

Evaluation of antioxidant, cytotoxic, and DNA damage protection activities of endophytic fungus *Aspergillus oryzae* isolated from *Ficus carica* Medicinal Plant

[Avaliação das atividades antioxidantes, citotóxicas e de proteção contra danos ao DNA do fungo endofítico *Aspergillus oryzae* isolado da planta medicinal *Ficus carica*]

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ABSTRACT

Endophytic fungi are friendly microorganisms that colonize plants and play a vital role in how plants interact with their surroundings. They produce useful secondary metabolites that are beneficial to humans and plants. Endophytic fungi with bioactive properties were recovered from *Ficus carica* medicinal plant's leaves. A potent isolate was identified as *Aspergillus oryzae*. Biological activity of this fungus' crude ethyl acetate (EtOAc) extract was investigated. *A. oryzae* extract effectively scavenges radicals against DPPH, with an IC₅₀ value of 29.8 µg mL⁻¹. It demonstrated significant cytotoxic effects against HepG-2 (IC₅₀ = 30.6 µg mL⁻¹), HeLa (IC₅₀ = 33.1 µg mL⁻¹), and MCF-7 (IC₅₀ = 20.9 µg mL⁻¹). Numerous bioactive functional groups are present in EtOAc of *A. oryzae* product, according to FT-IR and GC-MS spectral analysis. This validated feature of major different potent compounds, including Oleic acid methyl ester, behenic acid, methyl ester, linoleic acid ethyl ester, eicosanoic acid, methyl ester and cis-5,8,11,14,17- eicosapentaenoic acid. Because these chemicals are biologically relevant in a variety of ways, and *in vitro*, potential biological activity of fungus extract was acknowledged. *A. oryzae* seems to have potential sources of bioactive natural compounds. This might serve as a solid basis for pharmacological applications.

Keywords: natural products, bioactivity, endophytic fungi, GC-MS, *Ficus carica*

RESUMO

Os fungos endofíticos são microrganismos amigáveis que colonizam as plantas e desempenham um papel fundamental na forma como as plantas interagem com seu ambiente. Eles produzem metabólitos secundários úteis que são benéficos para os seres humanos e as plantas. Fungos endofíticos com propriedades bioativas foram recuperados das folhas da planta medicinal *Ficus carica*. Um isolado potente foi identificado como *Aspergillus oryzae*. A atividade biológica do extrato bruto de acetato de etila (EtOAc) desse fungo foi investigada. O extrato de *A. oryzae* elimina eficazmente os radicais contra DPPH, com um valor IC₅₀ de 29,8 µg mL⁻¹. Ele demonstrou efeitos citotóxicos significativos contra HepG-2 (IC₅₀ = 30,6 µg mL⁻¹), HeLa (IC₅₀ = 33,1 µg mL⁻¹) e MCF-7 (IC₅₀ = 20,9 µg mL⁻¹). Diversos grupos funcionais bioativos estão presentes no EtOAc do produto da *A. oryzae*, de acordo com a análise espectral FT-IR e GC-MS. Essa característica validada dos principais compostos potentes diferentes, incluindo éster metílico do ácido oleico, ácido behênico, éster metílico, éster etílico do ácido linoleico, ácido eicosanoico, éster metílico e ácido cis-5,8,11,14,17- eicosapentaenoico. Como esses produtos químicos são biologicamente relevantes de várias maneiras e *in vitro*, a atividade biológica potencial do extrato do fungo foi reconhecida. O *A. oryzae* parece ter uma fonte potencial de compostos naturais bioativos. Isso pode servir como uma base sólida para aplicações farmacológicas.

Palavras-chave: produtos naturais, bioatividade, fungos endofíticos, GC-MS; *Ficus carica*

INTRODUCTION

For many years, microorganisms and plants have been rich sources of effective drugs with strong anti-multi-disease activity (Aly *et al.*, 2008). Plants are natural sources of valuable molecules that can aid in developing novel treatments. As a result, the plant's antibacterial, antiulcer, anti-inflammatory, analgesic, antioxidant, and wound-healing qualities have been extensively researched throughout the world. It nevertheless requires time to grow the plants and harvest their bioactive metabolites. Novel resource research is gaining popularity as a result, especially endophytic fungi (endophytes), which are derived from medicinal plants and can synthesize compounds unique to plants (Aladesanmi *et al.*, 2006). Nearly every plant that has been investigated thus far has endophytes. These microorganisms reside within plant tissues without harming the plants (Tan and Zou, 2001), are linked to live plant tissues, and do not produce any signs of their presence in the plant (Mengistu, 2020). Endophytic fungi help to defend their hosts from harmful bacteria by competing for the food resources and habitat of their hosts (Sun *et al.*, 2011). Furthermore, they strive to hinder infection by hazardous organisms by promptly infecting their hosts and consuming resources required for pathogenic microbe growth (Liu *et al.*, 2010). Moreover, they can operate as protective systems for their hosts, protecting them from predators such as animals and insects (Alhakmani *et al.*, 2014; Meena *et al.*, 2017).

Fungi have been shown to produce more metabolites than other endophytes, suggesting that they could be sources of naturally occurring bioactive compounds (Schulz and Boyle, 2005). It is critical to choose an appropriate host plant that contains unique bioactive endophytes. Research indicated that plants grown in unique environmental conditions that are resistant to stress arouse interest in studying their indoor plants, as the biological activity and growth conditions of the plant are among the important criteria for choosing to examine the indoor plant (Strobel, 2006). It is highlighted that traditional herbal medicine plants are also a potential source of bioactive endophytes (Strobel, 2006).

Recognition of endophytic fungi as a group of organisms is growing that produce new

metabolites of industrial significance (Jin *et al.*, 2018). The pharmaceutical and agricultural industries are looking into them because they seem to be an undiscovered source of secondary metabolites. The secondary metabolites screened from the fungal endophytes like macrolides, phenolics, taxols, and terpenoids, could act as antileprotic, antibacterial, antifungal, and anti-malarial and as well possess cytotoxic impacts (Huang *et al.*, 2007). Common characteristics of endophytic fungi include the synthesis of extracellular enzymes for restricted colonization and penetration of selected plant cells. Thus, they are also potential sources of esterases, amylases, ligninases, cellulases, proteases, pectinases, and lipases (Huang *et al.*, 2007).

Ficus carica (Syn: *Ficus sycomorus*; family: Moraceae) is commonly referred to as "fig". The domesticated fig originated in the arid regions of Asia Minor. It develops into a low-branched deciduous tree or shrub. Large, wavy-margined leaves usually contain five lobed segments, though they might have only three or four (McGovern, 2002). Its fruit, root, and leaves are used in the traditional medicinal system to treat a range of ailments. *Ficus* is one of the most abundant genera of medicinal plants. Numerous biological activities, including anti-rheumatic, antioxidant, anti-inflammatory, and laxative properties, have been connected to it (Joseph and Raj, 2011). *Ficus* species have been shown in previously published research to be effective in treating tuberculosis. They have been applied topically to treat skin conditions like tinea and eczema (Abdel-Hameed, 2009; Di Pierro *et al.*, 2014). Interestingly, the genus *Ficus* has a wide range of applications in traditional medicine, including the treatment of gastrointestinal, respiratory, and cardiovascular illnesses, and the fruit paste was utilized to exert antispasmodic, analgesic, and anti-inflammatory effects (Mawa *et al.*, 2013; Barolo *et al.*, 2014). Additionally, *Ficus ingens* extract was found to have a hepatoprotective effect against liver injury in albino rats. Furthermore, cancer suppressive, antioxidant, antiviral, hypoglycemic, and anthelmintic activities of *F. carica* extract (Joseph and Raj, 2011; Herre *et al.*, 2008) and the anticancer effect of *Ficus benghalensis* extract (Abdel El Raheim *et al.*, 2013) were previously discovered.

Almustafa and Yehia (2023) report that Saudi Arabia's Al-Ahsa Oasis is one of the world's most productive agricultural areas. It also has many unexplored variants and is abundant in kinds recognized for its outstanding processing qualities. Numerous investigations revealed that the oasis is home to a sizable number of medicinal plants in addition to its significant economic significance (Almadini *et al.*, 2021).

Although *F. carica* is prevalent in the environment, few research have been conducted to investigate the endophytic fungi that coexist with it. This work aims to isolate fungal endophytes from the medicinal plant *F. carica* and assess their biological capabilities.

MATERIALS AND METHODS

Plant Samples Collections. Fresh, healthy *F. carica* leaves were gathered in March 2022 from Al-Ahsa Oasis in the eastern part of Saudi Arabia (25°38'43.00" N 49°58'52.20" E). Within 24 h after collection, the samples were rinsed under running tap water and then washed twice with deionized water that had been double-filtered to remove any surface particles such as dust and bird droppings. Segments of one centimeter by one centimeter were cut from the cleaned leaves. Following sequential washes in 4% sodium hypochlorite solution (90 s), 70% ethanol (30 s), and sterilized distilled water (20 s), the surface

was disinfected under aseptic conditions before being allowed in the laminar airflow chamber to air dry (Kamel *et al.*, 2020).

Isolation of fungal Endophyte isolates. To isolate endophytic fungi, sterilized sections were placed onto potato dextrose agar (PDA, 20g agar, 20g dextrose, 4g potato extract, and 1 L water, HiMedia, Mumbai, India) medium enriched with 100µg mL⁻¹ streptomycin to inhibit the growth of bacteria. Endophytic fungi often begin to generate hyphal filaments after 4-6 days at 28°C. Every day, the development of the fungal endophytic colonies from the segments was observed in Petri dishes, and the individual hypha tips were promptly selected and moved to a fresh PDA medium. This procedure was repeated multiple times until the sole results were pure fungal colonies. Following standard identification protocols (Beharka and Nagaraja, 1998; Seifert, 2008; Verma *et al.*, 2009; Kamel *et al.*, 2020), the recovered pure fungal isolates were first identified depending on their morphological and microscopic features. The codification of endophytic fungus found on *F. carica* leaves is SI-01–SI-21. The colonization frequency (CF) and dominance (DF) of endophytic fungal species have been calculated in the following ways (Hata and Futai, 1995; Kumar and Hyde, 2004) to approximate their abundance and preference distribution:

$$CF = \frac{\text{number of segments colonized by fungi}}{\text{total number of segments observed}} \times 100,$$

$$DF = \frac{\text{percentage colony frequency}}{\text{the sum of the percentage of colony frequency of all fungi}} \times 100.$$

Preliminary Examination of the Bioactive Characteristics of Fungal Isolates. Of the several fungal isolates obtained from surface-sterilized leaf fragments with a high colonization rate were the endophytic isolates utilized in this study.

Antifungal Activity. Using a dual culture strategy, the antifungal activity of four fungal isolates (SI -06, SI -08, SI -10, and SI -15) was assessed against pathogens (*Fusarium oxysporum*, *Candida albicans*, *Alternaria alternata*, *Pythium ultimum*, and *Botrytis cinerea*) that were gently donated by King Saud University, Department of Botany and Microbiology (Lu *et al.*, 2022). As a result, the middle of the PDA plates was injected with

pathogens, and three of the corners were seeded with typical endophytic isolates (5 mm) for examination. Every experiment was run three times, and at 28 °C, all plates were incubated for five to eight days. Antifungal action was demonstrated by the investigated fungal pathogen's mycelial growth being blocked in the pattern of an active endophyte. The fungal growth radius was subtracted from the fungal growth distance (mm) in the antagonist colony's direction to determine the degree of inhibition. Three classifications were created based on the breadth of the inhibitory zones: <2 mm (weak inhibition, +), 2–10 mm (moderate inhibition, ++), and >10 mm (strong inhibition, +++).

Fungal Metabolites Extraction. Up until the stationary phase was reached, each isolate (SI-06, SI-08, SI-10, and SI-15) was cultivated in a 500 mL conical flask with 150 rpm shaking for 5 to 7 days at 25°C that contained 200mL of sterilized potato dextrose broth (PDB (L⁻¹), potato extract 4g, dextrose 20g, Oxoid, UK). Following the incubation period, sterile Whatman No. 1 filter paper was used to filter the mycelial biomass that had formed in the flask to eliminate the mycelial mat. After that, the supernatant was firmly shaken in a separating funnel containing an equal volume of ethyl acetate (EtOAc) for 15 to 20 min. Before being evaporated using a rotary evaporator (Ika, Germany). After that, the metabolite-containing EtOAc phase was collected in a 500mL conical flask and the process was repeated twice. The crude extracts were then concentrated and maintained at 4°C.

Secondary Metabolites Screening. The crude extracts of endophytic fungal isolates were subjected to preliminary phytochemical screening according to the standard protocol described by Kokate *et al.* (2005) and Maobe *et al.* (2013).

Terpenoids. 3 mL of H₂SO₄ have been added to 2mL of chloroform, and 1 mL of crude fungal extract was added. The existence of terpenoids was shown by the development of a reddish-brown shading.

Tannins. The addition of FeCl₃ to the fungal crude extract produced a bluish-black hue that indicated the presence of tannins.

Flavonoids. A 20% NaOH solution was added in two to three drops to 1mL of fungal crude extract. The yellow hue suggested the presence of flavonoids.

Phenols. 5mL of distilled water were combined with 1mL of fungal crude extract, and two to three drops of a 5% ferric chloride solution were added last. Phenols were characterized by a dark green tint.

Saponins. 1mL of fungal extract was vigorously shaken for 10 min in a test tube. The saponin emulsion had some stability.

Steroids. 1mL of the fungal extract was combined with 3mL of chloroform, then filtered. Carefully, a few drops of H₂SO₄ were added. An interface with a blue-green ring showed an abundance of steroids.

Alkaloids. One mL of fungal crude extracts, 5mL of 2 M HCl solution, and a few drops of Mayer's reagent (3mL of potassium iodide combined with mercuric chloride solution) were combined. Turbidity or a cream-colored precipitate was thought to be an indicator of the existence of alkaloids.

Large-Scale Cultivation. A 1000 mL Erlenmeyer flask containing sterilized PDB was filled with pure fungal isolate after the most potent endophyte (SI-08) was selected based on its *in vitro* inhibitory activity against different pathogens and phytochemical investigation results. The cultivation was incubated at 28±2°C for four weeks. After the incubation period, the cultures were removed utilizing EtOAc in an equivalent volume. The combination was vacuum filtered through a Buchner funnel, and then it was continuously extracted with EtOAc until it was completely gone. Utilizing a rotatory evaporator (Buchi, Switzerland), the EtOAc extract was condensed to yield the crude fungal extract. Following that, 100µg µL⁻¹ of dimethyl sulphoxide (DMSO) was added to the crude extract to dilute it. It was a syringe that was employed for biological activity analysis.

Genotypic Identification. The ribosomal ribonucleic acid (RNA) gene's ITS1-5.8S-ITS2 and ITS sections were sequenced to support the SI-08 isolate's morphological identification. Direct extraction of the whole genomic DNA of the fungus was done from mycelium that was actively developing in potato dextrose broth (PDB, Oxoid, UK) using the SDS extraction methodology described by Plaza *et al.* (2004). The polymerase chain reaction (PCR, Applied Biosystem, USA) was performed on the extracted DNA using primers ITS1: TCCTCCGCTTGATATGC and ITS4: TCCGTAGGTGAACCTGCGG (White *et al.*, 1990). Subsequently, the amplified product was purified using the BigDye Deoxy Terminator cycle-sequencing kit (Applied Biosystems, Darmstadt, Germany) and sequenced using an automated DNA sequencer (ABI PRISM 3700). At <http://www.ncbi.nlm.nih.gov/blast>, the

obtained fungal sequence was compared to species sequences from the NCBI GenBank with prior knowledge. The similarity between amplified DNA sequences and those in the GenBank database served as the basis for the identification of endophytic fungus. The accession number of the pertinent isolate was obtained. The phylogenetic analysis of sequences was built with MEGA version 7.0.

Antioxidant Assay. Employing a radical scavenging experiment termed DPPH (Sigma-Aldrich, St. Louis, MO, USA), the antioxidant activity of the EtOAc extract was examined. With minor adjustments, the method was taken from Yehia (2022) and involved making a solution (0.1mM) by dissolving 1.9 mg of DPPH in 100mL of ethanol, letting it sit in the dark for one hour, and then combining 1mL of it with 2mL of fungal extract at varying concentrations (5, 10, 25, 50, and 100µg mL⁻¹). The mixture was agitated and left to stand at room temperature for 60 minutes. A Spectrophotometer Plus, Japan, was used to quantify the absorbance of the resultant solution at 517nm. Substituted for using DPPH solution, ethanol was used as a control. Instead of using a sample, distilled water was utilized to create a blank. Vitamin C was chosen as a positive control in the experiment (Vc, Merck, India). The following formula was implemented to determine how well DPPH radicals (DC) might scavenge free radicals:

$$DC = 1 - \frac{As - Ab}{A0} \times 100$$

where As, Ab and A0 correspond for the sample, background, and blank absorbance values, respectively.

Based on the DC (%) dependency curve, the IC₅₀ (µg mL⁻¹, test sample concentration that decreases the DPPH absorption by 50%) was established. Each trial (n = 3) was run in triplicate and findings were reported as mean values.

Cytotoxic Assay. Mahnashi *et al.* (2021) outline the way the MTT test was used to enumerate the reduction in MTT in viable cells (yellow to purple) by assessing the effect of EtOAc crude extract on the cell viability of three tumor cell lines; breast carcinoma (MCF-7), cervical carcinoma (HeLa), and liver carcinoma

(HepG-2) (Shanghai Bioleaf Technology Co. Ltd., Shanghai, China). 100µL of Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Gaithersburg, MD, USA) supplemented with 100 U mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin (Sigma-Aldrich, St. Louis, MO, USA) was used to propagate cell lines at a density of 5000 per well. The control cells were provided with only the media, whereas the seeded cell lines were exposed to different dosages of 5, 10, 25, 50, and 100µg mL⁻¹. The plates were incubated for two days at 37°C in a humidified atmosphere with 5% CO₂ (Thermo, Forma 370). After incubation, 10 µL of [MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well at a dose of 5 µg mL⁻¹, and a further hour was given to the mixture to incubate at 37°C. After 10 min of shaking in an automated shaker in the dark, 100µL of DMSO was added to each well to dissolve the purple formazan crystals. The data were then measured in a microplate reader (Infinite 200 Pro, Tecan, Switzerland). By dividing the sample's absorbance of 570 nm by the absorbance of the control and multiplying the result by 100, the percentage of cell viability was calculated. Additionally, the IC₅₀ values—the concentration of EtOAc crude extract that reduces cell viability by 50%—were ascertained. Each experiment was run in three replicates (n = 3).

DNA Damage Protection Assay. The ability of endophytic fungal EtOAc extract to protect oxidative λ-DNA (Merk, India) against the damaging impacts of hydroxyl radicals generated by Fenton's reagent was assessed, however with some adjustments to the procedure outlined by Ghanta *et al.* (2007). Before the addition of loading dye, 10µL of Fenton's reagent (1 mM FeSO₄, 25 mM H₂O₂ in Tris buffer 10mM, pH 7.4) was incubated with 3µL of 0.5µg of λ-DNA at a final reaction volume of 30µL at 37°C for 45 min, with or without varying extract dosages (5µL: 0.1, 1.0, and 10µg mL⁻¹). The proportional changes between native and oxidized DNA were examined using 1% agarose gel electrophoresis. The gel was seen and the band intensity was recorded using a Gel Doc system (Bio-Rad, Hercules, CA, USA). Quercetin was the positive control that was used.

FT-IR Analysis. Thermo Scientific, Waltham, Massachusetts, USA, provided a Nicolet 6700

spectrometer for the Fourier-Transform Infrared analysis of the crude EtOAc extract. 10 mg of anhydrous KBr powder and 1 mg of dry EtOAc fungal extract were combined to create a potassium bromide-KBr pellet. The spectrum with a 4000–500 cm^{-1} scan range. The resulting spectra were examined and documented.

Identification of Bioactive Constituents by GC-MS. Gas chromatography-mass spectrometry (GC-MS) on an Agilent 7820A Gas Chromatography (GC, Agilent Technologies Inc., Santa Clara, CA, USA) paired with a mass spectrometer (MS, ISQ Single Quadrupole, Waltham, MA, USA) was employed to investigate the fungal EtOAc crude extract to determine the presence of bioactive chemicals. 1 μL of the fungal extract was injected into the chromatographic column (HP-5MS column, 30 m $\times$ 0.25 mm, 0.25 μm film thickness) using the autosampler AS1300. After being held at 40°C for 2 min, the instrument's temperature was increased to 260°C. It was then progressively raised to 290°C for 2 min. The carrier was ultra-pure helium, applied at a flow rate of 1 mL min^{-1} . The ionization voltage was 70 eV in total. We selected a mass spectral scan range of 45–1000 m/z . The mass spectra and retention durations (RT) of the bioactive compounds were determined and compared to information from the National Institute of Standards and Technology library (NIST14, US) to determine their identity.

Statistical Analysis. The mean and standard deviation (SD) of the data were determined using three ($n = 3$) replicates. The data was studied using SPSS v21.0, a statistical program (SPSS, Inc., Chicago, IL, USA).

RESULTS

Isolation and morphological identification of fungal endophytes. This is most likely the first study in the Eastern section of Saudi Arabia describing the endophytic fungi that colonize *F. carica* leaves. To identify isolates at first, spore morphology, microscopic analyses, and

morphological characteristics were employed. After 380 leaf segments were successfully recovered, all the fungal isolates were categorized into 10 genera. 206 endophytic fungi were found, exhibiting a 50.9% total colonization frequency. The obtained fungi were morphotypically identified as belonging to 21 species, as illustrated in (Table 1). *Aspergillaceae* (32.5 %), *Pleosporaceae* (19.4%), *Glomerellaceae* (14.6%), *Chaetomiaceae* (8.2%), *Trichosphaeriaceae* (6.8%), *Davidiellaceae* (6.3 %), *Trichocomaceae* (5.8%), *Hypocreaceae* (4.4%) and *Nectriaceae* (2.8%) were the nine families constituted the class Ascomycota of fungi, which accounted for most endophytes. Starting from this point, the most common and frequently colonized species were *Aspergillus oryzae* (17.5%), *Colletotrichum gloeosporioides* (10.2%), *Alternaria tenuissima* (8.7%), and *Aspergillus tamari* (7.3%). Therefore, isolates were chosen for further preliminary screening examination.

Preliminary Antifungal Activity Screening. *A. oryzae*, *C. gloeosporioides*, *Alt. tenuissima*, and *A. tamari* were the four main fungal isolates that were assessed utilizing the dual culture technique to determine their possible antagonistic activity against fungal pathogens; *P. ultimum*, *C. albicans*, *F. oxysporum*, *Alt. alternata*, and *B. cinerea*, (Table 2). The findings of this investigation were coded as follows: no inhibition (–), weak inhibition (+), moderate inhibition (++) , or robust inhibition (+++) towards pathogens. Our results showed that all endophytic fungi inhibited at least two of the tested diseases. Further, moderate inhibition was shown by *Alt. tenuissima* for *P. ultimum* and *C. albicans*, whereas no inhibition for *Alt. alternata*, *F. oxysporum* and *B. cinerea*. Conversely, *C. gloeosporioides* documented no inhibition against *Alt. alternata*, *F. oxysporum*, and *B. cinerea*, but there was a weak inhibition against *C. albicans* and a substantial inhibition against *P. ultimum*. By comparing the inhibitory influence induced by other isolates, it was concluded that the *A. oryzae* isolate displayed remarkable antifungal potential against microbial infections.

Table 1. Colonization and dominant frequency of endophytic fungi from *F. carica* leaves

Endophytic fungi			No.	*CF%	*DF%
Genus	Sp.	Code of isolate			
<i>Penicillium</i>			12		
	<i>P. funiculosum</i>	SI -01	8	1.9	3.9
	<i>P. rubens</i>	SI -02	4	0.9	1.9
<i>Chaetomium</i>			17		
	<i>C. globosum</i>	SI -03	11	2.7	5.3
	<i>C. crispatum</i>	SI -04	6	1.5	2.9
<i>Aspergillus</i>			67		
	<i>A. niger</i>	SI -05	11	2.7	5.3
	<i>A. tamari</i>	SI -06	15	3.7	7.3
	<i>A. terreus</i>	SI -07	5	1.2	2.4
	<i>A. oryzae</i>	SI -08	36	8.8	17.5
<i>Colletotrichum</i>			28		
	<i>C. magnum</i>	SI -09	9	2.2	4.4
	<i>C. gloeosporioides</i>	SI -10	21	5.1	10.2
<i>Trichoderma</i>			9		
	<i>T. viridae</i>	SI -11	3	0.8	1.5
	<i>T. harzianum</i>	SI -12	6	1.5	2.9
<i>Nigrospora</i>			14		
	<i>N. sphaerica</i>	SI -13	14	3.4	6.8
<i>Alternaria</i>			21		
	<i>Alt. alternata</i>	SI -14	3	0.8	1.5
	<i>Alt. tenuissima</i>	SI -15	18	4.4	8.7
<i>Fusarium</i>			6		
	<i>F. moniliforme</i>	SI -16	4	0.9	1.9
	<i>F. oxysporum</i>	SI -17	2	0.5	0.9
<i>Cladosporium</i>			13		
	<i>C. sphaerosprum</i>	SI -18	3	0.8	1.5
	<i>C. herbarum</i>	SI -19	10	2.4	4.8
<i>Curvularia</i>			19		
	<i>C. lunata</i>	SI -20	13	3.2	6.3
	<i>C. pallescens</i>	SI -21	6	1.5	2.9
Total			206	50.9	

*CF%; percentage of colonialization frequency, *DF%, percentage of fungal dominance frequency.

Table 2. Screening for antifungal activity of endophytic fungi isolated from *F. carica* against different fungal pathogens via dual culture method

Pathogenic fungi	Endophytic isolate			
	<i>A. oryzae</i>	<i>C. gloeosporioides</i>	<i>Alt. tenuissima</i>	<i>A. tamari</i>
<i>P. ultimum</i>	+++	++	++	+
<i>B. cinerea</i>	+++	—	—	—
<i>F. oxysporum</i>	+++	—	—	+
<i>C. albicans</i>	+++	+	++	+
<i>Alt. alternata</i>	+++	—	—	++

Inhibition zones: not active (—), (weak inhibition, +); <2 mm, (moderate inhibition, ++); 2–10 mm and (strong inhibition, +++); >10 mm.

Extraction of Secondary Metabolites. After growing on PDB medium, *A. oryzae*, *A. tamari*, *C. gloeosporioides*, and *Alt. tenuissima* were

extracted with EtOAc, yielding crude secondary metabolites of 67.5mg, 15.2mg, 10.9mg, and 21.2mg, respectively. The metabolites produced

by EtOAc crude extracts were analyzed to identify a potent fungal isolate for the following investigation.

Qualitative Assessing for Secondary Metabolites. Chemical analysis was performed on fungal crude extracts to determine the presence or absence of chemical scaffolds, including saponins, steroid phenols, tannins, alkaloids, terpenoids, and flavonoids (Table 3). In the EtOAc extract of *C. gloeosporioides*, tannins, flavonoids, phenols, and steroids were found, but

only alkaloids and saponins were found in the phytochemicals of *Alt. tenuissima*. Conversely, *A. tamari* only revealed phenols, tannins, terpenoids, alkaloids, and flavonoids. *A. oryzae*, on the other hand, disclosed every bioactive metabolite. As previously stated, *A. oryzae* was a fungal endophyte with significant antifungal activity and the capacity to create a diverse spectrum of active metabolites. As a result, strategies to extract bioactive compounds from this endophytic fungus and study their biological characteristics will be created.

Table 3. Secondary metabolites screening of endophytic fungi isolated from *F. carica*.

Endophytic isolate	Tannins	Terpenoids	Steroids	Saponins	Flavonoids	Phenols	Alkaloids
<i>A. oryzae</i>	+	+	+	+	+	+	+
<i>C. gloeosporioides</i>	–	–	–	+	–	–	+
<i>Alt. tenuissima</i>	+	–	+	–	+	+	–
<i>A. tamari</i>	+	+	–	–	+	+	+

(+), mean present; (–) mean absent.

Molecular Identification of Promising Isolate. Besides morphological identification, genotypic approaches were utilized to verify the identification of SI-08—*A. oryzae*, the most promising endophytic fungal isolate, due to its significant metabolite synthesis and antifungal activity. The fungal sequence was deposited to the GenBank with accession number PP177442. The sequence was compared to homologous sequences using BLAST. for final identification. The BLASTn examination showed that the 614 bp had 99 to 100% homology with *A. oryzae* EF661560.1, EF661560.1, MH746006.1, EF634406.1, OQ446449.1, and OQ726519.1. The results of the ITS identification process agreed with the findings from the analysis of the microscopic and morphological aspects. Moreover, highly comparable sequences were chosen, and a phylogenetic tree according to the maximum likelihood technique was constructed using MEGA 7.0 (Figure 1). Based on BLAST analysis, the isolate is extremely associated with the *A. oryzae* cluster.

Antioxidant activity. *A. oryzae* EtOAc extract exhibited notable dose-dependent free radical scavenging ability, as illustrated in Figure 2. The fungal extract exhibited remarkable activities (12.5–92.9%) at dosages varying from 5 to 100 µg mL⁻¹, while the positive control, Vc, demonstrated a significant antiradical capability

of 97.8% at a concentration of 100 µg mL⁻¹. Conversely, the IC₅₀ values of EtOAc extract and Vc were calculated to be an impressive 29.8 and 16.4 µg mL⁻¹, respectively. The findings propose that *A. oryzae* might be a viable natural antioxidant source.

Cytotoxic Activity. The MTT assay, depicted in Figure 3, assessed the anti-proliferative effects of *A. oryzae* EtOAc extract at dosages between 5 to 100 µg mL⁻¹ on the breast cancer cell lines MCF-7, the cervical cancer cell line HeLa, and the liver cancer cell line HepG-2. Every cancer cell line tested showed cytotoxic action that was dependent on dosage. The *A. oryzae* EtOAc extract displayed significant cytotoxicity against the HepG-2, HeLa, and MCF-7 cell lines throughout our experiment, with values of 10.7, 4.2, and 12.6 correspondingly, at a concentration of 100 µg mL⁻¹. We were delighted to find that the IC₅₀ values on HeLa, HepG-2, and MCF-7 were, respectively, 20.9, 33.1, and 30.6 µg mL⁻¹. Based on our research, it appears that *A. oryzae* found in the leaves of *F. carica* can inhibit the growth and proliferation of HepG-2 HeLa, and MCF-7 cell lines.

DNA Protection Ability. The potential of *A. oryzae* EtOAc extract to guard λ-DNA from oxidative damage was investigated. The electrophoretic pattern of DNA is shown in

(Figure 4) with and without varying extract doses. In contrast to the control, lane 1, the formation of hydroxyl radicals by Fenton's reaction led to the loss of the DNA band completely, showing complete DNA degradation; in lane 3. Visible bands at dosages of 0.1, 1.0, and 10 $\mu\text{g mL}^{-1}$ were visible in lanes 4, 5, and 6, indicating that all fungal extract dosages successfully decreased oxidative stress and protected the DNA from OH radicals. The sharpness of the band shows the best link between extract content and DNA protective

action; a heavier band's intensity indicates the highest degree of action, while a faint band indicates the lowest amount of DNA damage protection. Of all fungal extract concentrations, 10 $\mu\text{g mL}^{-1}$ (lane 2) had the best DNA damage protection activity, whereas 0.1 $\mu\text{g mL}^{-1}$ (lane 3) had the lowest performance. Standard quercetin was utilized as a positive control (lane 2). The findings of this study indicate that the *A. oryzae* EtOAc extract may offer some degree of protection against OH radical damage to DNA.

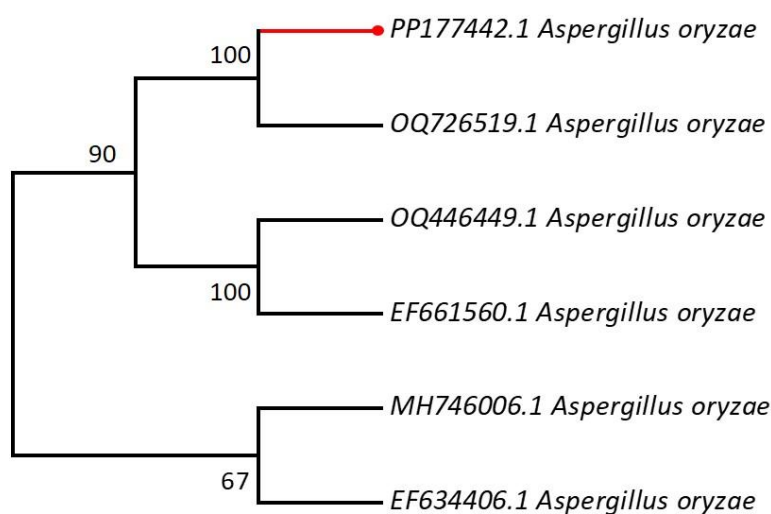


Figure 1. Phylogenetic tree based on the 5.8S-rRNA-ITS regions between the endophytic fungus *A. oryzae* and published data and evolutionary distances computed using the Maximum Likelihood method.

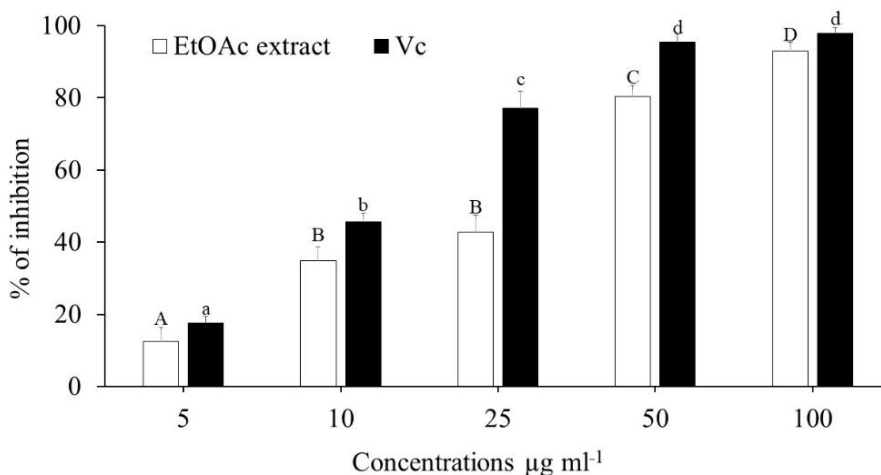


Figure 2. DPPH radical scavenging activity of EtOAc extract of *A. oryzae*, Vc; positive control at various dosages (5–100 $\mu\text{g mL}^{-1}$). Each value (n = 3) is expressed as mean $\pm$ SD.

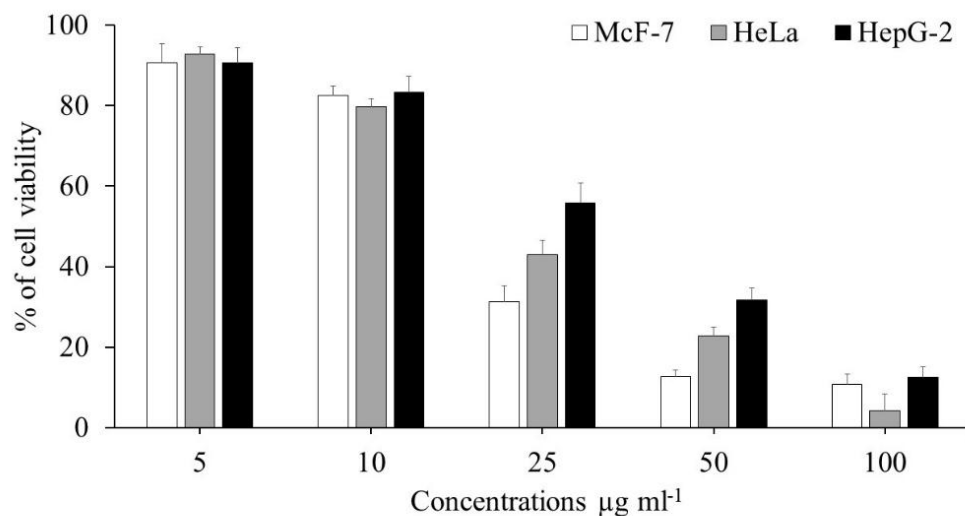


Figure 3. The impact of *A. oryzae*'s EtOAc extract on the viability of HeLa, HepG-2, and McF-7 cell lines in vitro. Different concentrations ranging from 5 to $100\mu\text{g mL}^{-1}$ were applied to tumor cells, and the MTT test was used to assess the viability of the cells. The graph's bars show the mean $\pm$ SD as a percentage of the proliferation of distinct experiments conducted in triplicate ($n = 3$).

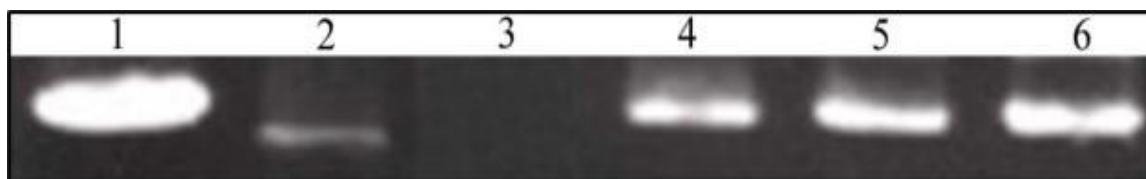


Figure 4. Endophytic fungal defense against DNA damage via EtOAc extract of *A. oryzae*. Lane 1: DNA native to λ Lane 2: Positive control, λ DNA + Fenton's reagent + quercetin Lanes 3 and 4 contain λ DNA and Fenton's reagent, respectively; Lane 5 has λ DNA and Fenton's reagent and extract ($1.0\mu\text{g mL}^{-1}$) and Lane 6 contains λ DNA and Fenton's reagent plus extract ($10\mu\text{g mL}^{-1}$).

FT-IR. In the present investigation, Fourier transform infrared (FT-IR) analysis was utilized to detect putative functional groups according to peak values utilizing the EtOAc extract of the endophytic fungus *A. oryzae*. The obtained peaks have intensity (cm^{-1}) of 3411.17 (OH group, aliphatic primary amine or phenol, N-H), 1727.32 (Carboxylic acid, C = O), 1616.10 (aromatic, C=C), 1509.25 (aromatic, C=C), 1446.30 (indicating a methyl C-H asymmetric bend or even a methylene C-H bend.), 1348.39 (indicating an OH-bend), 1184.28 (Ester carbonyl), 1039.43 (Polysaccharide), 875.97 (1,3 di-substituted, C-H), 834.64 (alkene, C=C), 748.13 (1,2 di-substituted, C-H) and 597.73 (alkyl halides, C-Br) (Figure 5). The detected peaks indicate the presence of several functional groups, which are related to the presence of bioactive compounds.

Bioactive Compound Identification using GC-MS Analysis. Furthermore, Gas chromatography-mass spectrometry (GC-MS) analysis was done on *A. oryzae* EtOAc extract to detect the metabolites profile (Figure 6). A number of resolved peaks of active chemicals, along with their retention time (RT), peak area, molecular weight, and molecular formula, are displayed in Table (4) and Figure (6) based on the NIST library. The chromatogram revealed that the extract of *A. oryzae* contains 11 different compounds, where major compounds were (1) Oleic acid methyl ester, (2) behenic acid, methyl ester, (3) linoleic acid ethyl ester, (4) Stearic acid methyl ester and (5) cis-5,8,11,14,17- eicosapentaenoic acid. On the other hand, minor compounds were hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid, Eicosanoic acid, methyl ester, Erucic acid,

Hexacosanoic acid, methyl ester and Stigmastan-3,5-diene with ratios 3.22, 0.91, 2.99, 2.48, 0.31, 0.56, respectively. The discovery of these

metabolites revealed that the endophytic fungus *A. oryzae* is capable of producing substances that closely resemble its bioactivities.

Table 4. Gas chromatography-mass spectrometry (GC-MS) analysis of *A. oryzae* endophytic EtOAc extract. The potential presence of bioactive substances as well as their molecular weight, peak area, retention time, and formula

S. no.	Retention Time	Peak area	Molecular Formula	Molecular weight	Compound Name (IUPAC)
1	8.41	3.22	C ₁₇ H ₃₄ O	270.4	Hexadecanoic acid methyl ester
2	9.49	5.86	C ₁₉ H ₃₈ O ₂	298.5	Stearic acid methyl ester
3	9.38	41.55	C ₁₉ H ₃₆ O ₂	296.5	Oleic acid methyl ester
4	10.20	12.94	C ₂₀ H ₃₀ O ₂	302.4	Cis-5,8,11,14,17- eicosapentaenoic acid
5	10.71	0.91	C ₁₈ H ₃₂ O ₂	280.4	9,12-Octadecadienoic acid
6	11.16	2.99	C ₂₁ H ₄₂ O ₂	326.5	Eicosanoic acid, methyl ester
7	12.53	2.48	C ₂₂ H ₄₂ O ₂	338.5	Erucic acid
8	12.81	8.85	C ₂₃ H ₄₆ O ₂	354.6	Behenic acid, methyl ester
9	14.29	7.44	C ₂₀ H ₃₆ O ₂	308.4	Linoleic acid ethyl ester
10	15.78	0.31	C ₂₇ H ₅₄ O ₂	410.7	Hexacosanoic acid, methyl ester
11	16.90	0.56	C ₂₉ H ₄₈	396.7	Stigmastan-3,5-diene

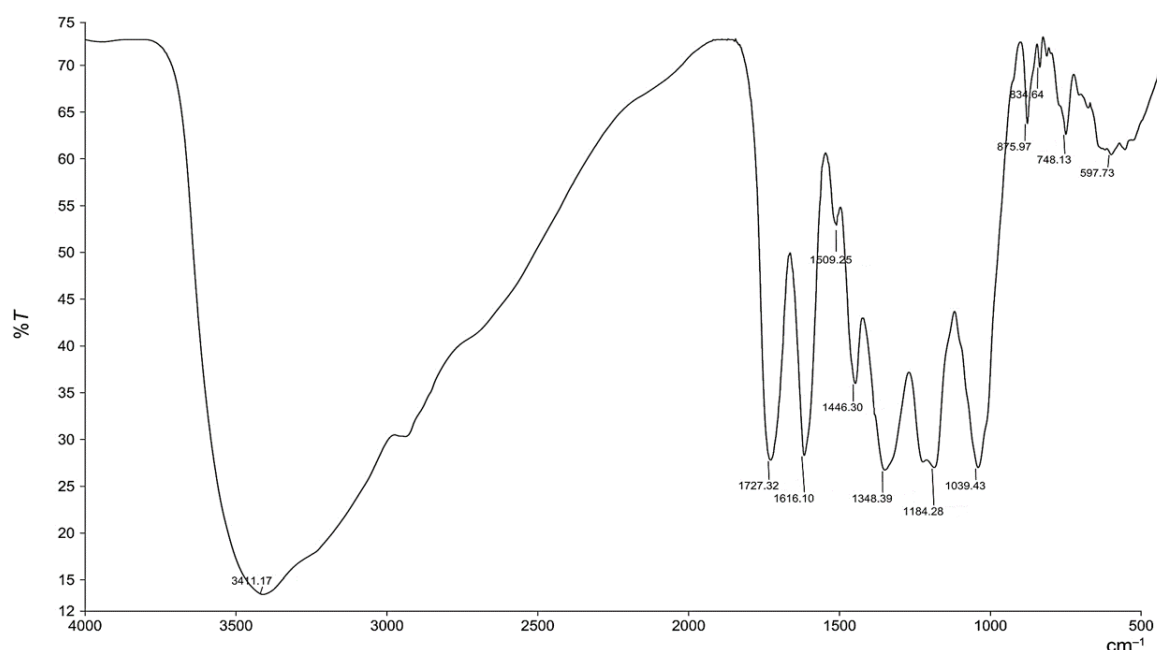


Figure 5. FTIR spectroscopic analysis of *A. oryzae* EtOAc extract.

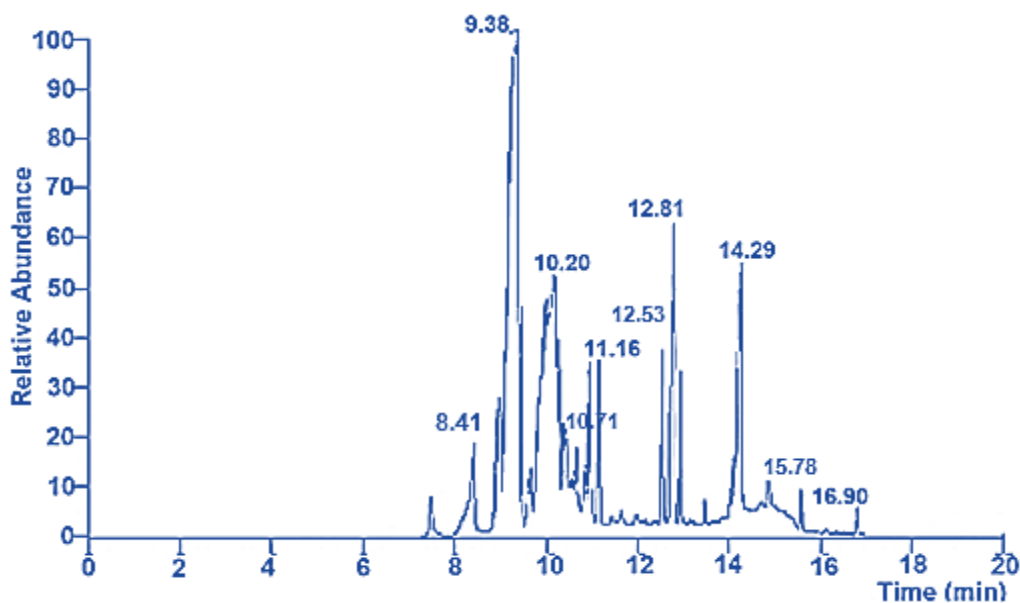


Figure 6. GC-MS chromatogram of EtOAc extract of *A. oryzae*.

DISCUSSION

Every part of the plant, including the bark, flowers, roots, fruits, leaves, stems, and scales, has been reported to have endophytes (Dar *et al.*, 2015). Huge populations of endophytes, or microorganisms, are found in plants (Almustafa and Yehia, 2023). Plants that grow in a variety of temperate, tropical, semi-tropical, cold, hot, and deep marine habitats are known to produce endophytes (Almustafa and Yehia, 2023). Moreover, seaweed and marine algae can be used to isolate endophytes (Boyle *et al.*, 2001). Hundreds of genera and species can be isolated from a single plant, making it clear from numerous studies conducted in the past century that endophytes are a natural source of biological diversity. It is noteworthy that over 300,000 plant species on Earth are capable of hosting endophytes (Carroll, 2004). As a result, they represent a significant source of biological diversity. Despite biological diversity, the abundance of isolated endophytic fungi increases the possibility of obtaining novel endophytic microbe strains and kinds.

There are 126 phytoconstituents found in the leaves of *F. carica*, with polyphenolic chemicals making up the majority. *F. carica* leaves have a higher polyphenolic content than red wine and tea, which means that they have more antioxidant activity than those beverages. The possible health

advantages of *F. carica* leaves have been demonstrated by several laboratory tests and clinical research. The strong hypoglycemic, antioxidant, and anti-inflammatory properties of the active ingredients have been demonstrated by scientific studies, supporting the antidiabetic impact of *F. carica* leaves (Li *et al.*, 2021).

The leaves of *F. carica* are a widely used traditional remedy, especially for the treatment of diabetes. Over the past ten years, several scientific investigations have been carried out to examine the chemistry, bioactivity, and molecular mechanisms. There's also tea created from the infusion of *F. carica* leaves. The Compendium of Herbology indicates that leaves are moderately poisonous, even though scientific studies have shown that the toxicity of *F. carica* leaves is significantly higher than the usual dosage. Therefore, caution should be used when eating *F. carica* leaves. In addition, *F. carica* leaves demonstrate renoprotective and hepatoprotective effects at significantly lower concentrations than dangerous dosages (Li *et al.*, 2021).

This investigation was conducted in Al-Ahsa Oasis, Saudi Arabia, and evaluated the various endophytic fungi associated with *F. carica*. Because leaves are a rich and abundant source of fungal endophytes, their populations were chosen

for examination in this study (Arnold *et al.*, 2000).

The study of the fungal endophytic community of *F. carica* leaves allowed the isolation of 203 filamentous endophytic isolates, which were produced from 380 *F. carica* leaf pieces, yielding a 50.3% colonization rate. The isolates were identified and categorized into 11 genera, which correspond to 21 distinct species, based on their physical characteristics. By examining their morphological characteristics, endophytic fungi such *Aspergillus* sp., *Alternaria* sp., *Fusarium* sp., *Chaetomium* sp., *Colletotrichum* sp., and *Curvularia* sp. that have been previously described as frequent endophytes in other plants were identified from the recovered isolates (Davis *et al.*, 2003; Kjer *et al.*, 2009; Demers *et al.*, 2015; Liu *et al.*, 2015; Yang *et al.*, 2015). *C. gloeosporioides*, *Alt. tenuissima*, *A. tamari*, and *A. oryzae* were the most common fungi, colonizing at rates ranging from 3.7% to 8.8%.

The study's comparatively low total colonization rate may have something to do with Saudi Arabia's desertification. Notably, Ascomycota make up most of the fungal taxa that were retrieved for this investigation. This is in line with some studies that have shown Ascomycetes to be the predominant endophytic fungi associated with medicinal plants (Barnett and Hunter, 2006; Bhardwaj *et al.*, 2015; Tan *et al.*, 2018).

Because microbes are developing new defense mechanisms against antimicrobial drugs, the rise of pathogenic bacteria and fungi resistant to commercial treatments is a significant issue for health services (Elbasuney *et al.*, 2021). Finding efficient antibacterial drugs is therefore necessary. Fungal endophytes can survive in plant tissues without showing any symptoms or damaging their hosts (Sharaf *et al.*, 2022). One of the major sources of bioactive chemicals with a variety of biological activities, including antiviral anticancer, antibacterial, and antioxidant properties, is thought to be fungus endophytes. Numerous powerful secondary metabolites, such as phenylpropanoids, alkaloids, quinones, peptides, terpenoids, lignans, flavonoids, steroids, phenolics, and isocoumarins, are produced by endophytic fungi and exhibit strong antimicrobial properties against a wide range of pathogenic microbes. Consequently, to

counteract antimicrobial resistance, innovative antimicrobials must be extracted from novel fungal endophytes.

Corresponding to the findings of this study, the endophytic fungus *A. oryzae* can suppress the formation of different test fungal pathogens. This indicates that ethyl acetate has the highest quantity and concentration of bioactive substances that either directly or indirectly affect pathogens. The ability of secondary metabolites to synthesize cell walls, depolarize the cell membrane, inhibit protein synthesis, and inhibit nucleic acid synthesis is linked to their activity. This is congruent with the observations of Beharka and Nagaraja (1998), who claimed that *A. oryzae* might be used as an antifungal agent. However, earlier research indicates that endophytic fungus; *Aspergillus* sp. *ASCLA* was isolated from leaf tissues of the medicinal plant *Callistemon subulatus* and Isoshamixanthone was isolated which has antimicrobial activity against pathogenic microorganisms (Kamel *et al.*, 2020). Moreover, the isocoumarin derivatives oryzaeins were obtained from *A. oryzae*, the endophyte hosted in the rhizome of *Paris polyphylla* var. *yunnanensis*. These indicated that antifungal activity is due to the presence of multiple compounds that have antifungal. RNA and protein synthesis, cell wall production, cell division, and the efflux-mediated pumping system were all reduced by flavonoids, which frequently inhibited fungal growth through a variety of underlying processes, such as disruption of the plasma membrane and the creation of mitochondrial failure (Al Aboody and Mickymaray, 2020). Terpenoids have been shown to have hypoglycemic properties, enhance transdermal absorption, reduce the risk of cardiovascular disorders, and have anticancer, anti-inflammatory, antibacterial, antiviral, and antimalarial properties (Yang *et al.*, 2020). Terpenoid phytochemicals are antibacterial agents that inhibit cell walls by affecting membranes (Sudha *et al.*, 2016).

In addition, I'd like to point out that *C. gloeosporioides*, *Alt. tenuissima*, and *A. tamari* isolates inhibited pathogenic fungal growth moderately to weakly. This demonstrates that the action spectrum of endophytes differed significantly among isolates, implying that a diversity of biologically active substances was

involved in the antifungal effect. This is consistent with Negi (2012) and Arivudainambi *et al.* (2011), who discovered antipathogenic bioactive compounds in *C. gloeosporioides*, *Alt. tenuissima*, and *A. tamari*. However, it should be noted that the cultivation of environmental microbes, including the fungal endophytes described here, under lab conditions frequently results in suppression or the synthesis of low amounts of bioactive molecules. As a result, numerous genomic and cultivation-based techniques have been developed to promote the expression of these metabolic pathways despite axenic culture conditions. Furthermore, the complicated chemical space in fungal crude extracts may obscure the effect of the bioactive component, leading to modest activity.

The phytochemical preliminary research of *A. oryzae* crude extract showed the existence of several fungal metabolites, like saponins, steroids, tannins, phenols, alkaloids, terpenoids, and flavonoids, in comparison to other fungal crude extracts. This corroborates a study by Gopiesh and Kannabiran (2008), which reports that endophytes contain some phytochemicals. Our results are reinforced by the fact that endophytes have been demonstrated to be able to synthesize some metabolites but not others (2011). Primary and secondary antioxidant activity are present in phenols and terpenes (Gülçin, 2006; Hajdú *et al.*, 2007). Moreover, flavonoids, phenol, tannin, alkaloids, and terpenoids contribute to bioactivity (Tran *et al.*, 2010; Muthukrishnan and Subramaniyan, 2012).

After creating the method, *A. oryzae*, a putative endophytic fungus. To confirm the fungal isolates presumed morphological identity, a molecular identification process was employed. This involved PCR amplification using primers ITS1 and ITS4, followed by BLAST searches of the obtained sequences and comparison with sequences downloaded from the public database GenBank (Kitamoto, 2015).

Wei *et al.* (2005) talked about the morphological traits' significance in terms of phylogeny. It was suggested that when defining new species of *Aspergillus* species, morphological features should be considered instead of host association and that molecular phylogenetic evidence is also necessary to show that the taxon is different from the currently known species. Although

Aspergillus is believed to be a pathogen that produces leaf spots, many studies have revealed that some species of the *Aspergillus* can also function as bioactive substance makers and antifungal agents (Son *et al.*, 2018; Ngo *et al.*, 2021).

Since ethyl acetate extraction is a successful method for recovering fungal secondary metabolites, we have chosen it for this instance (Garcia *et al.*, 2012). Ethyl acetate is a solvent for extraction that preferentially extracts low and high-molecular-weight phenolic compounds (Scholz and Rimpler, 1989).

The most popular method for assessing an antioxidant compound's capacity to scavenge free radicals in the extract of plants is called DPPH radical scavenging activity. Since DPPH is a persistent free radical, the assay is predicated on its tendency to decolorize in the existence of antioxidants. In the DPPH radical, an odd electron causes the absorbance at 517 nm. The absorbance values drop when an antioxidant chemical donates an electron to DPPH. This shift in absorbance can be quantitatively determined (Sri-Harsha *et al.*, 2013). By neutralizing these free radicals and blocking them from causing cellular damage, antioxidant chemicals guard against cellular harm. Due to their ability to interact with and neutralize free radicals, which are a major factor in the development of cancer cells, antioxidant compounds are also important in the prevention of cancer. Therefore, the development of novel antioxidant molecules is crucial to improve human health and avoid degenerative and other diseases by stabilizing free radicals and reducing cellular damage (Song and Yen, 2002). Numerous studies have been conducted on endophytic fungi, like *Penicillium* sp., *Torula* sp., *Phoma* sp., *Chaetomium* sp., and *Cladosporium* sp. as possible natural antioxidant sources (Song and Yen, 2002; Gebhardt *et al.*, 2007). Because of this, they are just as effective as synthetic antioxidants and generate a wide range of new metabolites with antioxidant activity. In our study, the EtOAc extract of *A. oryzae* demonstrated strong antioxidant activity in this investigation. This could be attributed to some processes, such as scavenging free radicals, binding of transition metal ion catalysts, preventing chain initiation, and breaking down peroxides (Bounatirou *et al.*, 2007). This activity is attributed to the presence of phenolic

compounds which are confirmed by phytochemical screening. Our results are intriguingly consistent with a review of the literature that reported that the crude extract of *A. oryzae* exhibited antioxidant activity, with DPPH free radical scavenging activity being inhibited by 59% and 62.1% (Güder and Korkmaz, 2012). We were pleased to find that the endophytic fungi *Cytospora rhizophorae*, and *Neopestalotiopsis protearum*, which showed strong antiradical action with IC₅₀ values of 330 and 1240 µg mL⁻¹, respectively, had much lower IC₅₀ values in this experiment (36.6 µg mL⁻¹) (Zhou *et al.*, 2012). Aspergilli are the most frequent fungal endophytes for producing antioxidants, according to recent studies. *A. flavus*, *A. fumigatus*, and *A. nidulans* have promising antioxidant activity; with IC₅₀ values ranging from 68.4 to 347.1 µg mL⁻¹. Additionally; *A. minisclerotigenes* AKF1 and *A. oryzae* DK7 from *Mangifera casturi* Kosterm were discovered to have antioxidant activity, with respective IC₅₀ values of 142.96 and 145.01 µg mL⁻¹ (Tran *et al.*, 2010). Research has demonstrated that endophytic fungi can produce a broad range of chemicals with potent antioxidant properties, some of which have been identified and isolated. These compounds include terpenoids polyketides, flavonoids xanthenes, and phenolic acids.

The initial stage in developing anti-cancer medications is to evaluate the cytotoxicity of natural products (Sharaf *et al.*, 2022). The production of bioactive compounds with therapeutic value or potential by endophytes has been documented in the literature (Zhao *et al.*, 2010; Kharwar *et al.*, 2011; Kusari *et al.*, 2013). It is plausible that fungal endophytes are the foundation of these metabolites (Strobel *et al.*, 2004; Chen *et al.*, 2016). *A. oryzae* crude extract was discovered to have considerable cytotoxicity against the MCF-7, HeLa 7, and HepG-2 cell lines in the current study. The IC₅₀ values were found to be lower than the National Cancer Institute (NCI) standard, at 20.9, 33.1, and 30.6 µg mL⁻¹, respectively. A crude extract with an IC₅₀ value of less than 30 µg mL⁻¹ is considered a promising anti-cancer medication, under NCI guidelines (Cui *et al.*, 2011). Furthermore, some investigations on endophytes have been conducted to find effective and innovative cancer treatments (Mahnashi *et al.*, 2021; Zhan *et al.*, 2007; Nascimento *et al.*, 2012). Finding creative

active natural chemicals from *A. oryzae* that may be utilized as more advanced cytotoxic drugs will be made easier with the support of our current study.

As free radicals can damage DNA strands, resulting in mutagenesis and cytotoxicity. When hydroxyl radicals interact with DNA, they either add bases or deplete from the sugar molecule. Fenton's, which are composed of H₂O₂, Fe³⁺, and ascorbic acids, can create extremely reactive OH through the Fenton reaction, according to Lee *et al.* (2002).

EtOAc extract of *A. oryzae* was examined for its potential to shield DNA from Fenton-induced damage. The current investigation found that 10 µg mL⁻¹ effectively prevented λ DNA damage caused by Fenton's OH radicals. Even while the EtOAc extract demonstrated cytotoxic activity toward HepG-2, HeLa, and MCF-7 cell lines. It also showed remarkable antioxidant activity. As a result, this contradictory trend may be linked to the *A. oryzae* extract's presence of several phytochemicals with multifunctional bioactivity. This is the first study to validate *A. oryzae*'s ability to defend against DNA damage. Undeniably, *Aspergillus* species have attracted interest due to their ability to create a great deal of secondary metabolites with structural bioactivity. Some of them might be significant as targets for pharmaceuticals in the future that are used to manage human illnesses, inhibit plant diseases, and produce anticancer and antioxidant medications (Moussa *et al.*, 2020).

Aspergilli are the most prevalent genus of fungal endophytes in recent years, and studies have shown that they may have remarkable anticancer properties. Pulchrarin, an anticancer drug, was produced by the endophytic *Aspergillus* TRL1 that was isolated from *Tabebuia rosea*. This chemical demonstrated excellent suppression of human tumor cells, such as liver (HepG2) and breast (MCF-7) cell lines (Moussa *et al.*, 2020). Furthermore, *Aspergillus* ASCLA was used to isolate novel pyrano xanthenes, which show anticancer potential against human cervix carcinoma (Kamel *et al.*, 2020).

Gas chromatographs coupled to mass spectrometers are consequently among the most often used methods for exploring phytochemical substances of natural origin because of their

great efficiency, sensitivity, and stability (Almustafa and Yehia, 2023). The findings of a study illustrating the GC–MS analysis of *A. oryzae*'s extract show that it contains five distinct bioactive compounds, with the main constituents being oleic acid methyl ester, with ratios of 41.55. It has antibacterial, antioxidant, anticancer, anti-inflammatory, and insecticidal effects in addition to being an inhibitor of cancer enzymes (Dilika et al., 2000; El-Fayoumy et al., 2021). On the contrary, the following molecules: Stearic acid methyl ester, and cis-5,8,11,14,17-eicosapentaenoic acid have been shown to have antibacterial, antimicrobial, and anti-colorectal cancer properties; however, linoleic acid ethyl ester and Behenic acid, methyl ester have not been found to have any biological activity (Dilika et al., 2000; El-Fayoumy et al., 2021). On the other hand, minor compounds were hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid, Eicosanoic acid, methyl ester, Erucic acid, Hexacosanoic acid, methyl ester and Stigmastan-3,5-diene have different biological activities such as hypocholesterolemic, antihistamine, hepatoprotective, anticorectal cancer activity, antiandrogenic, nematicide, hypocholesterolemic, antimicrobial, and antioxidant.

It is noteworthy that our fungal extract has demonstrated significant in vitro antifungal, antiradical, and cytotoxic activity. As a result, the existence of certain important compounds may be the cause of such activities. To our knowledge, this is a pioneering study on bioactive chemicals produced from *A. oryzae*. As a result, the endophytic fungus *A. oryzae* investigated here may offer novel sources for generating metabolites of relevance in biotechnological uses.

CONCLUSIONS

An endophytic fungus *A. oryzae* was isolated from *F. carica* leaves in the current study and deposited in the gene bank with accession number PP177442. Through GC-MS and phytochemical investigations, the bioactive compound generated by *A. oryzae* was identified and evaluated. *A. oryzae* crude extract exhibits encouraging antifungal activities against different fungal pathogens. Moreover, this extract does not harm normal cell lines and may

have antioxidant properties while effectively shielding the DNA from damage caused by free radicals. Eventually, *A. oryzae* crude extract is suggested as a bioactive substance for various biological uses.

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