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Original Article

Antibacterial, antioxidant and neuroprotective activities of crude extract from the endophytic fungus *Fusarium* sp. isolate *OQ-Fus-2-F* from *Euphorbia* sp. plant

[Actividades antibacterianas, antioxidantes y neuroprotectoras del extracto crudo del hongo endofítico *Fusarium* sp. aislado OQ-Fus-2-F de *Euphorbia* sp. planta]

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Abstract

Context: Human life is contested with the emergence of new infectious diseases and antibiotic-resistant pathogens, cellular deterioration due to oxidative stress, and age-related neurodegenerative disorders.

Aims: To evaluate the antibacterial, antioxidant, and acetylcholinesterase inhibitory activities of crude extract from the endophytic fungus *Fusarium* sp. isolate OQ-Fus-2-F as a basis for medical application.

Methods: *Fusarium* sp. isolate OQ-Fus-2-F was isolated from the stem of *Euphorbia* sp. plant. It was identified morphologically and based on 18S rRNA gene partial sequence. The isolated was cultivated in a malt extract glucose medium. Cell filtrate was extracted with ethyl acetate, and the crude extract was dried *in vacuum*. The crude extract was assessed for its antimicrobial activity through agar diffusion and micro-broth dilution assays; antioxidant activity was measured using DPPH and ABTS methods, and the anticholinergic activity was evaluated in terms of inhibition in acetylcholinesterase (AChE) activity.

Results: Crude extract of *Fusarium* sp. isolate OQ-Fus-2-F was more potent in inhibiting the growth of *B. subtilis* with a MIC value of 125 µg/mL. Its antioxidant activity was highly detected in ABTS than DPPH assay with IC_{50} values 37.5 ± 3.5 µg/mL and 191.3 ± 17.6 µg/mL, respectively. Moreover, the extract inhibited the AChE activity with IC_{50} 177.0 ± 13.7 µg/mL.

Conclusions: Crude extract of *Fusarium* sp. isolate OQ-Fus-2-F has potential biomedical applications as it contains secondary metabolites with antimicrobial, antioxidant, and neuroprotective activates.

Keywords: acetylcholinesterase inhibition; Alzheimer's disease; antibacterial activity; antioxidant activity; *Fusarium* sp.

Resumen

Contexto: La vida humana se enfrenta a la aparición de nuevas enfermedades infecciosas y patógenos resistentes a los antibióticos, el deterioro celular debido al estrés oxidativo y los trastornos neurodegenerativos relacionados con la edad.

Objetivos: Evaluar las actividades antibacterianas, antioxidantes e inhibidoras de la acetilcolinesterasa del extracto crudo del hongo endofítico *Fusarium* sp. aislado OQ-Fus-2-F como base para aplicaciones médicas.

Métodos: Fusarium sp. el aislado OQ-Fus-2-F se aisló del tallo de *Euphorbia* sp. planta. Se identificó morfológicamente y se basó en la secuencia parcial del gen ARNr 18S. El aislado se cultivó en medio de glucosa con extracto de malta. El filtrado celular se extrajo con acetato de etilo y el extracto crudo se secó al vacío. Se evaluó la actividad antimicrobiana del extracto crudo mediante ensayos de difusión en agar y dilución en microcaldo; La actividad antioxidante se midió utilizando métodos DPPH y ABTS y la actividad anticolinérgica se evaluó en términos de inhibición de la actividad acetilcolinesterasa (AChE).

Resultados: Extracto crudo de *Fusarium* sp. aislado OQ-Fus-2-F fue más potente para inhibir el crecimiento de *B. subtilis* con un valor de MIC de 125 µg/mL. Su actividad antioxidante fue altamente detectada en ABTS que en el ensayo DPPH con valores de CI_{50} de 37,5 ± 3,5 µg/mL y 191,3 ± 17,6 µg/mL, respectivamente. Además, el extracto inhibió la actividad de AChE con IC_{50} 177,0 ± 13,7 µg/mL.

Conclusiones: Extracto crudo de *Fusarium* sp. aislado OQ-Fus-2-F tiene aplicaciones biomédicas potenciales ya que contiene metabolitos secundarios con activos antimicrobianos, antioxidantes y neuroprotectores.

Palabras Clave: acetilcolinesterasa inhibición; actividad antibacterial; actividad antioxidante; enfermedad de Alzheimer; *Fusarium* sp.

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INTRODUCTION

Endophytes are microorganisms that colonize the internal tissues of plants. De Bary defined the term in 1866 for variable life-history strategies of the microorganism, ranging from facultative saprobes to parasites and from exploitative to mutualistic organisms (Patil et al., 2015). Endophytic fungi are a highly polyphyletic group characterized by their occurrence within the plant host tissues without causing any noticed effects (Hyde and Soytong, 2008). They produce chemicals that induced plant growth, confer resistance to diseases and herbivores, as well as bioactive metabolites with medical significance (Jia et al., 2016). In fact, the biological activities of their metabolites include antioxidant, antimicrobial, antitumor, and anti-inflammatory (Devi et al., 2020). Moreover, several reports have indicated the microbial origin of many plant-derived secondary metabolites (Yan et al., 2018). The production of paclitaxel (Taxol®) as an anticancer drug from the fungus *Taxomyces andreanae* inhabiting the North American yew tree *Taxus brevifolia* represented the first proof that this fungus is the actual producer of the metabolite and not the plant *per se* (Hassan et al., 2007).

The emergence of new infectious diseases and resistant pathogens to available antibiotics, cellular deterioration due to oxidative stress associated with neurodegenerative disorders (i.e., Alzheimer's and Parkinson's diseases) mandates the need to explore new sources for therapeutic compounds from nature; endophytic fungi represent untapped reservoirs of highly diverse metabolites, structurally and functionally (Kjer et al., 2009). Among these fungi are the *Fusarium* group, which belong to the imperfect fungi and include over 300 species that are thriving terrestrial and marine environments (Li et al., 2020). They produce structurally diverse metabolites (polyketides alkaloids, peptides, amides, terpenoids, quinones, and pyranones) with incredible biological activities; they demonstrated great pharmaceutical importance (Jakubczyk and Dussart, 2020; Toghueo, 2020). Therefore, in the present study, a *Fusarium* sp. isolate OQ-Fus-2-F was purified from *Euphorbia* sp. plant and appraised to produce a biologically active crude extract, mainly with antibacterial, antioxidant, and acetylcholinesterase inhibitory potential.

MATERIAL AND METHODS

Plant samples collection and fungal isolation

Euphorbia sp. was collected from the Al-Qatrana area in Al-Karak Governorate, South Jordan (31° 13'04.9"N 36°01'31.4"E, GPS 31.218027, 36.025392). The plant was identified by Dr. Feryal Al-Khraisat, Department of Biology, Mutah University. Isolation of the endophytic fungi was carried out aseptically and described in Hazalin et al. (2009). Briefly, plant areal parts were surface sterilized and cut aseptically into 1 cm long segments that were placed on potato dextrose agar plates (Oxoid, UK) supplemented with 100 µg/mL of streptomycin and penicillin G (Bio Basic Inc, Canada). The agar plates were incubated at room temperature and observed for fungal growth for 2-4 weeks. The grown mycelia with different morphological appearance were picked and transferred to malt extract agar (Oxoid, UK).

Cultivation of *Fusarium* sp. isolate OQ-Fus-2-F

Cultivation of *Fusarium sp.* isolate OQ-Fus-2-F was performed in 2 L Erlenmeyer flask containing 1 L of malt extract glucose broth of the following composition % (w/v): 2% malt extract, 0.4% yeast extract, and 1% glucose, pH 5.6. The culture was incubated on an orbital shaker (Forma Orbital Shaker, Thermo electron cooperation, USA) with 130 rpm agitation and at 27°C. During the cultivation process, 50 mL samples and thereafter-daily samples were taken and used to measure the glucose content using glucose test strips (Combur test, Germany) and pH values using pH-meter (pH 523, WTW, Germany). The decrease in glucose content was determined quantitatively using a fully automated chemistry analyzer (Cormay Accent 200, Switzerland). The culture was harvested when glucose was totally consumed. The mycelia were separated by filtration, and the filtrate was extracted with an equal volume of ethyl acetate. The or-

ganic phase was dried over sodium sulfate (anhydrous) and concentrated *in vacuum* at 45°C. The resulting crude extract was dissolved in methanol to a final concentration of 50 mg/mL and stored at 4°C.

Antibacterial activity

The antibacterial activity of the crude extract was determined by agar diffusion test, and the minimum inhibitory concentration (MIC) was determined by micro-broth dilution assay according to Clinical and Laboratory Standards Institute guidelines and as described in Al-Zereini (2014). Different concentrations of the crude extract were applied on sterile 6 mm blank discs. The discs were placed on Muller Hinton agar plates seeded with an overnight culture of tested bacteria at cell density 10^6 cell/mL. Tested bacterial strains were the Gram-negative bacteria [*Pseudomonas aeruginosa* (ATCC 13048) and *Escherichia coli* (ATCC 25922)] and the Gram-positive bacteria [*Staphylococcus aureus* (ATCC 43300) and *Bacillus subtilis* (ATCC 6633)]. Streptomycin (50 µg/disc, Bio Basic Inc, Canada) was used as a positive control, and the results are presented as means of three independent tests. Type of inhibition, bacteriostatic or bactericidal, was determined by platting the preparations in micro-broth dilution assay, where there were no visible growths, on agar plates with proper medium and incubation for 24 h.

Antioxidant assay

Antioxidant activity was measured in terms of the radical scavenging ability of the tested crude extract to decolorize 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, Germany) or 2,20-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich, Germany). The scavenging ability of DPPH radical was estimated following Ashram et al. (2019) with minor modifications. Different concentrations of the tested crude extract were added to 1 mL methanolic solution of 0.1 mM DPPH (OD_{517nm} 0.7-0.8). The mixture was shaken vigorously and left at 37°C for 20 min. The decrease in the starting absorbance of the resulting solution, OD_{517nm} 0.7-0.8, was measured at 517 nm by UV/Vis spectrophotometer (Novaspec Model. 80-2088-64, Pharmacia Biotech, UK).

Meanwhile, the ability of the crude extract to neutralize and decolorize the $ABTS^{\bullet+}$ radical cation was evaluated, as mentioned in Al-Zereini (2014). Briefly, 7 mM ABTS and 2.45 mM potassium persulfate (final concentration in distilled water) were reacted together and incubation at room temperature in darkness for 16 h to generate $ABTS^{\bullet+}$ radical cation. The resulting $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.005 at 734 nm UV/VIS Spectrophotometer (Novaspec Model. 80-2088-64, Pharmacia Biotech, UK). To 2 mL of diluted $ABTS^{\bullet+}$, 10 µL of different extract concentrations was added and mixed vigorously. The reactive mixture was allowed to stand at room temperature for 6 min, and the absorbance was recorded immediately at 734 nm.

The scavenging activity of tested crude extract and neutralization of DPPH or $ABTS^{\bullet+}$ radicals was calculated according to the formula [1].

$$\text{Scavenging effect (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100 \quad [1]$$

Where $A_{control}$: control absorbance and A_{sample}: sample absorbance.

Methanol (99.5%) was used as a blank, and Trolox standard (concentrations from 0 to 1.5 µg/mL) in ethanol was prepared and assayed using the same conditions. All results were expressed in terms of the mean value of Trolox® equivalent antioxidant capacity (TEAC). The assay was performed in triplicate for each sample and each concentration of standard.

The extract concentration that caused 50% inhibition in formed radicals (IC_{50}) was interpolated from a dose-response curve plotted for percentage inhibitions against respective extract concentrations.

Acetylcholinesterase (AChE) inhibition assay

AChE inhibitory activity was measured by the colorimeter method of Ellman et al. (1961) with slight modification. Briefly, the reaction mixture contained 300 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of different sample concentrations (2-50 µg/mL) and 5 µL AChE (5 U/mL,

EC 3.1.1.7, type VI-S from electric eel, Sigma-Aldrich, Germany). The preparation was incubated for 20 min at 25°C; then 10 µL of 0.01 mM DTBN [5,5-dithiobis(2-nitrobenzoic acid)] and 2 µL of 0.71 mM acetylthiocholine iodide substrate (Sigma-Aldrich, Germany) were added. The final preparation was incubated for 20 min at 25°C, and the developed color was measured at 412 nm using a 96-well microplate reader (BioTek ELx800 UV, USA). A mixture where 5 µL of 0.1 M phosphate buffer (pH 8.0) replaced AChE (5 U/mL) was prepared as a blank, a preparation with 10 µL of 0.1 M phosphate buffer (pH 8.0) instead of the sample served as control (100% enzyme activity), and 10 µL of 1 mg/mL galantamine instead of the sample was used as a positive control. The experiments were carried out in triplicate, and inhibition in enzymatic activity was calculated according to the formula [2].

$$\text{Enzyme inhibition (\%)} = [(AE_{blank} - AE_{sample})/\ AE_{blank}] \times 100 \quad [2]$$

Where, AE_{blank}: Activity of the enzyme without the sample and AE_{sample}: Activity of the enzyme with the sample.

The extract concentration that caused 50% inhibition in AChE activity (IC_{50}) was interpolated from a dose-response curve plotted for percentage activity inhibitions against respective extract concentrations.

Identification of fungal isolate OQ-Fus-2-F

Identification of fungus of interest was performed morphologically according to Watanabe (2010) and by amplifying the 18S rRNA gene partial sequence using ITS-1 and -4 primers (White et al., 1990). Morphological identification was made for a one-week grown culture on malt extract agar as well as on potato dextrose agar plates using a stereomicroscope (Nikon, Japan). Several characteristic features were examined, hyphae form and branching, presence and production of conidia, septa formation in hyphae, a form of conidia or spore, among others.

Fungal DNA was extracted from 100 mg hypha samples using the E.Z.N.A.® Fungal DNA Mini Kit (Omega BioTek, USA). The purified genomic DNA was used to amplify the internal transcribed spacers with the 5.8S rRNA regions of 18S rRNA fragment by PCR. The forward and reverse primer sequences were 5'TCCGTAGGTGAACCTGCGG3' and 5'TCCTCCGCTTATTGATATGC3', respectively. The PCR reaction constituted of 300 ng of genomic DNA, 25 µL iMaxII master mix, 3 µL of each primer (10 pmole/µL), 3 µL $MgCl_2$ and complete with sterile deionized water up to a final volume of 50 µL. The amplification condition was denaturation step (5 min at 94°C), followed by 35 cycles (denaturation at 94°C for 30 s, annealing at 57°C for 30 s, 1 min of elongation at 72°C) and a final extension step of 10 min at 72°C. The amplification product was analyzed by 1% agarose gel electrophoresis at 5 V/cm and visualized under UV.

Preliminary chemical characterization of crude extract

Preliminary identification of main chemical classes in the crude extract of *Fusarium* sp. isolate OQ-Fus-2-F was carried out through thin layer chromatography (TLC) and as described by Jork et al. (1990). The crude extract was spotted on aluminum plates impregnated with silica gel 60 (20 × 20 cm) (Macherey-Nagel, Germany) and separated using (toluene:acetone:acetic acid, 70:30:1) as a mobile phase. The resulting bands were detected under UV light at 260 and 360 nm. The TLC plate was sprayed with 37% formaldehyde/sulfuric acid for detection of alkaloids, p-anisaldehyde/sulfuric acid for detection of phenols, terpenes, sugars, steroids, and 1% ethanolic solution of aluminum chloride for detection of flavonoids.

Statistical analysis

All results are reported as means ± standard deviation (SD**)**. The dose-response relation was appraised using PROBIT regression analysis with a 95% confidence limit (Finney, 1978). Results were analyzed by one-way ANOVA and Tukey HSD *post hoc* test using the Statistical Package for the Social Sciences software (SPSS, version 16). Data were considered significant at $p<0.05$ (one-way ANOVA). Tukey HSD *post hoc* test was carried out on data where $p<0.01$ to identify which of tested factor pairs are significantly different from each other.

RESULTS

During the screening process for the isolation of endophytic fungi with potential production of bioactive crude extract, fungal isolate OQ-Fus-2-F was selected for further investigation. The isolate grew as colonies with fluffy cottony aerial mycelia, white with purple shade pigmentation. The conidiophores were hyaline and arose laterally with flexible irregular secondary conidiophore branches and hyaline phialidic grouped slender cells (sporodochia). The microconidia are kidney-shaped, 0-1 septate cells with 3.7-11.3 µm long and 1.5-5.0 µm wide. The macroconidia were oval, straight to slightly curved, and relatively slender in shape; they were 2–4 septate cells, 23.7-47.5 µm long and 3.7-6.3 µm wide. Chlamydospores were rarely observed (Fig. 1).

Amplification of 18S rRNA gene partial sequence (ITS1-5S rRNA-ITS4) produced ~620 bp amplicon. Relating the sequenced amplicon with those on GenBank database accessed through using online BLAST interface (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that the fungal isolate OQ-Fus-2-F belong to the genus *Fusarium* (phylum *Ascomycota*, family *Nectriaceae*, class *Sordariomycetes*) and is closely related to *Fusarium tricinctum* (Accession no. MK843921.1) (Fig. 2). The partial sequence of 18S rRNA of isolate OQ-Fus-2-F was deposited in the GenBank under the accession number MW047105.1 (https://www.ncbi.nlm.nih.gov/nuccore/MW047105.1).

Cultivation of *Fusarium* sp. isolate OQ-Fus-2-F in malt extract glucose medium required 6 days till all glucose was consumed. The culture pH fluctuated between 5.47 and 6.6, and it was 6.15 at the end of cultivation. The production of antibacterial metabolites was started on the 2nd day of culturing and coincided with the beginning of a decrease in available sugar (Fig. 3). The maximum antibacterial activity was attained on the 4th day and reached a 13 mm inhibition zone against *B. subtilis* at 500 µg/disc. Minimal changes in pH during the cultivation course indicating that the isolated was consuming both the sugar and nitrogen compounds as energy sources.

Chemical analysis of the extract components on TLC revealed different bands that on spraying with chromogenic indicators showed the absence of flavonoids and the presence of alkaloids, terpenoids, and steroids (data not shown). The extract exhibited moderate activity against *B. subtilis* and had a weak effect on *P. aeruginosa*. It caused 13 mm and 10 mm inhibition zones, respectively, at the highest tested concentration (Table 1). The Gram-negative *E. coli* and Gram-positive methicillin-resistant *S. aureus* were insensitive to the tested extract. The antibacterial activity of the tested extract was significantly lower than the bioactivity of streptomycin control ($p<0.05$).

Intriguingly, the extract possessed antioxidant activity; it caused scavenging activity in a concentration-dependent manner at a concentration 40 times higher than Trolox concentration required to cause equivalent effect to DPPH radicals with IC_{50} = 191.3 ± 17.6 µg/mL (40.2 ± 3.7 µg crude extract/µg Trolox) and a concentration 14 times higher than Trolox concentration required to cause equivalent effect to $ABTS^{\bullet+}$ radicals with IC_{50} = 37.5 ± 3.5 µg/mL (14.0 ± 1.3 µg crude extract/µg Trolox). In addition, the extract revealed activity as an AChE inhibitor and caused a reduction in the enzyme activity with IC_{50} = 177.0 ± 13.7 µg/mL (Table 2), a concentration 32 times higher than the galantamine positive control.

DISCUSSION

Human health is challenged with various diseases due to the emergence of new microbial infections with widespread antibiotic resistance, cellular destruction by generated oxygen radicals, and age-associated neurodegenerative disorders. These ailments are encountered with exploring new sources for bioactive metabolites from unconventional habitats. Worth noting, therapeutic agents of microbial origins have intensely been involved in improving human health in the past years (Dos Santos Dias et al., 2019).

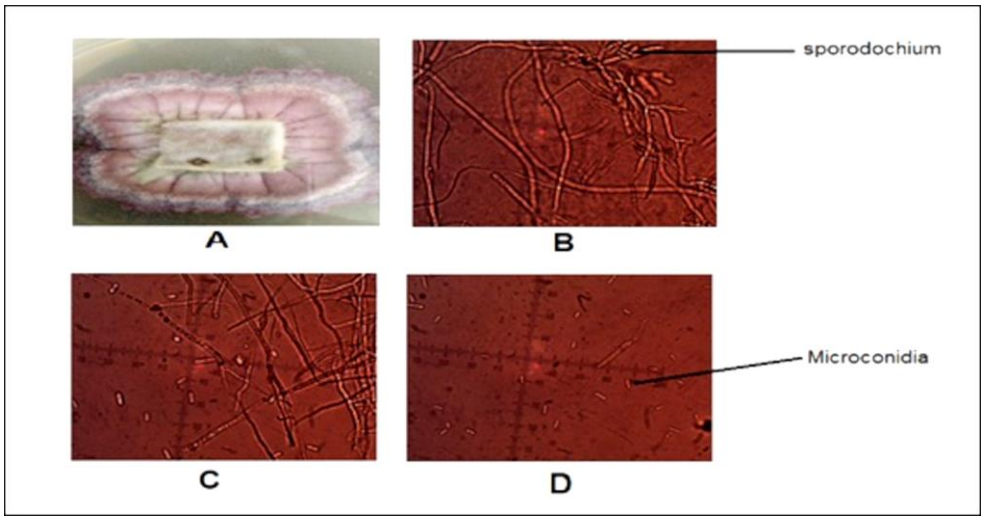

Figure 1. Microphotograph of *Fusarium* sp. isolate OQ-Fus-2-F.

(A) colonies morphology and pigmentation on potato dextrose agar; **(B, C)** Hyaline mycelial growth showing irregular branching and aggregate of conidia (sporodochium); and **(D)** microconidia. Microphotographs were performed: **(A)** using Nikon stereomicroscope (4×); **(B-D)** using Nikon microscope at 400×.

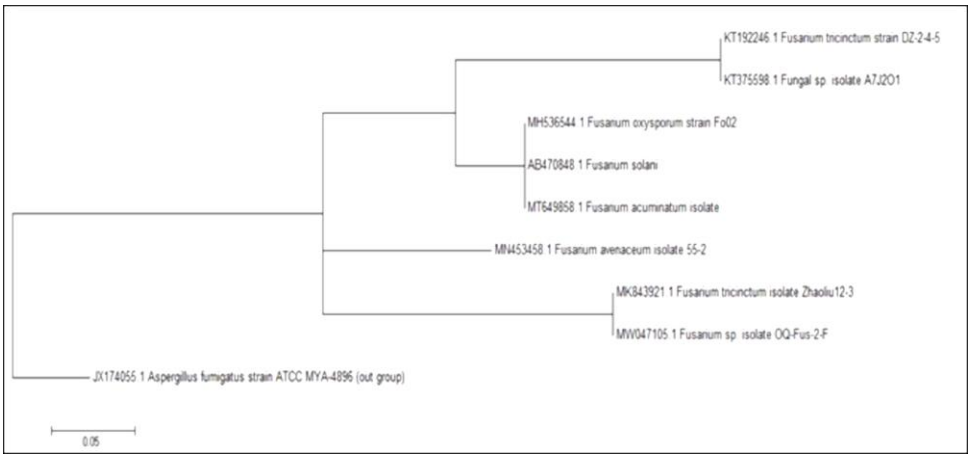

Figure 2. Phylogenetic tree of fungal isolate OQ-Fus-2-F.

Constructed by Maximum Likelihood Algorithm using MEGA7 software (Kumar et al., 2018). The scale bar indicates 5% base substitution per nucleotide position.

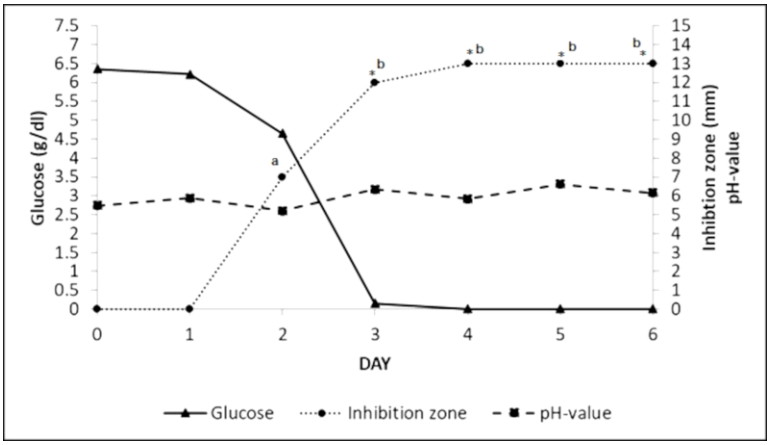

Figure 3. Growth kinetics of *Fusarium* sp. isolate OQ-Fus-2-F in malt extract glucose medium.

Asterisks indicated statistically significant values from 6 mm (diameter of the disc) (*p<0.05). Average inhibition zones, caused by 500 µg/disc against *B. subtilis,* with similar letters are not significantly different from each other based on the *post hoc* Tukey HSD test at p<0.01. Monitored parameters are changes in pH, glucose content, and increased antibacterial activity in daily samples during cultivation.

Table 1. Antibacterial activity of *Fusarium* sp. isolate OQ-Fus-2-F crude extract against tested bacterial strains.

Bacterial strain	Zone of inhibition [mm ± SD] (µg/disc)			Streptomycin (50 µg/disc)	MIC (µg/mL)	
	100	300	500		Crude extract	Streptomycin
Gram-positive						
B. subtilis	7.0 ± 1.0^{a}	10.6 ± 0.6$^{a, b}$	13.4 ± 0.6^{b}	32.3 ± 0.3*	125s	<0.4**
S. aureus	NA	NA	NA	28.6 ± 1.2**	>1000	1.6s**
Gram-negative						
E. coli	NA	NA	NA	25.3 ± 1.5**	>1000	0.8s**
P. aeruginosa	NA	9.3 ± 0.7^{c}	10 ± 1^{c}	22.6 ± 0.6*	1000s	0.8s**

Data are expressed as means ± SD (n = 3), asterisks indicated statistically significant inhibition zone or MIC values due to activity of positive control compared to tested concentrations of crude extract (*0.01<p<0.05, **p<0.01). The mean inhibition zones with similar letters are not significantly different from each other based on the *post hoc* Tukey HSD test at p<0.01, MIC: minimum inhibitory concentration was observed when there was no visible growth, NA: not active at tested concentration, s: biostatic.

Table 2. Antioxidant and acetylcholinesterase inhibitory activities of *Fusarium* sp. isolate OQ-Fus-2-F crude extract.

Test	% inhibition ± SD (µg/mL)					IC_{50} ± SD (µg/mL)
	20	50	100	200	300	
Antioxidant						
DPPH	17.0 ± 3.9^{a}	27.9 ± 1.6^{a,b}	35.4 ± 0.3^{b}	55.5 ± 5.1^{c}	63.7 ± 0.7^{c}	191.3 ± 17.6**
Trolox®						4.76 ± 0.7
ABTS	41.5 ± 3.9^{d}	59.0 ± 3.3^{d,e}	78.2 ± 5.3^{e}	NT	NT	37.5 ± 3.5*
Trolox®						2.67 ± 0.1
AChE	8.0 ± 2.0^{f}	9.9 ± 1.1^{f}	32.5 ± 3.3^{g}	67.1 ± 4.3^{h}	83.5 ± 3.5^{h}	177.0 ± 13.7**
Galantamine						5.5 ± 0.8

Results are expressed as means ± SD (n = 3), asterisks indicated statistically significant IC_{50} values compared to the positive control (*0.01<p<0.05, **p<0.01). Mean % inhibitions with similar letters are not significantly different based on the *post hoc* Tukey HSD test at p<0.01. IC_{50}: concentration that caused 50% inhibition in the absorbance of DPPH or ABTS radicals or acetylcholinesterase activity, NT: not tested.

Fusarium species are a group of filamentous fungi that are widely distributed in various habitats, aquatic, soil, and plant associated. They are prolific species in terms of the production of new metabolites with various biological activities (Sun et al., 2018).

Herein, the fungal isolate OQ-Fus-2-F from the stem of *Euphorbia* plant exhibited promising antibacterial activity against *B. subtilis*, revealed antioxidant efficacy in ABTS assay, and correlated such activity with anti-AChE potential. These bioactivities are attributed to the detected chemical classes of compounds in the crude extract of the isolate. Terpenoid and terpene-derived metabolites were originally identified as plant metabolites. However, they are considered interesting bioactive compounds produced by fungi and invertebrates (Kaaniche et al., 2019). Fusariumin A and asperterpenoid A, terpenoid-derivatives iso-

lated from the endophytic *Fusarium* sp. YD-2 derived from *Santalum album* Chinese tree exhibited moderate to significant anti-Gram-positive and negative bacterial activities with MIC = 6.3-25.2 µg/mL (Yan et al., 2018). Furthermore, the sesquiterpenoid ether fusartricin isolated from the fungal isolated *F. tricinctum,* endophytes in *Salicornia bigelovii* aerial parts, revealed antibacterial and antifungal activities with MIC = 19 µg/mL (Zhang et al., 2015).

Intriguingly, the nortriterpenoid helvolic acid isolated from fungal species such as *Xylaria* sp. and *Aspergillus* sp. had activity against Gram-positive bacteria and was ineffective against Gram-negative bacterial test species (Devi et al., 2020). It was isolated with two other derivatives, helvolic acid methyl ester and hydrohelvolic acid, from the endophytic fungus *Fusarium* sp. in *Ficus carica* leaves, and all had displayed anti-Gram positive and negative bacterial as well as antifungal activities (Liang et al., 2016). In addition, numerous studies demonstrated the bioactivity of sterol compounds, a major component of fungal membrane, and their significant pharmaceutical property (Huang et al., 2009; Dos Santos Dias et al., 2019). For example, 5α, 8α-epidioxyergosta-6, 22-dien-3β-ol purified from *Fusarium* sp. Ppf4 isolated from *Paris polyphylla* var. *yunnanensis* rhizome exhibited antimicrobial activity against bacteria and pathogenic fungi with MIC = 150-1000 µg/mL (Huang et al., 2009).

All the previously literate antimicrobial activities of terpenoid and steroidal derivatives coincided with the antibacterial activity of *Fusarium* sp. isolate OQ-Fus-2-F crude extract detected herein and highlighted that such activity might be due to the terpenoid, and steroid metabolites detected by the TLC. It is proposed that terpenoid and ergosterol compounds exert their antibacterial activity by affecting cellular membrane integrity or function. Ergosterol derivatives affect the bacterial membrane nature and fluidity, as amphipathic lipids with detergent properties. They can interact with the bacterial cell membrane and create transient or permanent pores, in addition to their ability to interfere with the electron transport chain and cause the destruction of oxidative phosphorylation (Tintino et al., 2017). However, terpenoid metabolites interfere with the bacterial membrane and cause the leaching of nucleic acids and proteins from the cell (Sumayya et al., 2020). Furthermore, low susceptibility and resistance of Gram-negative tested bacterial strains to the applied extracts could be attributed to the presence of the outer membrane and negatively charged LPS in the wall of Gram-negative bacteria that confer resistance to the applied compounds repelling them. Different mechanisms of drug resistance in *S. aureus* are known, which might explain its resistance to the tested crude extract.

Worth noting, antioxidants act as radical scavengers and protect the body from several diseases involving asthma, inflammation, arthritis, cardiovascular diseases, diabetes, and neurodegeneration (e.g., Alzheimer's and Parkinson's diseases). Chemical profile screening for the type of metabolites included in the crude extract indicated that the antioxidant metabolites might be alkaloids in nature. In agreement with the recorded biological activity of such compounds, the [-(a-oxyisohexanoyl-N-methyl-leucyl)2-] alkaloid produced by the *F. tricinctum* SYPF 7082, endophytes in the root of the Chinese medicinal plant *Panax notoginseng*, inhibited the NO production by Murine macrophage cell line with IC_{50} = 18.10 ± 0.16 µM (Sun et al., 2018). Recently, the cyclopyazonic acid indole alkaloid derivatives from deep-sea *Aspergillus*. sp. SCSIO 41024 demonstrated antioxidant activity in DPPH assay with IC_{50} = 31.9 to 228.4 µg/mL (Chen et al., 2020). The mechanism behind such activity might be due to the ability of these alkaloids to donate the hydrogen of their OH and NH functional group to neutralize the radicals (Dalimunthe et al., 2018). Remarkably, ABTS was more sensitive in detecting the antioxidant capacity of the tested crude extract than DPPH. It could be attributed to the fact that ABTS detects the antioxidant capacity of hydrophobic and hydrophilic extracts. At the same time, DPPH predicts the antioxidant capacity for hydrophobic antioxidants, and ABTS is soluble in both aqueous and organic solvents and reacts relatively rapidly compared to DPPH (Rivero-Pérez et al., 2007).

Because of their antioxidant efficacy, alkaloids were proposed to have anticholinergic activity and are promising candidates in Alzheimer's disease (AD) treatment through inhibiting the AChE enzyme. According to the cholinergic hypothesis of AD pathogenicity, the level of acetylcholine neurotransmitters in the brain and nerve synapsis decreased due to its hydrolysis by the AChE enzyme. It also accelerates the deposition of extracellular β-amyloid plaque characterizes the disease (Houghton et al., 2006). Synthetic and plant-derived alkaloid drugs (tacrine, donepezil, rivastigmine, and galantamine) were approved as AChE inhibitors for the treatment of AD by USA Food and Drug Administration. However, they are non-selective and have adverse health side effects (Liu et al., 2014). Therefore, AChE inhibitors from natural microbial sources represent an interesting alternative.

The herein documented AChE inhibitory potential of crude extract from *Fusarium* sp. isolate OQ-Fus-2-F might be referred to the included alkaloid metabolites in the extract; a finding that is in line with reported anti-AChE activities of vizoltricine alkaloid from *F. tricinctum* (Solfrizzo and Visconti, 1994), extract of the marine *Aspergillus ochraceus* strain SH0701 (Liu et al., 2014) and huperzine A alkaloid from *Penicillum* spp. LDL4.4 (Le et al., 2019). The proposed mechanism of AChE inhibition by alkaloids was through binding the positively charged nitrogen to the oxyanion in amino acid residues of the enzyme's gorge and binding sites (Houghton et al., 2006).

CONCLUSIONS

The endophytic *Fusarium* sp. OQ-Fus-2-F from *Euphorbia* sp. plant produces bioactive crude extract with interesting antibacterial activity against *B. subtilis* and promising antioxidant activity with AChE inhibitory potential. It could have a pharmaceutical advantage as a drug precursor for treating bacterial infection and oxidative stress-related neurodegenerative diseases; a candidate in treating Alzheimer's and Parkinson's diseases.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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AUTHOR CONTRIBUTION:

Contribution	Al-Zereini W	Al-Mustafa A	Al-Qaralleh O
Concepts or ideas	x	x	
Design	x	x	x
Definition of intellectual content	x	x	
Literature search			x
Experimental studies	x	x	x
Data acquisition			x
Data analysis	x	x	x
Statistical analysis	x		x
Manuscript preparation	x		
Manuscript editing	x		
Manuscript review	x	x	x

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