H, s, 5-OH), 6.16 (1H, s, H-8), 4.86 (1H, dd, *J=19*.3, H-1A), 4.55 (1H, d, *J*=17.8, H-1B), 3.91 (3H, s, 7-MeO), 3.30 (3H, s, 3-MeO), 3.00 (1H, m, H-4A), 2.65 (1H, dd, *J*= 18.1, H-4B), , 1.53 (3H, s, H-11).

Anhydrojavanicin (**4**). Red crystals (1.5 mg); HPLC retention time: 37.77 min; UV λmax (PDA): 224.5, 299.2 and 480.2 nm; ESI-MS: 275.1 [M+1] and 570.9 [2M+23]; 273.2 [M-1]; NMR (300MHz, MeOH-d4) δ_H: 6.19 (1H, s, H-7), 5.15 (1H, m, H-2), 3.90 (3H, s, 8-MeO), 3.37 (1H, m, H-3A), 2.84 (1H, dd, *J* = 19.5, H-3B), 2.24 (3H, s, 4-Me), 1.52 (3H, d, *J* = 6.3, 2-Me).

Compound **1** was isolated as a dark orange-coloured crystal. It showed UV λ_{max} at 225.7, 304 and 502 nm (Figure S1 in supplementary data), which is characteristic of naphthoquinones or anthraquinones skeletons. The ESI-MS exhibited strong peaks at *m/z* 291.1 [M+1]; 313 [M+23] and 602.9 [2M+23] in the positive mode and at *m/z* 289.3 [M-1]$^-$ in the negative mode. These pseudo-molecular ions supported the molar mass of 290 g/mol and molecular formula of $C_{15}H_{14}O_6$. The proton NMR spectrum showed characteristic signals, which included an aromatic proton singlet at δ_H 6.37 (s, 1H) assigned to H-3, a methyl singlet at δ_H 2.31 (s, 3H) suggestive of methyl group attached to aromatic ring and assigned to H-9, a methylene singlet at δ_H 4.04 (s, 2H) assigned to H-10, a methyl singlet at δ_H 2.23 suggestive of methyl group attached to carbonyl assigned to H-12 and another methyl singlet at δ_H 3.86 (s, 3H) assigned to aromatic methoxy group, MeO-2. The MS and NMR data (Table 1) compared favourably well with that of the previously reported naphthoquinone, javanicin (Figure 1).[10]

Compound **2** was also isolated as a dark orange-coloured crystal. It showed a UV λ_{max} at 226.2; 304 and 503.5 (Figure S2 in supplementary data), which is very similar to that of compound **1**. The major difference was the presence of a spin system composed of signals at δ_H 2.93 (d, *J*=7.1, 2H) assigned to H_2-10, 4.08 (m, 1H) assigned to H-11 and 1.24

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Table 1: ^{1}H-NMR Data of Compounds 1 and 2 and Reference Compounds

Position	δ_H Compound 1 (CD_3OD)	δ_H Javanicin ($CDCl_3$)[10]	δ_H Compound 2 (CD_3OD)	δ_H Solaniol ($CDCl_3$)[10,27]
1	-			
2	-			
3	6.37 s (1H)	6.21 (s, 1H)	6.34 s (1H)	6.21 (s, 1H)
4	-			
5	-			
6	-			
7	-			
8	-			
9	2.31 s (3H)	2.30 (s, 3H)	2.34 s (3H)	2.35 (s, 3H)
10	4.04 s (2H)	3.91 (s, 2H)	2.93 d (2H, *J*= 7.1)	2.97–2.93 (m, 2H)
11	-	-	4.08 m (1H)	4.21–4.05 (m, 1H)
12	2.23 s (3H)	2.24 (s, 3H)	1.24 d (3H, *J*= 6.2)	1.32 (d, J = 6.2 Hz, 1H)
5-OH	-	13.26 (s, 1H)	-	13.34 (s, 1H)
8-OH	-	12.86 (s, 1H)	-	13.07 (s, 1H)
2-MeO	3.96 s (3H)	3.94 (s, 3H)	3.94 s (3H)	3.94 (s, 1H)

(d, *J*=6.2, 3H) assigned to CH_3-12. This COSY system is consistent with a substructure arising from the reduction of 11 carbonyl group in **1** to a hydroxyl group. Compound **2** was thus deduced as a dihydro derivative of compound **1** and the proton NMR compared favourably well with that of the previously reported naphthoquinone, solaniol (Figure 1).[10]

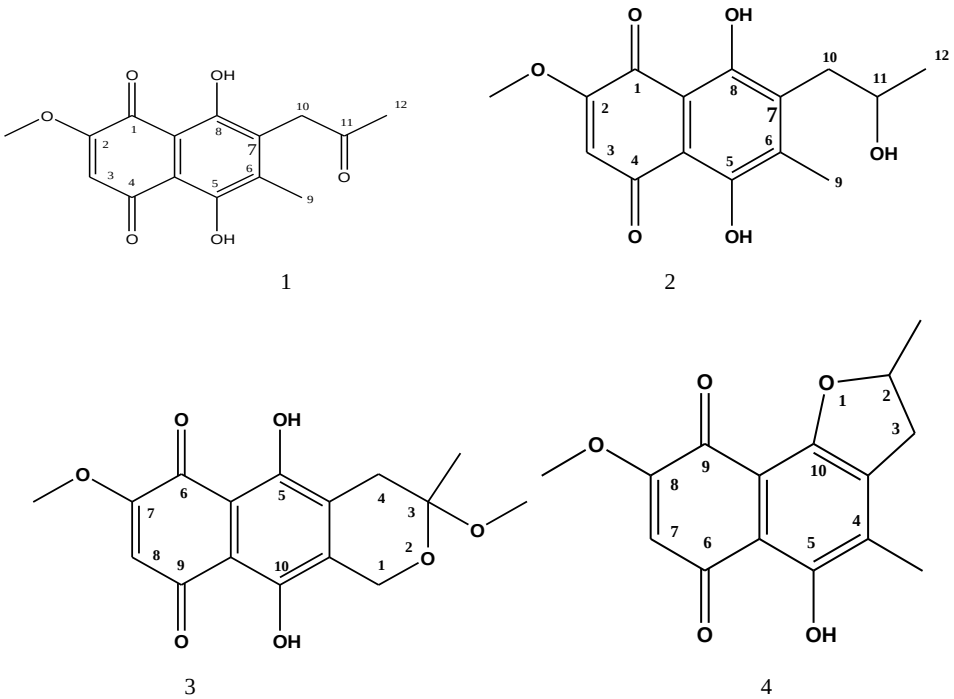

Figure 1: Chemical Structures of the isolated compounds

Compound **3** was isolated as wine-red coloured crystals. It showed UV λ_{max} at 224.4; 301.5 and 595.3 (Figure S3 in supplementary data), which is also characteristic of naphthoquinone skeleton. The NMR spectrum of compound **3** showed signals suggestive a pyran moiety fused with the naphthoquinone substructure. The pyran moiety was confirmed by the presence of 2 diasotropic methylene protons. The first resulted in the deshielded signals observed at δ_H 4.86 dd (1H, *J*=19.3) assigned to H-1A and 4.55 (d, *J*=17.8, 1H) assigned to H-1B. The second diasotropic methylene protons were observed at δ_H 3.00 (m, 1H, 1H) assigned to H-4A and 2.65 (dd, *J*= 18.1, 1H) assigned to H-4B. Other important signals include a methyl singlet at δ_H 1.53 (s, 3H) assigned to CH_3-11, two methoxy singlets at δ_H 3.30 (s, 3H) assigned to 3-MeO and 3.91 (s, 3H) assigned to 7-MeO and an aromatic proton singlet at δ_H 6.16 (s, 1H) assigned to H-8. The NMR data (Table 2) compared

favourably well with that of the previously reported fusarubin, the major difference being the presence of a methoxy singlet signal at position 3. Thus compound **3** is elucidated as 3-*O*-methyl fusarubin (methyl ether fusarubin) (Figure 1).[14]

Table 2: NMR Data of compound 3 and reference compound

Position	δ_H Compound 3 ($CDCl_3$)	δ_H Fusarubin methyl ether[14] ($CDCl_3$)
1	4.86 dd (1H, *J=19*.3, H-1A) 4.55 (d, *J*=17.8, 1B)	4.85 dd (1H, *J=17.8*, H-1A) 4.54 (1H, (d, *J*=17.8, 1B)
2	-	
3	-	
4	3.00 m (1H, H-4A) 2.65 dd (1H, *J*= 18.1, H-4B)	2.99 dd (1H, (1H, *J*= 18.0, H-4A); 2.65 dt (1H, (1H, *J*= 18.0, H-4B)
5	-	
6	-	
7	-	
8	6.16 s (1H)	6.15 (1H, s, H-8)
9	-	
10	-	
11	1.53 s (3H)	1.53 s (3H)
5-OH	12.64 s (1H)	12.63 s (1H)
10-OH	12.92 s (1H)	12.91 s (1H)
3-MeO	3.30 s (3H)	3.30 s (3H)
7-Meo	3.91 s (3H)	3.91 s (3H)

Compound **4** was isolated as red crystals. It showed UV λ_{max} at 224.5, 299.2 and 480.2 (Figure S4 in supplementary data), which is characteristic of naphthoquinones. The ESI-MS exhibited strong pseudo-molecular ion peaks at *m/z* 275.1 [M+1] and 570.9 [2M+23] in the positive mode and at *m/z* 273.2 [M-1] in the negative mode, which are consistent with the molar mass of 274 g/mol and molecular formula of $C_{15}H_{14}O_5$. The NMR spectrum of compound **4** showed signals suggestive of a furan moiety fused with the naphthoquinone substructure. The furan moiety was confirmed by the presence of a diasotropic methylene proton signal at δ_H 3.37 (m, 1H) assigned to H-3A and 2.84 (dd, *J* = 19.5, 1H) assigned to H-3B which showed spin correlation (HH-COSY) with a highly deshielded methine proton signal at δ_H 5.15 (m, 1H) assigned to H-2. There was also the presence of methyl doublet signal at δ_H 1.52 (d, *J* = 6.3, 3H) which showed spin correlation with the H-2 and H-3A/B and assigned to CH_3-2. The other signals associated with the naphthoquinone substructure include an aromatic proton singlet at δ_H 6.19 (s, 1H) assigned to H-7 and the methoxy singlet at δ_H 3.90 (s 3H). The NMR data as shown in Table 3 is in strong agreement with that previously reported for 2,3-dihydro-5-hydroxy-8-methoxy-2,4-dimethyl-naphtho-[1,2-b]-furan-6,9-dione (anhydrojavanicin) (Figure 1).[22]

These compounds have been previously isolated from fungal endophytes as well as soil fungi. We have also proposed a biosynthetic route for the isolated compounds as shown in Figure 2. All the compounds have the same base nucleus of javanicin and as such must have their biogenic origin from compound **1** and this compound, in turn, would have arisen from any of the possible biosynthetic routes to the naphthoquinone nucleus.[23] Understanding the biogenetic origin of secondary metabolites isolated from fungi endophytes is very crucial for several reasons particularly in the context of natural product drug discovery and development. From the knowledge of biogenetic origin, one can gain insight into plausible approaches to adopt in modifying or enhancing the scalable production of novel compounds for improved pharmaceutical or industrial applications. One can also gain insight into the possibility of a metabolic partnership between the isolated fungal endophyte and the host plant.

Javanicin originally isolated from *Fusarium javanicum*, a soil fungus, has been discovered in other *Fusarium* species.[24] Javanicin from *F. solani* of *Glycyrrhiza glabra*, showed good antibacterial, but moderate anti-tuberculosis activity.[25] This is consistent with the earlier report of the moderate antimycobacterial activity of javanicin isolated from *Fusarium sp*. BCC14842, which in addition showed good antifungal activity and moderate cytotoxicity against human cancer cell lines.[14] Javanicin has also been isolated from *Phomopsis sp*. obtained from fresh stem of *Radix Stephaniae Japonicae*, and it showed anti-HIV and cytotoxic activities.[26] *Chloridium sp*. from the root of *Azadirachta indica* has also yielded javanicin, which gave good antimicrobial activities against human pathogens.[24]

Solaniol was first isolated from the culture of a strain of *F. solani* as dark-red crystals, in the company of other coloured naphthoquinones − fusarubin, javanicin (red crystals) and bostrycoidin (dark-brown crystals) − and was given the name solaniol.[27] *F. solani* JS-0169 from the leaves of *Morus alba* has also yielded Solaniol, together with javanicin and other naphthoquinones, and Solaniol showed no cytotoxicity against the human cancer cell lines.[28]

3-*O*-methyl fusarubin belongs to a group of compounds known as pyranonaphthoquinones, which possess a wide range of biological activities.[29,30] It has earlier been isolated from *F. solani* of *Glycyrrhiza glabra*, and exhibited good antibacterial and moderate antimycobacterial activities.[25] Similarly, 3-*O*-methylfusarubin obtained from *Fusarium sp.* BCC14842 showed moderate antimycobacterial as well as cytoxic activities.[14] In another report, a soil *Fusarium* sp yielded 3-*O*-methyl fusarubin, which exhibited significant antiplasmodial activity against a strain of *P. falciparum*.[31] 3-*O*-methyl fusarubin, anhydrofusarubin and its analogue fusarubin have been reported to exhibit an outstanding broad-spectrum antimicrobial activities against human pathogens as well as good radical scavenging properties.

Table 3: NMR Data of Compound 4 and Reference Compound

Position	δ_H (MeOD) compound 4	δ_H (CDCl3) Anhydrojavanicin[22]
1	-	-
2	5.15 m (1H)	5.20 s (1H)
3	3.37 m (1H, H-3A)[#] 2.84 dd (1H, *J* = 19.5*, H-3B)	3.30 dd (1H, *J*=17.9, H-3A) 2.75 dd (1H, *J*= 17.0, 6.35, H-3B)
4	-	-
5	-	-
6	-	-
7	6.19 s (1H)	6.06 s (1H)
8	-	-
9	-	-
10	-	-
2-Me	1.52 d (3H, *J* = 6.3)	1.59 d (3H, *J*= 6.0)
4-Me	2.24 s (3H)	2.25 s (3H)
8-MeO	3.90 s (3H)	3.88 (3H)

#Slight overlap with the solvent peak

**Calculated manually (Figure S13B in Supplementary documents)*

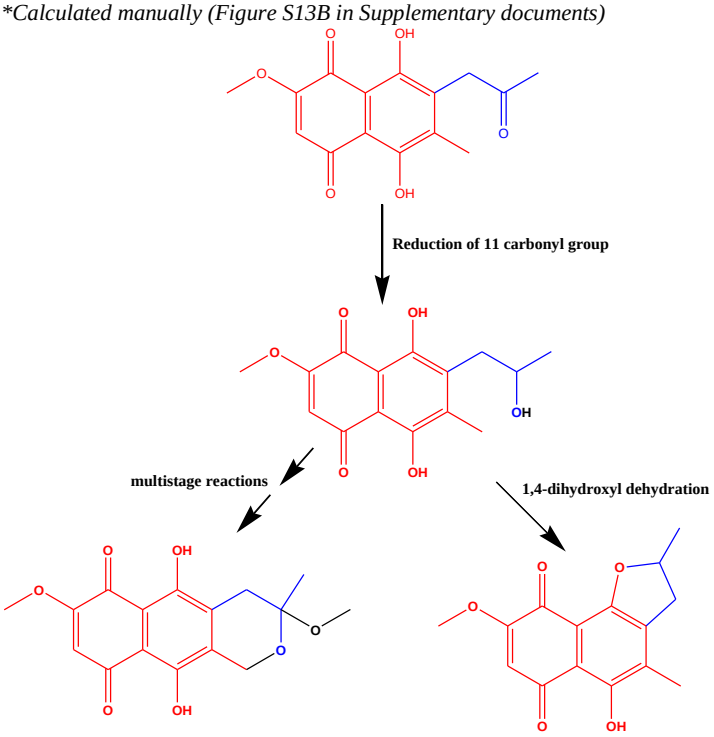

Figure 2: Proposed biosynthetic routes of the isolated compounds

Anhydrojavanicin has previously been isolated from a strain of *F. solani* inhabiting *Chlorophora regia* root,[32] as well as from *Fusarium proliferatum* of the green Chinese onion, and showed good antibacterial activity.[33] Other studies reported a good number of structural analogues of anhydrojavanicin, isolated from the root of *Bulbine capitata*, which showed good antioxidant activities.[34] Anhydrojavanicin has also been isolated along with javanicin and other naphthoquinones from a fungal endophyte of *Radix Stephaniae Japonica*.[26]

Fusarium species are not only known for their production of bioactive compounds, they are also very popular for the variety of pigments they produce, which are already in use in some commercial products like food, cosmetics and textile, as colourants.[11,35] *F. solani* BRM054066 was reported to produce a red pigment structurally identified as fusarubin, dihydrofusarubin, and bostrycoid, which showed antioxidant and anti-inflammatory properties.[36] Some naphthoquinone pigments which were obtained from *F. oxysporum* have their colours range from red to purple.[35] It has been found that the generation of pigments by these microbes is dependent on the culture conditions, such as pH, temperature, and presence/absence of some nutrients like phosphate or nitrogen; they often produce their pigments under stressed conditions.[37] The naphthoquinone dyes are synthesized by these organisms through the polyketide pathway.[38] *Fusarium solani* has been a good source of structurally variable naphthoquinones,[39] which possess different biological and physical properties.

Naphthoquinones are very reactive compounds that could exist as monomers, dimers and tetramers, or be found as furano- and pyrano-derivatives.[40] Pharmacological activities of naphthoquinone derivatives are mainly due to their possession of the 1,4- or less popular 1,2-naphthoquinone core skeleton, which accords them redox and acid-base properties; the ability to accept one or two electrons to form the naphthoquinone mono- or dianion, respectively.[40]

Conclusion

The outcome of this research corroborates the fact that naphthoquinones are one of the most common secondary metabolites of *F. solani* which are popular for their diverse biological activities and pigmentation properties. Some species of *Fusarium* have been identified among the pathogenic fungi from the seed of *Cola nitida* (kola nut). Our present study further contributes to the plethora of endophytic fungi isolated from the leaves of this plant. The isolated naphthoquinones have the potential for development into novel therapeutic agents as well as applications as pigments in food, cosmetics, pharmaceutical, and textile industries.

Conflict of Interest

The authors declares no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Nwobodo DC, Okoye NN, Sifir Mudkhur M, Ikem JC, Eze PM, Okoye FBC, Saki M, Esimone CO. In vitro antiplasmodial and anticancer analyses of endophytic fungal extracts isolated from selected Nigerian medicinal plants. Sci. Rep. 2024; 14(01): 19765. doi: 10.1038/s41598-024-66456-5.
2. Tiwari P, Bae H. Endophytic Fungi: Key Insights, Emerging Prospects, and Challenges in Natural Product Drug Discovery. Microorganisms. 2022; 10(02): 360. https://doi.org/10.3390/microorganisms10020360
3. Wang H, Eze PM, Höfert S, Janiak C, Hartmann R, Okoye FBC, Esimone CO, Orfali RS, Dai H, Liu Z, Proksch P. Substituted L-tryptophan-L-phenyllactic acid conjugates produced by an endophytic fungus *Aspergillus aculeatus* using an OSMAC approach. RSC Adv. 2018; 8(14):7863–7872.
4. Okoye FBC, Lu S, Nworu CS, Esimone CO, Proksch P, Chaldi A. Debbab A. Depsidone and Diaryl Ether Derivatives from the Fungus *Corynespora cassiicola*, an Endophyte of *Gongronema latifolium*. Tetrahedron Lett. 2013; 54(32): 4210–4214.
5. Okoye FBC, Nworu CS, Debbab A, Esimone CO, Proksch P. Two new Cytochalasins from an endophytic fungus, KL-1.1 isolated from *Psidium guajava* leaves. Phytochem. Lett. 2015; 14: 51-55.
6. Fasinu PS, Okoye FBC, Abiodun OO, Kamdem RST, Ogbole OO. Editorial: Fungal Bioactive Metabolites of Pharmacological Relevance. Front. Pharmacol. 2022; 13:912068. doi: 10.3389/fphar.2022.912068
7. Obidiegwu CO, Abba CC, Okoye NN, Okolo CC, Nwachukwu CU, Ujam NT, Okoye FBC. ACL-4, an Endophytic Fungus Isolated from *Ageratum conyzoides* Leaves Possesses the Unique Potential of Generating Low Molecular Weight Bioactive Lead Compounds. Trop. J. Nat. Prod. Res. 2022; 6(12): 2041-2046
8. Gupta S, Chaturvedi P, Kulkarni MG, Van Staden J. A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. Biotech. Adv. 2020; 39(01):107462. doi: 10.1016/j.biotechadv.2019.107462
9. Liao C, Doilom M, Jeewon R, Hyde KD, Manawasinghe IS, Thilini Chethana KW, Balasuriya A, Thakshila SAD, Luo M, Mapook A, Htet ZH, Koodalugodaarachchi V, Wijekoon N, Saxena RK, Senanayake IC, Kularathnage ND, Alrefaei AF, Dong W. Challenges and update on fungal endophytes: classification, definition, diversity, ecology, evolution and functions. Fung. Divers. 2025; 131(08): 301–367. https://doi.org/10.1007/s13225-025-00550-5

10. Maharjan S, Lee SB, Kim GJ, Cho SJ, Nam JW, Chin J, Choi H. Isolation of Unstable Isomers of Lucilactaene and Evaluation of Anti-Inflammatory Activity of Secondary Metabolites Produced by the Endophytic Fungus *Fusarium sp*. QF001 from the Roots of Scutellaria baicalensis. Molecules 2020; 25(04):923. doi: 10.3390/molecules25040923
11. Adione NM, Onyeka IP, Abba CC, Okoye NN, Eze PM, Umeokoli BO, Anyanwu OO, Okoye FBC. Antimicrobial Activities of the Endophytic Fungus, *Fusarium equiseti*, Isolated from the leaves of *Ocimum gratissimum.* J. Adv. Med. Pharm. Sci. 2022; 24(07): 11-23
12. Ebrahim W, Özkaya FC, Ebada SS. Antifungal metabolites from endophytic fungus *Fusarium verticillioides* strain WF18. South Afr. J Bot. 2020; 133(01): 40–44.
13. Ngwoke KG, Tochukwu N, Ekwealor C, Nwankwo V, Obi-Okafor U, Izundu C, Okoye FBC, Esimone C, Proksch P, Situ C. Antibacterial, anti-inflammatory and peroxidase-mediated cyclooxygenase-1 inhibitory properties of *Fusarium solani* extract. Pharm. Bio. 2019; 57(01): 310-317.
14. Khan N, Afroz F, Begum MN, Roy Rony S, Sharmin S, Moni F, Mahmood Hasan C, Shaha K, Sohrab MH. Endophytic *Fusarium solani*: A rich source of cytotoxic and antimicrobial napthaquinone and aza-anthraquinone derivatives. Toxicol. Rep. 2018; 5: 970–976.
15. Ravichandiran P, Sheet S, Premnath D, Kim AR, Yoo DJ. 1,4-Naphthoquinone Analogues: Potent Antibacterial Agents and Mode of Action Evaluation. Molecules 2019; 24(07): 1437.
16. Liu H, Yan C, Li C, You T, She Z. Naphthoquinone Derivatives with Anti-Inflammatory Activity from Mangrove-Derived Endophytic Fungus *Talaromyces sp*. SK-S009. Molecules 2020; 25(03): 576.
17. Adesanwo JK, Ogundele SB, Akinpelu DA, McDonald AG. Chemical Analyses, Antimicrobial and Antioxidant Activities of Extracts from *Cola nitida* Seed. J. Explor. Res. Pharmacol. 2017; 2(03): 67–77.
18. Ekalu A, Habila JD. Phytochemistry, pharmacology and medicinal uses of Cola (Malvaceae) family: a review. Med. Chem. Res. 2020; 29(12): 2089–2105.
19. Nwobodo DC, Ihekwereme CP, Okoye FBC. Screening of endophytic fungal metabolites from *Cola nitida* leaves for antimicrobial activities against clinical isolates of *Pseudomonas aeruginosa*. The EuroBiotech J. 2020; 4(03): 161–166.
20. Damour H, Okoye FBC, Proksch P, Hakiki A, Mosaddak M, Hegazy MF, Debbab A. Pretrichodermamide A and nafuredin from Trichoderma sp, an endophyte of *Cola nitida*. J. Mat. and Environ. Sci. 2015; 6(03): 779–783.
21. Nwobodo DC, Ugwu MC, Chuka M, Okoye FBC, Esimone CO. Antimicrobial and Antioxidant Compounds Produced by Fungal Endophytes Isolated from Selected Nigerian Medicinal Plants. Int. J. Pharm. Phytopharmacol. Res. 2023; 13(03): 6-15
22. Tatum JH, Baker RA, Berry RE. Metabolites of *Fusarium solani*. Phytochem. 1989; 28(01): 283-284
23. Wu, W., Wang, S., Zhang, H, Guo W, Lu H, Xu H, Zhan R, Fidan O, Sun L. Biosynthesis of Novel Naphthoquinone Derivatives in the Commonly-used Chassis Cells *Saccharomyces cerevisiae* and *Escherichia coli*. Appl. Biochem. Microbiol. 2021; 57 (S1), S11–S26. https://doi.org/10.1134/S0003683821100124
24. Kristensen SB, Pedersen TB, Nielsen MR, Wimmer R, Muff J, Sørensen JL. Production and Selectivity of Key Fusarubins from *Fusarium solani* due to Media Composition. Toxins 2021; 13(06): 376; https://doi.org/10.3390/toxins13060376
25. Shah A, Rather MA, Hassan QP, Aga MA, Mushtaq S, Shah AM, Hussain A, Baba SA, Ahmad Z. Discovery of anti-microbial and anti-tubercular molecules from *Fusarium solani* : an endophyte of *Glycyrrhiza glabra*. J. Appl. Microbiol. 2017; 122(05): 1168–1176.
26. Yang Z, Ding J, Ding K, Chen D, Cen S, Ge M. (2013). Phomonaphthalenone A: A novel dihydronaphthalenone with anti-HIV activity from *Phomopsis sp*. HCCB04730. Phytochem. Lett. 2013; *6*(02):257–260.
27. Arsenault GP. Fungal metabolites—III. Quinones from *Fuasrium solani* D2 Purple and Structure of (+)-Solaniol. Tetrahedron 1968; 24(13): 4745–4749.
28. Choi HG, Song JH, Park M, Kim S, Kim CE, Kang KS, Shim SH. Neuroprotective γ-Pyrones from *Fusarium Solani* JS-0169: Cell-Based Identification of Active Compounds and an Informatics Approach to Predict the Mechanism of Action. Biomolecules 2020, 10(01): 1–11.
29. Meyer GW, Bahamon Naranjo MA, Widhalm JR. Convergent evolution of plant specialized 1,4-naphthoquinones: metabolism, trafficking, and resistance to their allelopathic effects. J. Experim. Bot., 2021; 72(02):167–176.
30. Naysmith BJ, Hume PA, Sperry J, Brimble MA. Pyranonaphthoquinones – isolation, biology and synthesis: an update. Nat. Prod. Rep. 2017; 34(01): 25–61.
31. Kumar KP, Javvaji K, Poornachandra Y, Allanki AD, Misra S. Antimicrobial, Anti-plasmodial and Cytotoxicity Properties of Bioactive Compounds from *Fusarium sp*. USNPF102. J. Microbiol. Res. 2017; 7(02): 23–30.
32. Kyekyeku JO, Kusari S, Adosraku RK, Bullach A, Golz C, Strohmann C, Spiteller M. Antibacterial secondary metabolites from an endophytic fungus, *Fusarium solani* JK10. Fitoterapia 2017; 119: 108–114. https://doi.org/10.1016/j.fitote.2017.04.007
33. Jiang CX, Li J, Zhang JM, Jin XJ, Yu B, Fang JG, Wu QX. Isolation, Identification, and Activity Evaluation of Chemical Constituents from Soil Fungus *Fusarium avenaceum* SF-1502 and Endophytic Fungus *Fusarium proliferatum* AF-04. J. Agric. Food Chem. 2019; 67(07): 1839–1846.
34. Bezabih M, Abegaz BM, Dufall K, Croft K, Skinner-Adams T, Davis TME. Antiplasmodial and Antioxidant Isofuranonaphthoquinones from the Roots of *Bulbine capitata*. Planta Med. 2001; 67(04): 340–344.
35. Lebeau J, Petit T, Clerc P, Dufossé L, Caro Y. Isolation of two novel purple naphthoquinone pigments concomitant with the bioactive red bikaverin and derivates thereof produced by *Fusarium oxysporum*. Biotech. Progr. 2019; 35(01): 1–13.
36. Menezes BS, Solidade LS, Conceição AA, Santos Junior MN, Leal PL, de Brito ES, Canuto KM, Mendonça S, de Siqueira FG, Marques LM. Pigment production by *Fusarium solani* BRM054066 and determination of antioxidant and anti-inflammatory properties. AMB Express 2020; 10(01): 117. doi: 10.1186/s13568-020-01054-y.
37. Di Salvo E, Lo Vecchio G, De Pasquale R, De Maria L, Tardugno R, Vadalà R, Cicero N. Natural Pigments Production and Their Application in Food, Health and Other Industries. Nutrients. 2023; 15(08):1923. doi: 10.3390/nu15081923.
38. Valenzuela-Gloria MS, Balagurusamy N, Chávez-González ML, Aguilar O, Hernández-Almanza A, Aguilar CN. Molecular Characterization of Fungal Pigments. J. Fungi 2021; 7(05): 326.
39. Guo R, Cai X, Li Q, Huang Y, Chen B, Guan P, Tang J, Zou X. An efficient high-speed countercurrent chromatography method for preparative separation of javanicin from Fusarium solani, a fungus isolated from the fruiting body of the mushroom *Trametes trogii*. Biomed. Chromat. 2019; 33(09):e4574. doi: 10.1002/bmc.4574.
40. Mone NS, Bhagwat SA, Sharma D, Chaskar M, Patil RH, Zamboni P, Nawani NN, Satpute SK. Naphthoquinones and Their Derivatives: Emerging Trends in Combating Microbial Pathogens. *Coatings*. 2021; 11(04):434. https://doi.org/10.3390/coatings11040434