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Antifungal Potential of *Cladosporium* sp. (Endophytic fungi) Associated with *Olea europaea* L. Leaves

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Abstract

In the leaves of *Olea europaea* L. Olive trees an endophytic fungus was discovered. *Cladosporium* sp. was identified to be the fungus based on its morphological characteristics and nuclear ribosomal DNA ITS sequence analysis and was registered in NCBI as the *Cladosporium* genus has been registered under the number (0P939922.1) The species was not specified, and it was considered of unknown species after comparing it to global isolates. In comparison to olive leaf extract, *Cladosporium* sp. including total flavonoid, total phenolic, total terpenoid, and total saponins, Which were 121.9%, 198.1%, 89.13%, and 29.87 % respectively compared to its content in olive leaf extract, which was 61.54 %, 67.88 % , 17.1%, and 20.19% respectively. The *Cladosporium* sp. extract inhibited the growth of 27 isolates belonging to different species of *candida* which were *Candida albicans* , *C. lypolitica* , *C. tropicalis* , *C. sphaerica* , *C. krusei* , *C. guilliermondii* , *C. parapsilosis* , *C. norvegicus* , *C. glabrata* , and *C. kefyr* , the inhibition effects increased with increasing concentration to reach the highest level to suppress fungal growth when concentrated 30 mg/ml. This proves the antifungal potential of endophytic fungi in the future.

Keywords: Antifungal, *Candida*, *Cladosporium*, Endophytic, Olive trees.

Introduction

Endophytes are defined as microorganisms that live asymptomatically in plant tissues, causing no damage to their hosts, and are isolated from surface-sterilized plant explants. The term endophytes means "in the plant" (from the Greek endon = within, phyton = plant), and most endophytes originated in the rhizosphere and phyllosphere [1]. Endophytic fungi are regarded as the unseen inhabitants of the microbial world and represent an underutilized source of novel therapeutics and compounds. Fungi create secondary metabolites, which are characterized as low molecular weight molecules adapted for specialized tasks in nature but not necessary for growth. New, highly effective medications are desperately needed to treat diseases like cancer, bacterial drug resistance, and fungal infections [2], Numerous active chemicals with a wide range of biological characteristics are produced by endophytic fungus [3].

Plants with endophytic fungus have been classified separately [4-7], One of the most extensively sold and important botanical medicines is Olive and it is a rich source of secondary metabolites with broad-spectrum therapeutic properties [8]. Anti-diabetic, anti-inflammatory, heart disease, respiratory and urinary tract infections, stomach and intestinal illnesses, antimicrobial for many fungal and bacterial pathogens, and more have all been demonstrated for it, and it has been used to treat these conditions since ancient times [9-11], There is little information

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regarding the endophytic fungal community inhabiting olive tree tissues. However, several endophytic fungi isolated from the olive plant were shown to have major pharmacological effects, such as *Phomopsis columnaris, Fusarium oxysporum*, and *Trichoderma gamsii* [12], *Penicillium commune* , *Penicillium canescens* and *Alternaria alternate* [13], *Alternaria* sp., *Epicoccum* sp. , *Fusarium* sp. , *Aspergillus* sp., *Anthrinium* sp., *Chaetomium* sp., *Diaporthe* sp., *Nigrospora* sp. [14,15] . The purpose of this study is to determine the antifungal capability of endophytic fungus *Cladosporium* sp. associated with the leaves of *Olea europaea* L.

Materials and Methods

Collection of samples

Leaves of 16 distinct olive *Olea europaea* L. were collected from farms in Al-Duluiya city, Salah-AlDin Governorate, Iraq. The samples were obtained at random in April of 2022. They were transported to the lab in sterile polypropylene bags.

Endophytic fungi isolation

To eliminate dirt and dust, the olive leaves were sliced and thoroughly cleaned under running tap water for 20-30 seconds. The leaf fragments were sterilized three times with 75% ethanol for 2 minutes before being washed twice with sterile distilled water. Endophytic fungi were isolated from olive leaves using potato dextrose agar (PDA) media[16]. The PDA plates were then incubated at 25 ± 2°C for seven days to allow endophytic fungi to proliferate. Purification of fungal isolates was accomplished by transferring a portion of the colony's edge to a fresh PDA medium.

Morphological and Molecular diagnosis of endophytic fungi

Using the adhesive tape fixation technique and the lactophenol blue cotton stain, isolated endophytes were characterized microscopically, and observed with a compound microscope at 10X and 40X magnification. Observations were made regarding the shape, color, margin, elevation, spore structure, and other morphological characteristics of endophytic isolates [17].

The CTAB approach was used to extract DNA from *Cladosporium* sp. for the purposes of molecular screening and phylogenetic analysis [18]. The ITS domain of rDNA was amplified with the universal primers ITS1 (50-TCCGTAGGTGAACCTGCGG-30) and ITS4(50-TCCTCCGCTTATTGATATGC-30) as described by [19,20] ,With deionized water, the total volume was brought up to 50 mL. The ITS region was amplified in the following way: 95°C for 5 minutes, then 40 cycles of 94°C for 60 seconds, 50°C for 60 seconds, and 72°C for 60 seconds, with a final stretch at 72°C for 5 minutes. The PCR products were cleaned up with the Gel Extraction Kit (TianGen, China), attached to the sequencing vector pGEM-T easy vector, and read by Shanghai Sangon Biologic Engineering Technology and Service Co. Ltd. For a study of phylogeny BLAST from the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) was used to look at the ITS gene sequence of an isolated fungus. MEGA program version 4.0 was used to predict phylogenetic relationships [21].

Quantitative evaluation of active compounds

Total phenol content (TPC): The Folin-Ciocalteu method reported by [22] with some slight changes was used to determine the TPC of the fungal endophytes and olive leaves. Based on the Gallic acid calibration curve, the TPC was determined. The data was reported in terms of gallic acid equivalents (GAEs)(µg), which were calculated for each milligram of dry extract.

Total flavonoid content (TFC): used the NaNO2-Al(NO3)3-NaOH colorimetric test reported by [23]. To calculate the TFC.Based on the Rutin calibration curve, the TFC was determined. The data was reported as the amount (µg) of Rutin Equivalents (RAEs) per milligram of dry extract .

Each extract solution was steeped in 9 mL of ethanol for 24 hours to determine its total terpenoid content as shown in [24]. Using the formula (wi-wf/wi100), were able to calculate the yield (in percent) of total terpenoid contents.

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Following the specified procedures, determined the total alkaloid content as shown in [25], the percentage of bromothymol blue in a sample is calculated as Y% = (NVM2)/W 100%, where N is the bromothymol blue solution concentration (mol/L), V is the volume of bromothymol blue solution eaten (L), M is the molecular weight of securinine (217.26), and W is the sampling weight.(g). Measurements of total saponin content followed the guidelines established by[25].

Assay for antifungal activity

Extracts of the endophytic fungus *Cladosporium* sp. were tested for their antifungal activity using the disk method. *Candida albicans* (6 samples), *Candida lypolitica* (2 samples), *Candida tropicalis* (4 samples), *Candida sphaerica* (3 samples), *Candida krusei* (3 samples), *Candida guilliermondii* (2 samples), *Candida parapsilosis* (1 sample), *Candida norvegicus* (1 sample), *Candida glabrata* (4 samples), and *Candida kefyr* (1 sample) were used as indicator organisms. Identified and preserved isolates were obtained fungi laboratory in the College of Education for Pure Sciences, University of Tikrit.

Sabouraud's agar medium (Himedia, Mumbai) was freshly prepared and sanitized for the antifungal assay (SA), and a pinch of streptomycin was added and thoroughly mixed together. The media was then put into each petri dish to a depth of 10 ml and allowed to be set. A sterile cotton swab was used to distribute the test fungal cultures (1×105cfu/ml) across the SA surface. Then, using a sterile cork borer, drilled a 0.5-centimeter-deep hole in the medium and deposited 10-20-30 µg/ml of *Cladosporium* sp. extracts into individual wells. After that, the plates went into an incubator at 27 Co for 48-72 hours. The drug fluconazole 25 µg (One of the most commonly prescribed antifungal drugs for *Candida* infections) served as a control [26]. At the end of the incubation time, the diameter of the inhibitory zone surrounding each well was measured.

Statistical analysis

Statistical analysis of the results obtained was done using the SPSS-27 (Statistical Packages for Social Sciences-version 27). The difference in percentages (qualitative data) was tested using P-value and was considered statistically significant when it was equal to or less than 0.05.

Results and Discussion

Morphological and Molecular diagnosis of endophytic fungi

The result of isolating endophytic fungi from the olive tree was determined through microscopic and morphological analysis, which showed that all fungal isolates belonged to *Cladosporium* sp. Fungus. An examination of the rDNA ITS region and its relationship to other genes. The use of rDNA genes has proven effective in tracing the origins of previously unknown species [27]. The sequence was 99% identical to that of *Cladosporium* sp, according to a homology search against GenBank**.** (GenBank accession number 0P939922.1) Through the process of alignment, a phylogenetic connection was created as shown in Fig. 1. Molecular analysis placed the isolates within the genus *Cladosporium*. and linked them to the *Cladosporium* genus.

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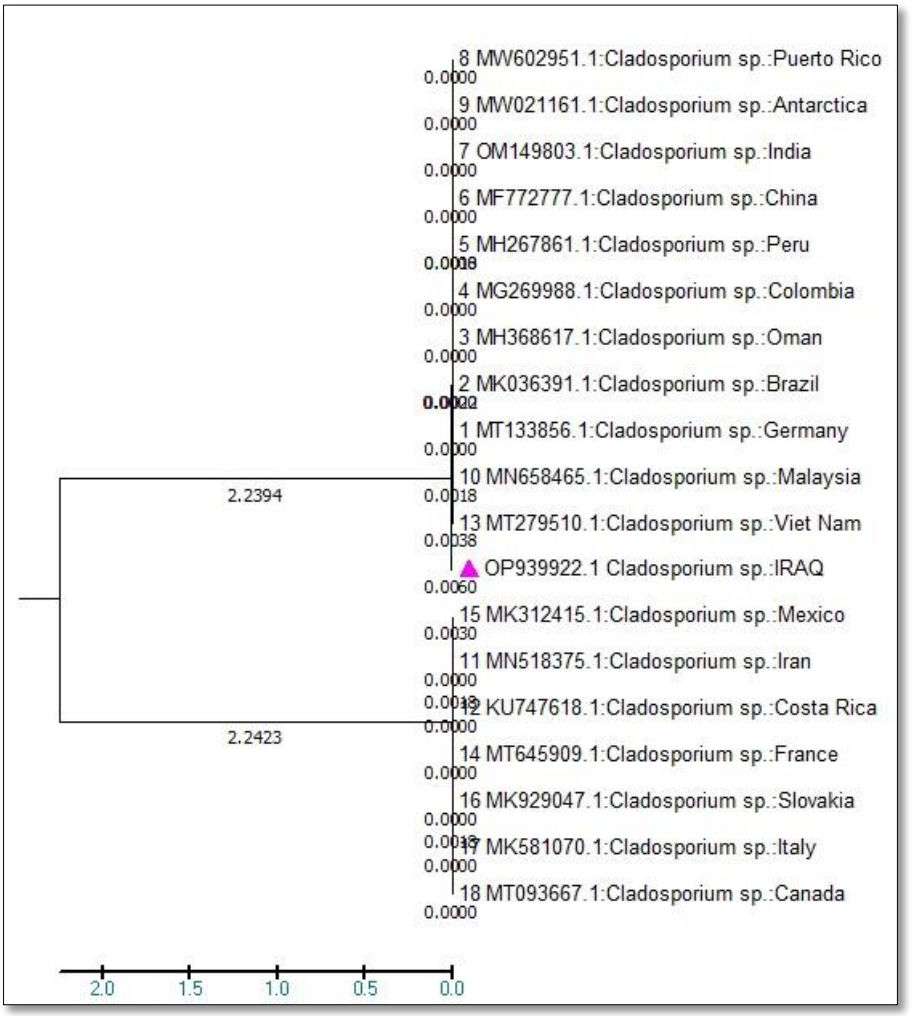

Figure 1. Phylogenetic tree demonstrating the relationship between *Cladosporium* sp. and other related fungi retrieved from GenBank based on ITS sequence homologies.

Determination of secondary metabolites

To determine the percentage yield, ethyl acetate used to extract the secondary metabolites from a fungal strain of *Cladosporium* sp. that was cultured in PD broth and Olive leaves as shown in Fig. 2. The results demonstrated that fungal extract had higher levels of secondary metabolites than olive leaf extract, with total flavonoid content rising to 121.9, a rise of 50.48% over the plant extract's 61.54, total phenolic content rising to 198.1, a rise of 34.27% over the plant extract's 67.88, and total terpenoid content rising to 89.13, compared 17.1to olive leaf extract a rise of 19.19%, for total saponin an increase was 29.87 % for fungal extract which was 67.6 compared to 20.19 in the olive leaf extract, as for alkaloids, the results show that their content in olive leaf extract was higher, and it was 2.67 $mg.ml^{-1}$, compared to 1.3 $mg.ml^{-1}$ for fungal extract.

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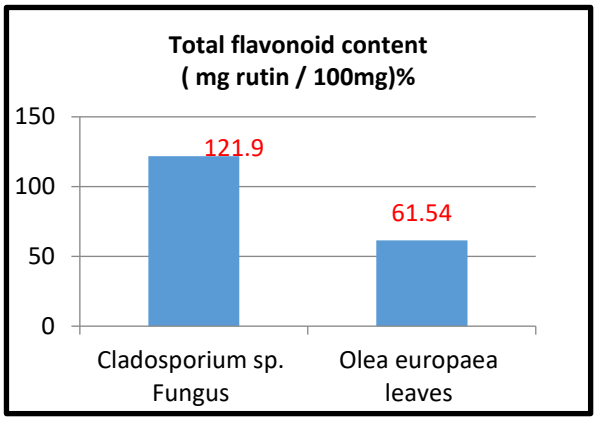
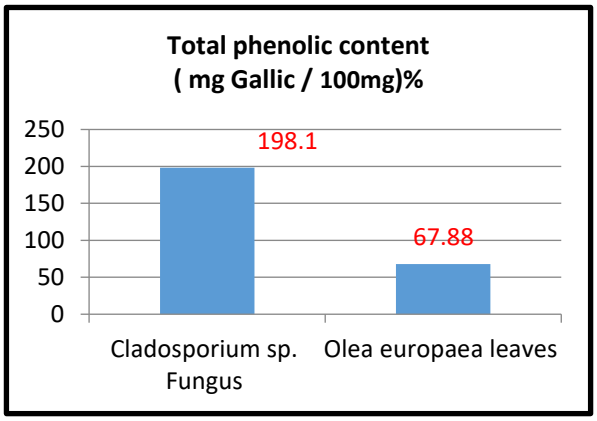


P-value ≤ 0.05(0.00)



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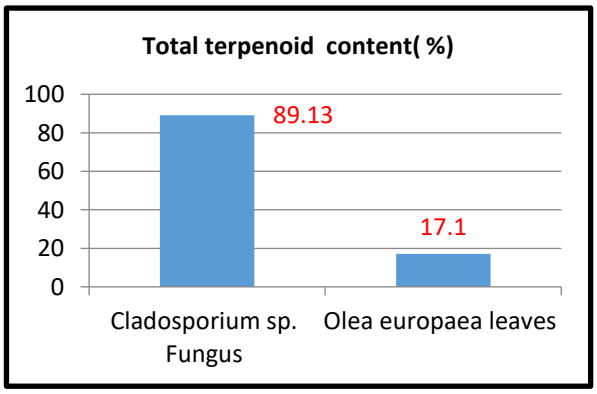

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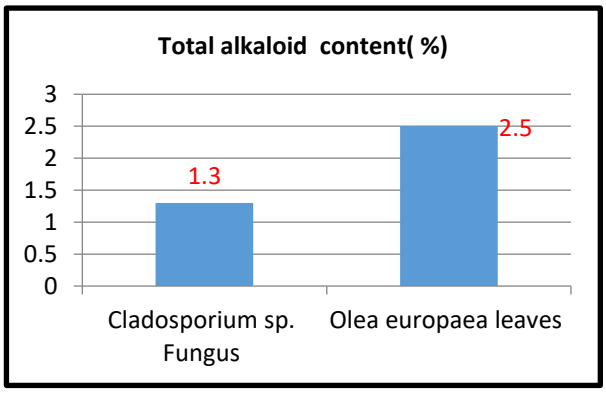
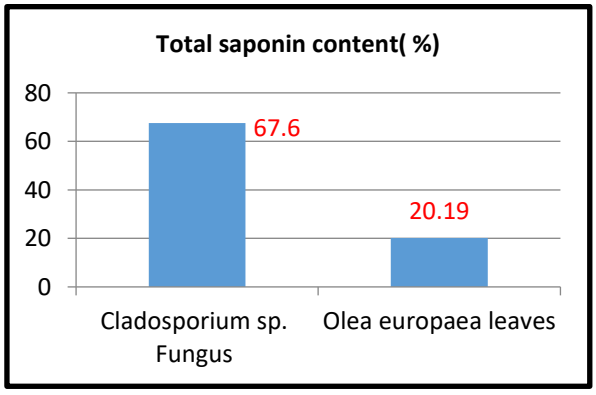


P-value ≥ 0.05(0.216)



P-value ≤ 0.05(0.001)

Figure 2. Comparing the amount of secondary metabolites in an olive plant leaf extract and an endophytic fungus called *Cladosporium* sp.

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According to the findings in Table 1, the 27 isolates of *candida* sp. from various species that were obtained from various clinical sources could not grow due to the endophytic fungus *Cladosporium* sp. As demonstrated in the Table below, all candida sp. isolates exhibited strong sensitivity to *Cladosporium* sp. extract, which increased with increasing concentration to reach the highest level to Inhibit the fungal growth when concentrated 30 mg.ml^{-1}. Fig. 3 illustrates, as an example, the inhibitory effect of *Cladosporium* sp. extracts on the growth of a *C.albicans* isolate.

Table 1. Antifungal ability of Endophytic fungus *Cladosporium* sp. Extract against different isolated of *candida* sp.

Isolate N.	*candida* sp.	Inhibition zone (mm)			
		0 mg.ml^{-1}	**10** mg.ml^{-1}	**20** mg.ml^{-1}	**30 mg.ml^{-1}**
1	*C.albicans*	0	17.4	20.6	**25**
2	*C.albicans*	0	14.3	17.8	**27.6**
3	*C.albicans*	0	10.8	14.8	**18.9**
4	*C.albicans*	0	18.1	24.5	**39**
5	*C.albicans*	0	13.7	18.5	**25.3**
6	*C.albicans*	0	21	25.1	**28.6**
7	*C.lypolitica*	0	20.9	23.5	**28**
8	*C.lypolitica*	0	19.1	19.8	**23**
9	*C.tropicalus*	0	12.11	20	**35.1**
10	*C.tropicalus*	0	12.4	19.7	**24.4**
11	*C.tropicalus*	0	18.4	22.6	**30.4**
12	*C.tropicalus*	0	12.4	18	**25.3**
13	*C.sphaerica*	0	15.6	18.1	**23**
14	*C.sphaerica*	0	19.2	25.6	**28**
15	*C.sphaerica*	0	16.5	17.8	**25.4**
16	*C.krusei*	0	11.6	19.2	**26**
17	*C.krusei*	0	11	15.7	**19.8**
18	*C.krusei*	0	13	21.2	**33**
19	*C.guilliermondii*	0	15.3	16	**23.5**
20	*C.guilliermondii*	0	16.3	24.7	**23**
21	*C.parapsilosis*	0	15.2	21.3	**29.3**
22	*C.norvegenesis*	0	12.4	13.6	**28.2**
23	*C.glabrata*	0	10.7	17	**21.4**
24	*C.glabrata*	0	20.1	28	**37.1**
25	*C.glabrata*	0	18.3	25	**28.4**
26	*C.glabrata*	0	11.5	21	**22**
27	*C.kefyr*	0	20	21	**23**
		P-value ≤ 0.05 (0.001)			

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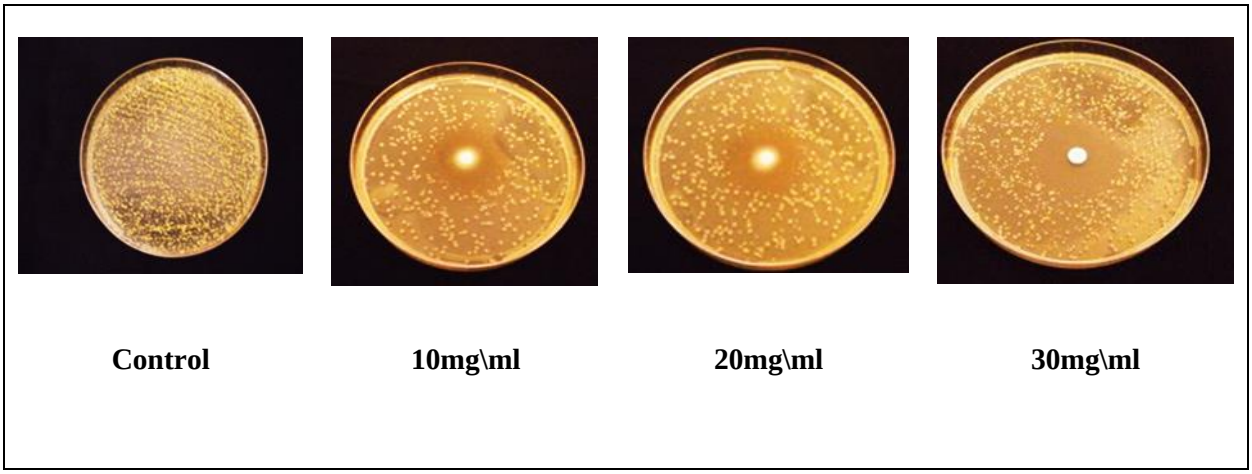

Figure 3. Antifungal activity of *Cladosporium* sp. extracts using *C.albicans* isolate No. 5 .

Discussion

All higher plants are hosts to one or more endophytic microbes on this earth. Endophytic fungi are microbes that reside in living plant tissues without causing any immediate harm to their host [28] in this study *Cladosporium* sp. was endophytic fungus that was isolated from olive plant leaves which identified according to morphological characteristics and nuclear ribosomal DNA ITS sequence analysis. Endophytic fungi are microorganisms that reside in the tissues of living plants and do not cause any immediate damage to their host [29], *Cladosporium* species are extremely widespread around the globe, and it is possible to isolate them from a wide variety of organic materials [30] .In comparison to the olive plant, which the fungus was isolated from, the results shown above indicate that *Cladosporium* extract is a rich source of secondary metabolites. These secondary metabolites include total flavonoid, total phenolic content, total terpenoid, and alkaloids. The antifungal activity of *Cladosporium* sp. was a reflection of its large content of secondary metabolites, each of which has its own unique set of biological features. *Cladosporium* sp. extract, in any concentration, was able to suppress the growth of numerous fungal isolates of Candida sp. that had been isolated from a variety of clinical sources. These potentially useful endophytes can be further utilized to expedite plant growth and amplification through the processes of isolating, genetically manipulating, and industrially scaling up their production. Additionally, the employment of endophytes as alternative sources of bioactive chemicals reduces the exploitation of the host plant, which results in the conservation of biodiversity [31].

Conclusion

The results indicate that the endophytic fungi isolated from *Olea europaea* L. leaves may have a bright future in medicine due to their capacity to generate physiologically active compounds with characteristics similar to those of the plant where the endophytic fungus was isolated. Therefore, further research is required to identify the active ingredients produced by endophytic fungi and investigate their diverse medicinal properties in an effort to find new sources of medications and therapies.

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Authors' Declaration

- Conflicts of Interest: None.

- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Diyala.

Authors' Contribution Statement

All authors contributed to the completion of the research by participating in collecting plant samples, isolating and diagnosing endophytes, and writing and reviewing the manuscript, The research tasks were distributed equally among the three authors.

References

1. Aharwal RP, Kumar S, Sandhu SS. Endophytic mycoflora: antibacterial secondary metabolites and their therapeutic potential. Curr Pharmacol Rep. 2021 Sep; 7(4): 150-70. https://doi.org/10.1007/s40495-021-00261-w
2. Toppo P, Kagatay LL, Gurung A, Singla P, Chakraborty R, Roy S, et al. Endophytic fungi mediates production of bioactive secondary metabolites via modulation of genes involved in key metabolic pathways and their contribution in different biotechnological sector. 3 Biotech. 2023 Jun; 13(6): 191. https://doi.org/10.1007/s13205-023-03605-z
3. Jiang Q, Wei N, Huo Y, Kang X, Chen G, Wen L. Secondary metabolites of the endophytic fungus *Cladosporium* sp. CYC38. Chem Nat Compd. 2020; 56: 1166-9. https://doi.org/10.1007/s10600-020-03257-y
4. Nicoletti R. Endophytic fungi of citrus plants. Agriculture. 2019 ; 9(12): 247. https://doi.org/10.3390/agriculture9120247
5. Le TT, Hoang AT, Nguyen NP, Le TT, Trinh HT, Vo TT, et al. A novel huperzine A-producing endophytic fungus *Fusarium* sp. Rsp5. 2 isolated from *Huperzia serrate*. Biotechnol Lett. 2020 ; 42(6): 987-995. https://doi.org/10.1007/s10529-020-02836-x
6. Ismail MA, Amin MA, Eid AM, Hassan SE, Mahgoub HA, Lashin I, et al. Comparative Study between exogenously applied plant growth hormones versus metabolites of microbial endophytes as plant growth-promoting for *Phaseolus vulgaris* L. Cells. 2021 ;10(5): 1059. https://doi.org/10.3390/cells10051059
7. Mamaghani NA, Saremi H, Javan-Nikkhah M, De Respinis S, Pianta E, Tonolla M. Endophytic *Cephalotrichum* spp. from *Solanum tuberosum* (potato) in Iran–a polyphasic analysis. Sydowia. 2022 ;74: 287-301. https://www.verlag-berger.at/detailview?no=2999
8. Malik LA, Aziz GM, Ad'hiah AH. The potential of some plant extracts as radical scavengers and dipeptidyl peptidase-4 inhibitors. Baghdad Sci J. 2019 Jan 2; 16(1): 162-168. https://doi.org/10.21123/bsj.2019.16.1(Suppl.).0162
9. Omar SH, Scott CJ, Hamlin AS, Obied HK. Biophenols: Enzymes (β-secretase, Cholinesterases, histone deacetylase and tyrosinase) inhibitors from olive (*Olea europaea* L.). Fitoterapia. 2018; 128: 118-129. https://doi.org/10.1016/j.fitote.2018.05.011
10. Syed RU, Moni SS, Alfaisal RH, Alrashidi RH, Alrashidi NF, Wadeed KM, et al. Spectral characterization of the bioactive principles and antibacterial properties of cold methanolic extract of *Olea europaea* from the Hail region of Saudi Arabia. Arab J Chem.. 2022; 15(8): 104006. https://doi.org/10.1016/j.arabjc.2022.104006
11. Rezagholizadeh L, Aghamohammadian M, Oloumi M, Banaei S, Mazani M, Ojarudi M. Inhibitory effects of *Ficus carica* and *Olea europaea* on pro-inflammatory cytokines: A review. Iran J Basic Med Sci. 2022; 25(3): 268. https://doi.org/10.22038/IJBMS.2022.60954.13494
12. Martins F, Pereira JA, Bota P, Bento A, Baptista P. Fungal endophyte communities in above-and belowground olive tree organs and the effect of season and geographic location on their structures. Fungal Ecol. 2016 ; 20: 193-201. https://doi.org/10.1016/j.funeco.2016.01.005
13. Varanda CM, Materatski P, Landum M, Campos MD, Félix MD. Fungal communities associated with peacock and cercospora leaf spots in olive. Plants. 2019 Jun 12; 8(6): 169. https://doi.org/10.3390/plants8060169
14. Malhadas C, Malheiro R, Pereira JA, de Pinho PG, Baptista P. Antimicrobial activity of endophytic fungi from olive tree leaves. World J Microbiol Biotechnol. 2017; 33: 1-12. https://doi.org/10.1007/s11274-017-2216-7
15. Lateef AA, Garuba T, Abdulkareem KA, Olayinka BU, Olahan GS, Adeyemi SB, et al. Molecular

Characterization of Potential Crop Pathogens Associated with Weeds as Endophytes in Uniilorin Plantations, Nigeria. Baghdad Sci J. 2022 Dec 1; 19(6): 1201. https://doi.org/10.21123/bsj.2022.5999
16. Gupta VK, Tuohy MG, Ayyachamy M, Turner KM, O'donovan A. Laboratory protocols in fungal biology. Springer International Publishing; 2nd Ed, 2022: 261. https://doi.org/10.1007/978-3-030-83749-5
17. Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, et al. Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. Stud Mycol. 2007 Jan 1; 58: 105-56. https://doi.org/10.3114/sim.2007.58.05
18. Zhang P, Zhou PP, Yu LJ. An endophytic taxol-producing fungus from Taxus media, *Cladosporium cladosporioides* MD2. Curr Microbiol. 2009 Sep; 59: 227-32. https://doi.org/10.1007/s00284-008-9270-1
19. White T,j. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990:315-22. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
20. Badotti F, de Oliveira FS, Garcia CF, Vaz AB, Fonseca PL, Nahum LA, et al. Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). BMC Microbiol. 2017 Dec; 17(1): 1-2. https://doi.org/10.1186/s12866-017-0958-x
21. Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 2004 Jun 1; 5(2): 150-63. https://doi.org/10.1093/bib/5.2.150
22. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 2007 ;101(1): 140-147. https://doi.org/10.1016/j.foodchem.2006.01.014
23. Zhu H, Wang Y, Liu Y, Xia Y, Tang T. Analysis of flavonoids in *Portulaca oleracea* L. by UV–vis spectrophotometry with comparative study on different extraction technologies. Food Anal Methods. 2010 ; 3: 90-97. https://doi.org/10.1007/s12161-009-9091-2
24. Datta D, Behera L, Chaudhary VT, Kumar S, Bisen K. Endophytes: rendering systemic resistance to plants. InRhizosphere Microbes: Biotic Stress Management 2022 Oct 8 (pp. 175-195). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-5872-4_9
25. Liu HG, Li T, Zhao YL, Zhang J, Wang YZ. Determination of some metabolites of Cordyceps sobolifera. Afr J Microbiol Res.2011; 5(30): 5518-5522.
26. Berkow EL, Lockhart SR. Fluconazole resistance in Candida species: a current perspective. Infect Drug Resist. 2017 Jul 31: 237-45. https://doi.org/10.2147/IDR.S118892
27. Petrini O. Fungal endophytes of tree leaves. InMicrobial ecology of leaves. Springer New York. 1991 : 179-197. https://doi.org/10.1007/978-1-4612-3168-4_9
28. Grinn-Gofroń A, Nowosad J, Bosiacka B, Camacho I, Pashley C, Belmonte J, et al. Airborne *Alternaria* and *Cladosporium* fungal spores in Europe: Forecasting possibilities and relationships with meteorological parameters. Sci Total Environ. 2019 Feb 25; 653: 938-46. https://doi.org/10.1016/j.scitotenv.2018.10.419
29. Khalil MI. Identification of Cladosporium sp. Fungi by in-silico RFLP-PCR. Baghdad Sci. J. 2020 Jan 2;17(1):220-6. https://doi.org/10.21123/bsj.2020.17.1(Suppl.).0220
30. Barzee TJ, Cao L, Pan Z, Zhang R. Fungi for future foods. J Future Foods. 2021 Sep 1; 1(1): 25-37. https://doi.org/10.1016/j.jfutfo.2021.09.002
31. Gauchan DP, Kandel P, Tuladhar A, Acharya A, Kadel U, Baral A, et al. Evaluation of antimicrobial, antioxidant and cytotoxic properties of bioactive compounds produced from endophytic fungi of Himalayan yew (*Taxus wallichiana*) in Nepal. F1000Res. 2020 May 19; 9: 379. https://doi.org/10.12688/f1000research.23250.2

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التأثير المضاد للفطر *Cladosporium* sp. (فطريات داخلية) المعزول من اوراق نبات الزيتون *Olea europaea* L.

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الخلاصة

تم عزل الفطر الداخلي من أوراق اشجار الزيتون وتم التعرف على الفطر على أنه *Cladosporium* sp. وفقًا لخصائصه المظهرية وتحليل تسلسل الحمض النووي الريبوسومي النووي باعتماد منطقة ITS حيث تم تسجيل الفطر في NCBI على انه الجنس *Cladosporium* وسجلت العزلة تحت الرقم التعريفي (0P939922.1) , ولم يتم تحديد النوع واعتبر على انه مجهول وذلك بعد مقارنته بالعزلات العالمية . اعطى مستخلص الفطر *Cladosporium* sp اعلى نسبة من مركبات الايض الثانوية وذلك بالمقارنة بمستخلص أوراق الزيتون التي تضمنت الفلافونويد الكلي ، المحتوى الفينولي الكلي ، التربينويدات الكلية ، الصابونين الكلي والتي كانت 121.9% و 198.1% و 89.13 % و 29.87% على التوالي مقارنة بمحتواها في مستخلص اوراق الزيتون والتي كانت 61.54% و 67.88 %و 17.1% و 20.19% على التوالي . كما اظهر مستخلص الفطر قدرة عالية على تثبيط نمو 27 عزلة تنتمي إلى أنواع مختلفة من *Candida* وهي *Candida albicans* , *C. lypolitica* , *C. tropicalis* , *C. sphaerica* , *C. krusei* , *C. guilliermondii* , *C. parapsilosis* , *C. norvegicus* , *C. glabrata* , *C. kefyr* . هذه النتائج تؤكد المستقبل الواعد للفطريات الداخلية كمضادات للفطريات.

الكلمات المفتاحية: مضاد فطري، *Candida*، *Cladosporium* sp، الفطريات الداخلية، اشجار الزيتون.