Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5209 - 5223
Received 25 April 2021; Accepted 08 May 2021.

Screening of Some Iraqi Plants for Fungal Species Producing Paclitaxel Anti-Cancer Drug

Eman H. B. Alfartosy[1], Abdullah H. Al-Saadoon[2], Asia Selman Abdullah[3*]

[1]Department of Biology, College of Science, University of Basrah, Iraq
[2]Department of Pathological analysis, College of Science, University of Basrah, Iraq
[3]Department of Pharmacology and Toxicology, College of Pharmacy, University of Basra, Iraq
*Corresponding author Asia S. Abdullah E-mail : < asiaselman2016@gmail.com>Tel: 00967-7719532558

Abstract

Researchers exploring the eukaryotic fungi because of their production of various plant related secondary metabolites with an extensive diversity of biological actions, such as paclitaxel, which produced at the beginning from yew bark**.** Sixty wild and cultivated plant samples collected from Basrah**.** The pure isolates screened for paclitaxel production, among thirty-two fungal endophytic species isolated from mature leaves, stems, flowers and fruits of different Iraqi plants, two important strains named *Alternaria alternata* (HKB1) and *Chaetomium globosum* (HKB2) are capable for paclitaxel production. The pure isolates of fungi screened for paclitaxel production by thin layer chromatography (TLC) using paclitaxel- standard, UV spectroscopic analysis and infrared (IR) spectrum investigated using FT-IR. Genomic DNA of the two strains HKB1 and HKB2 extracted, rRNA amplified by PCR, the amplicons sequenced, and the retrieved sequence of HKB1 and HKB2 strains deposited in GenBank with accession numbers LC621226 and LC621227, respectively. The mcf 7-cell line used to estimate the anti-tumor activity of isolated paclitaxel and the cytotoxic results showed that paclitaxel produced from *Alternaria alternata* was the best and most effective against MCF-7 cell line compared with the efficacy of Paclitaxel produced from *Chaetomium globosum*, which showed less efficacy but comparable to standard.

Key words: Iraqi plants, eukaryotic fungi Paclitaxel, *Alternaria alternata*, *Chaetomium globosum*

Introduction

Fungi are ubiquitous, eukaryotic microorganisms, which found in many different environments wherever organic material is available. They are found in a wide range of environments due to their capacity to utilize a variety of substrates and to their relative tolerance to low pH, low water activity and low temperature [1]. Pharmaceutical together with agricultural productions are exploring the eukaryotic fungi because of their production of a various plant related secondary metabolites with an extensive diversity of biological actions [2]. Amongst the plant-resulting

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natural yields, paclitaxel (anticancer drug), known economically as taxol, it is widely used in therapeutic application against various types of cancer [3]. Furthermore, paclitaxel has been considered for its important therapeutic effects against other non-cancer diseases, for the inhibition of restenosis [4]. Also for polycystic kidney disease and neurodegenerative illnesses [5]. Later, the attention in paclitaxel study will continue to increase [6]. Initially, paclitaxel produced from yew bark, the Pacific tree (*Taxus brevifolia*) by extraction. The content of paclitaxel in yew was very low [7]. Numerous alternative plans developed for the production of paclitaxel during the previous two decades. Semi-synthesis of paclitaxel (chemical alteration of precursors) accomplished, but the precursors extraction is costly, consuming long time and have a difficult purification procedure [8]. Moreover, chemical production is available, but the low yield and the big number steps of reaction limit its practicability [9]. For the large-scale production of paclitaxel, the tissue culture method of plant is developed effectively, but the incubation time is long and the yield is low [10]. Thus, it is interesting to find other options for production of paclitaxel, to rise its availability and lower its price.

The first endophytic fungus reported as microbial taxol –producing is *Taxomyces andreanae*, isolated from Pacific trees, yew in 1993 [11]. The benefits of microbial production of taxol include a rapid growth, easy genetic management and it is possible to scaling-up for manufacturing level [12]. Furthermore, the microbial source of production protects the natural plant resource [13]. Afterward, research groups from all over the world described more than 50 fungal isolates producing paclitaxel. These strains mostly isolated from different Taxus types like endophytes [14]. The paclitaxel yield in fungus strains is still little not enough for profitable determination. Therefore, it is important to explore various natural places for identification of good strains giving greater yield of paclitaxel [14]. Present studies focuses on exploring endophytes microbes that produces paclitaxel [11], engineering of genetics [15], refining the yield of paclitaxel using the genome shuffling [16], amendments of plant culture to enhance paclitaxel production [17] and heterologic expression of the precursor of paclitaxel in the microorganisms [18]. Therefore, it is emerging to find plants with a greater yield of paclitaxel.
In this study, various Iraqi plant samples investigated for fungal endophytes, to find promising endophytes for production of paclitaxel with a greater yield. To our knowledge, there have been no studies in Iraq on the production of the anti-cancer compound Paclitaxel from endophytic fungi inside Iraqi plants.

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Materials and Methods

Samples collection

During this study, 60 wild and cultivated plant samples collected from different regions in Basra Governorate, for the period from 10/3/2019 to 11/1/2020. Samples taken from different parts of plants, such as mature leaves, stems, flowers and fruits. The samples brought in clean plastic bags. To the fungi laboratory at Basra University / College of Science / Department of Life Sciences for isolation process.

Isolation of Fungi from Plants

Fungal endophytes isolated from the parts of the plants by two methods: The first method using Solid Culture Media according to [19]. By cutting, the plant part into small pieces 5x5 cm, then washing it twice with tap water to get rid of dust and impurities. Afterwards, the plant pieces were sterilized by immersing them in a Sodium hypochlorate solution (5% NaOCl) for a period of 3-4 minutes, then the plant pieces were transferred to ethanol at a concentration of 70% for a period of 3-4 minutes. Next, the plant cuttings were washed with sterile distilled water twice to remove traces of the sterilizer, and then the pieces were dried between two sterile Watman filter papers. Then the plant cuttings were planted on two types of culture media, potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) media, by placing 5-7 pieces from each plant for each plate and 4-5 replicates were made per plant. The dishes were incubated at 25 ± 2ºC for with periodic follow-up.

The second method using moist chamber, the parts of the plant were cut into small pieces 7 x 7 cm, washed with tap water twice, and the plant pieces sterilized as in the first method. After drying, the pieces were transferred to large Petri dishes (15 cm) containing 2-3 sterile filter papers and moistened with sterile distilled water. The dish was incubated at a temperature of 25 ± 2 °C with periodic follow-up.

Identification of fungi

After 7 days of incubation, the dishes were examined using a dissecting microscope to view the phenotypic features. After that, the fungi were examined by light microscope, by placing a drop of lactophenol Cotton Blue on glass slide, then a part of the developing fungal colony was transferred with a sterile needle and cover slip placed on it. Pure isolates of the fungi were made on slant culture media and incubated 7 days at a temperature of 25 ± 2ºC, after growth, the pure endophytic fungal isolates were stored in 15% (v/v) glycerol at − 4 °C as a suspension of spores and mycelium. All fungi isolated in this study were diagnosed according to [20 - 26].

Paclitaxel-producing fungal strains

The pure isolates of fungi were screened for paclitaxel production by the development of thin layer chromatography (TLC) using paclitaxel-authenticated standard (supplied by Xi'an Xinlu Biotech Co. Ltd., China). TLC sheets (supplied by Merck) with 8cm x 11cm dimensions and 2mm thickness and TLC plate

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was developed using the solvent system composed of chloroform/methanol (7:1, v/v) according to [27, 28]. After that, the plate was dried by air and sprayed by a reagent of vanillin-H_2SO_4 (containing vanillin (2 % w/v) in 1 mL sulphuric acid and 100 mL methanol), then incubation for 15min at 110 °C. After that Rf value of the bands were calculated, the spots of paclitaxel appeared dark blue [29].

Molecular identification of fungi

Identification of the two strains producing paclitaxel confirmed by molecular characterization based on the sequence of PCR-amplified rRNA-gene analysis, carried out according to the method of [30]. Universal primers were used, ITS1-ITS4, and special primers, NL1 – NL4, as in table1. The two strains of fungi sent to (Daejeon, South Korea) for rRNA gene sequencing. The fungal strains grown on PDA and SDA media, incubated for 7-14 days at 25 ± 2°C and a loop full transporter takes fungal cells to the micro tube, dissolved in 100µL of autoclaved distilled water and then boiled for 15 min at 100°C, after that, the genomic DNA extracted using Solgent purification bead. Thereafter, rRNA gene amplified by the use of PCR technique, two universal fungal primers incorporated in the mixture of reaction. The configuration of these primers was Forward ITS1 (F-5`- CTT GTT CAT TTA GAG GAA GTA AG -3`) and Reverse ITS4 (R-5`- CGC TAT CAA GCG GAG GAA AAG GC -3`). The PCR purified products (amplicons) checked by the use of marker a nucleotide size (100 base pairs) by 2% agarose gel electrophoresis. The DNA-amplification PCR sequences of fungal samples were analyzed at Macrogen in South Korea. Each sample sequenced in the sense and antisense orders using ITS1 and ITS4 primers [30].

Paclitaxel production by selected fungi by fungal fermentation

Fungal isolates activated with subcultures on PDA and SDA media and incubated at 25 ± 2 ° C for 7-14 days. One milliliter from the growing and pure fungal colony placed in a 250 ml glass beaker containing 125 ml of PDB and yeast extract peptone dextrose broth YPDB media and the pH of the media adjusted to 6. The fermentation carried out and all glass flasks were incubated in a Shaker Incubator, for a period of 14-21 days, at 25-28° C and a speed of 120 rpm. After the incubation period, the samples centrifuged for 10 minutes, at 10,000 rpm, to separate the fermentation product, the filtrate containing the organic compounds was taken and kept in the refrigerator until other operations are performed on it.

Extraction of paclitaxel

The filtrate of culture analyzed for paclitaxel by the use of method approved by [27]. To prevent contamination with fatty acid in the extraction of paclitaxel, 0.025%, w/v Na2CO3 added to the culture filtrate and then paclitaxel extracted by the addition of an equivalent volume dichloromethane. After that the organic layer was taken, filtered using the anhydrous sodium sulfate and evaporated to remove the solvent at 35°C under lower pressure by the use of a rotary evaporator. Then the absolute methanol used to dissolve dried extract and the paclitaxel was investigated. TLC qualitative investigation was done as described above, to confirm the purity of extracted paclitaxel. The sample spot proximal to the standard

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compound Rf was removed using a sterile medical blade by silica gel scraping and placed in a sterile cup, then washed by methanol. Then the paclitaxel quantified using ultraviolet spectroscopy analysis recorded in an aqueous solution on a PG T90U UV-visible spectrophotometer by conservative quartz cell with 1 cm optical path length at 298 K according to [31] and the concentration was then obtained after recording the absorption at 273 nm against a standard curve. The infrared spectrum of the compounds purified from the two fungal isolates measured, using FTIR spectra, and recorded in the range of 500- 4000cm^{-1} on Shimadzu spectroscopy, according to [31].

Anticancer action of paclitaxel

Maintenance of cell culture

The mcf 7 cell line obtained from the IRAQ Biotech Cell Bank Unit in Basrah and maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 50% confluence twice a week and incubated at 37 °C and 5% Co2 [32].

Combination Cytotoxicity Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell line mcf 7 were seeded at 1×10^4 cells/well. After 24h or a confluent monolayer was achieved, cells were treated with the tested compounds with final concentrations of (15, 30, 50, 100 and 200)µg/ml. Cell viability was measured after 72h of treatment by removing the medium, adding 28µL of 2 mg/mL solution of MTT (and incubating the cells for 2h at 37°C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 100µL of DMSO (Dimethyl Sulphoxide) followed by 37°C incubation for 15 min with shaking [33]. The absorbency was determined on a microplate reader at 620nm wavelength; the assay performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) calculated according to [34], as the following equation:

Proliferation rate as (PR)= B/A*100 where A is the mean optical density of untreated wells and B is the optical density of treated wells and IR= 100- PR .

Statistical analysis

The results of the cytotoxicity assays were expressed statistically using the SPSS program, version 22. The mean ± standard deviation (SD) used and statistical significance was calculated and evaluated using analysis of variance (ANOVA).

Results

Screening, isolation and purification of fungal endophytes producing paclitaxel

The most common genera in all plant samples belonged to *Aspergillus* (73.3%), *Alternaria* (53.3%), while the genus *Cladosporium* and *Fusarium* , *Penicillium* (36.6%), appeared by, and

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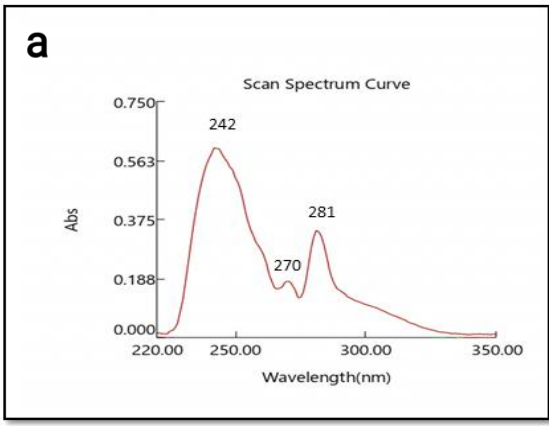
the genus *Chaetomium* was 26.6%. The least appearing genera in this study are *Zygosporium*, *Sepedonium*, *Microascus*, *Lasiodiplodia*, *Halinospora*, *Bipolaris* and *Acremonium*. There are nine species of fungi were recorded inside plants for the first time in Iraq, new reports for the Iraqi mycobiota. All the isolated species were registered in the Japanese Genebank.

All fungal isolates were screened to test their ability to produce paclitaxel, using PDB and YPDB and the crude extracts of these fungi were investigated by a thin layer chromatographic technique comparing with the standard paclitaxel compound. The results of TLC revealed that *Alternaria alternata* HKB1 strain (isolated from the flowers of the *Nerium oleander* plant in Garmat Ali, Basra, Iraq) and *Chaetomium globosum* HKB2 strain (isolated from the parts of medicinal plants *Tamarix aphylla* (the Arabic name Athel) plant in Al-Zubair and Abu Al-Khasib, Basra, Iraq) were paclitaxel-producing fungi. Because of their flow rate is identical or close to paclitaxel standard. Using the cultivation medium (PD broth), the producing capability of these fungal strains were 1g L^{-1} and 0.5g L^{-1} culture filtrate, respectively.

UV spectroscopic analysis confirmed the presence of paclitaxel in the crude extracts of the two fungal strains. UV absorption spectrum for the paclitaxel obtained from HKB1 and HKB2 matched the authenticated standard paclitaxel, the maximum absorption at 273 nm (Figure 1a–c). Therefore, HKB1 and HKB2 strains were used in this study to determine promising fermentation conditions to improve the production of paclitaxel.

The infrared (IR) spectrum of the purified paclitaxel obtained from HKB1 and HKB2 were investigated using FT-IR and recorded in the range of 500-4000 cm^{-1}. IR spectrum for the paclitaxel obtained from HKB1 and HKB2 matched the authenticated standard paclitaxel, (Figure 2 a–c).



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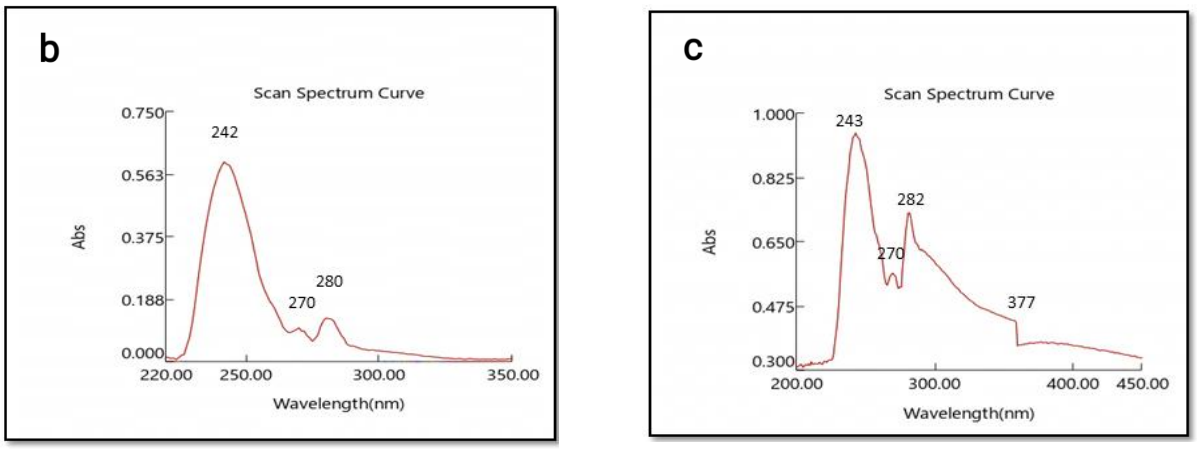

Figure 1: UV spectral analysis of paclitaxel separated from *Alternaria alternata* HKB1 and *Chaetomium globosum* HKB2 strains. (a) UV spectral analysis of standard (supplied by Xi'an Xinlu Biotech Co. Ltd., China). (b) UV spectral analysis of paclitaxel from *Alternaria alternata* HKB1 strain. (c) UV spectral analysis of paclitaxel from *Chaetomium globosum* HKB2 strain.

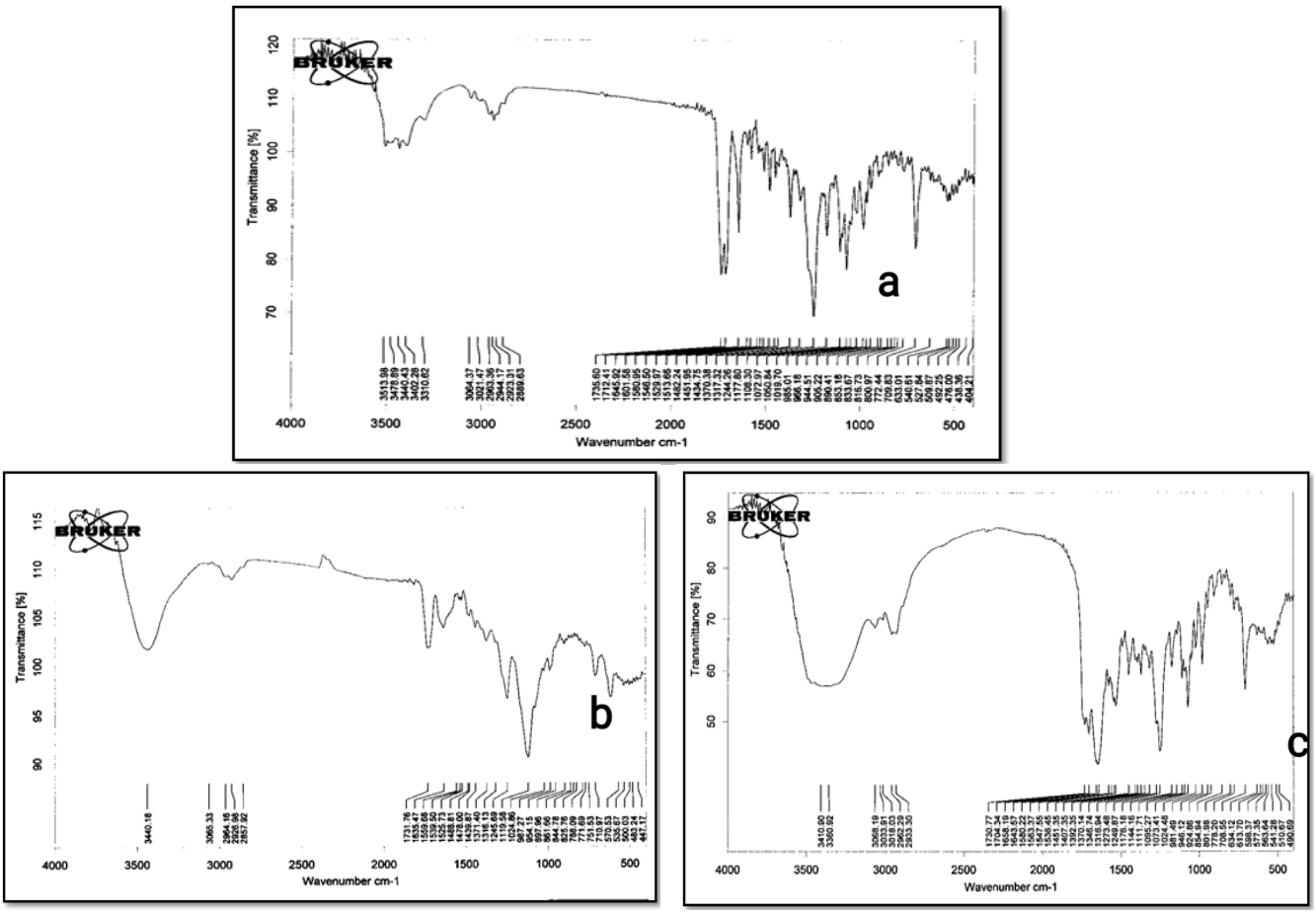

Figure 2: Infrared (IR) spectrum analysis of paclitaxel separated from *Alternaria alternata* HKB1 and *Chaetomium globosum* HKB2 strains. (a) IR spectrum analysis of standard (supplied by Xi'an Xinlu Biotech Co. Ltd., China). (b) IR spectrum analysis of paclitaxel from *Alternaria alternata* HKB1 strain. (c) IR spectrum analysis of paclitaxel from *Chaetomium globosum* HKB2 strain.

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Table 1: Important frequencies in FT-IR to spectra for standard and the isolates

Assignment	Frequency (cm^{-1})		
	Standard	HKB1	HKB2
N-H/O-H Stretching vibration	3513.98 - 3310.62	3440.18	3410.95 - 336092
Aromatic C=C-H Stretching vibration	3064.37, 3021.47	3065.33	3068.19, 3033.91, 3018.03
Aliphatic C-H Stretching vibration	2963.36-2889.63	296416-2857.92	2962.29,2933.30
C=O Stretching vibration	1735.60,1712.41	1731.76	1730-1643
C=C Stretching /N-H bending vibration	1645.92	1635.47	
Aliphatic C-H bending vibration	1370	1245.69	1249.87
C-O Stretching vibration	1072.97		1073.41
C=C-H bending vibration	709.83	710.97	708.55

Morphological properties of *A. Alternata* HKB1and *C. globosum* HKB2

The macroscopically and microscopically bases of the selected strains (HKB1 and HKB2) were observed. HKB1 fungus forms dark olive to black colonies on the center of the PDA reaching 5 cm in diameter during 7 days of incubation at 25°C with dense and irregular fungal strands. Conidiophores are unbranched, with one or few scars. It reaches 50µm in length, 3-6µm in width. Conidia are obclavate, with a short beaks, 27- 45 × 12-17µm, brown in color, rough-walled, with muriform septa, often arising in unbranched chains of 10 or more (figure 3).

Colonies of HKB2 strain are without aerial mycelium or tiny white aerial hyphae in the center. They produce orange exudates in the center. Reverse is yellow to yellow-brown, but dark beneath the fruiting bodies. Fruiting bodies are superficial with ostioles, olive green or somewhat dark olive-orange to gray in color, spherical to ovoidal, 250µm in height, 135 to 250µm in diameter. The terminal hairs are abundant, slightly rough, brown, flexuous, tapering to the ends. Asci 45-60 ×12-17µm, 8 spores, bi seriate. Ascospores are olive-brown at maturity, lemon-shaped, with an apical germ spore, 10-12 × 6-7µm (figure 4).

A pure culture of these two strains was kept in the Fungal Research Laboratory, Department of

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Biology, College of Science, University of Basra.

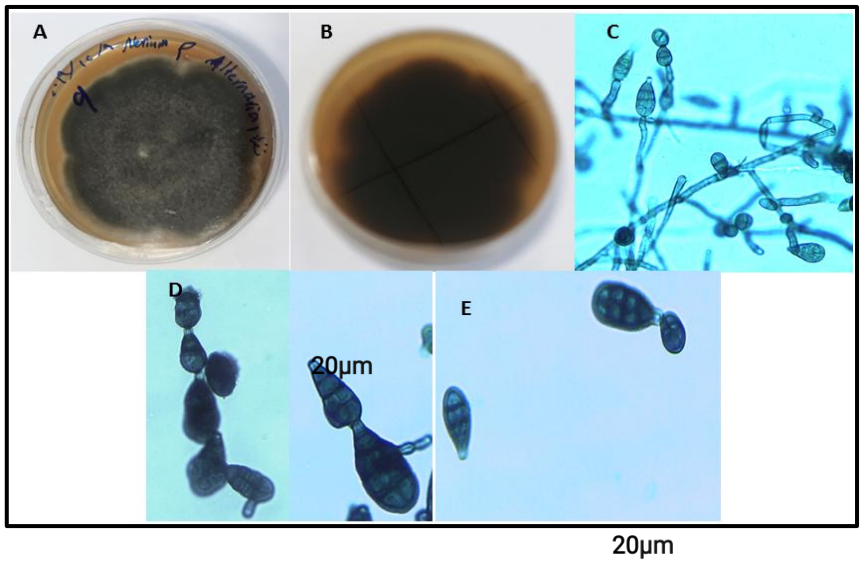

Figure 3: *Alternaria alternata* HKB1, (A) colony obverse on PDA; (B) reverse; (C) conidia and conidiophores; (D) chain of conidia; (E) single cells (bars C = 25µm, D and E = 20µm).

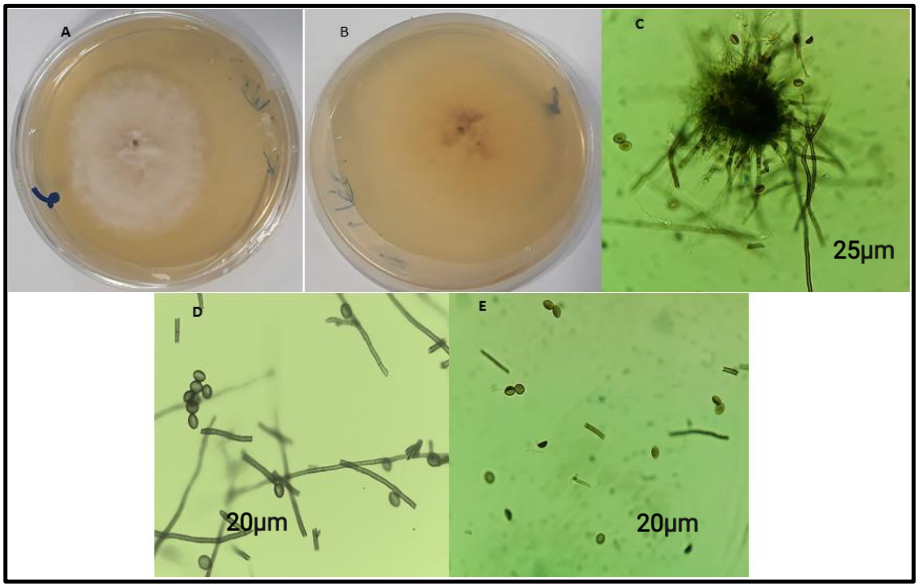

Figure 4: *Chaetomium globosum* HKB2; (A) colony obverse on PDA; (B) reverse; (C) fruity body; (D) and (E) Ascospores (bars C = 25µm, D and E = 20µm).

Molecular identification of *A. Alternata* HKB1 and *C. globosum* HKB2

Identification of HKB1 and HKB2 strains confirmed by molecular characterization based on the sequence of PCR-amplified rRNA-gene analysis. Genomic DNA of the two strains HKB1 and HKB2 were extracted, rRNA was amplified by PCR, the amplicons were sequenced, and the retrieved sequence of HKB1 and HKB2 strains was deposited in GenBank with accession numbers LC621226 and LC621227, respectively.

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Anticancer action of paclitaxel

The mcf 7-cell line was used to estimate the anti-tumor activity of paclitaxel separated from *Alternaria alternata* and *Chaetomium globosum* cultures. Cells were treated with the standard and tested compounds with final concentrations of (15, 30, 50, 100 and 200)µg/ml for 24 hours and the effect on cell viability was measured with the MTT assay. The results of the cytotoxicity test on cancer cells showed that the paclitaxel compound produced from fungal isolates has efficacy against breast cancer cells by inhibiting the growth of cells in all concentrations used in this test. It was found that the lowest concentration was able to inhibit and prevent cell proliferation, while high concentrations of paclitaxel showed a significant decrease in the proliferation of cancer cells, that is, the higher the concentration of Paclitaxel, the greater its effectiveness in inhibiting and killing cancer cells. Furthermore, the activity of isolated paclitaxel similar to the activity of standard paclitaxel, as illustrated in (Table 2).

The cytotoxic results showed that paclitaxel produced from *Alternaria alternata* was the best and most effective against MCF-7 cell line compared with the efficacy of Paclitaxel produced from *Chaetomium globosum*, which showed less efficacy but comparable to that of the standard compound.

Table 2: Anticancer activity of standard and separated fungal paclitaxel against mcf 7- cell line (breast cancer cells)

Paclitaxel concentration µg/ml	Cell viability (100%)		
	Standard	*Alternaria alternata*	*Chaetomium globosum*
0	98.470	98.470	98.470
15	61.208	77.769	54.158
30	61.432	58.746	67.474
50	62.103	59.306	66.020
100	66.243	58.746	63.894
200	61.767	57.403	68.817

MTT assay used for determining the cytotoxic effects of paclitaxel at 570 nm by the use of MTT solution under conditions described above; means in the same column with different letters considered statistically significant (LSD test, $P \leq 0.05$)

Discussion

Fungi within plants are of great importance due to their ability to produce biologically active compounds that have various medical, agricultural and industrial applications [35]. The current study proved that all plants used to isolate fungi are hosts for one or more fungi, as 233 fungal isolates were isolated from 60 plant samples, and this corresponds to many studies conducted on

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isolating fungi inside the plant. These findings are in scope with previous studies that were isolated the fungi from plants and found fungi in many healthy plants [35-37].

In this study, fungi were isolated using chemical disinfectants, namely sodium hypochlorate and ethanol 70%, and this isolation method is consistent with the method used by [19, 38] in isolating fungi, and unlike the method of [39], they used hydrogen peroxide. As a chemical sterilizer for plant surfaces as well as a studies of [40, 41] when silver chloride was used as a chemical sterilizer to isolate fungi inside the plant.

The results of the phenotypic diagnosis showed that all the fungi that were isolated during the study belonged to the Ascomycota group and this result in cope with other studies. The reason for this attributed to the ability of ascomycets to form large numbers of propagules, in addition to their high enzymatic ability to decompose organic materials and use them in growth and formation of reproductive units [42 – 44].

The results of the study showed that *Aspergillus* is the predominant genus and the most visible of the other fungi and its frequency was 73.3%, followed by other genus *Alternaria* 53.3%, then *Cladosporium* and the genus *Fusarium* , *Penicillium* 36.6%, this result in agreement with the results of [42]. The most common species of the genus *Aspergillus* is *Aspergillus terreus* and *Aspergillus niger*, the reason for this is that they are widespread fungi and are found in all environments in addition to the ability of these fungi to form reproductive units and grow in different environments [45].

The aim of the current study was to test fungal endophytes to find out their ability to produce anti-cancer compound paclitaxel. This study agrees with many studies conducted on the fungal endophytes to find paclitaxel producing fungi, such as [42 and 46 – 48]. This study resulted in isolating two strains capable of producing paclitaxel, namely *Alternaria alternata* and *Chaetomium globosum*. To our knowledge, this is the first study conducted in Iraq on endophytic fungi producing paclitaxel isolated from Iraqi plants.

The results showed that the paclitaxel compound produced from fungal isolates has efficacy against breast cancer cells growth in all concentrations used, the higher the concentration of paclitaxel, the greater its effectiveness in inhibiting and killing cancer cells. This result is consistent with the results of studies [35, 48]. The cause of cell death may be depend on the type

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of cells used, paclitaxel concentration and incubation period [49].

The results showed the possibility of producing paclitaxel in a pure and a high concentration from fungal endophytes inside the plant. This is consistent with the study of [50] obtained paclitaxel at a concentration of 84.5 g /L. The production of paclitaxel from the two fungal strains *Alternaria alternata* and *Chaetomium globosum* was confirmed using UV, IR diagnostic methods.

We conclude from this study that fungal endophytes are found in all plants on earth, and there may be types and genera of fungi inside the plant that have not yet been discovered.

Because of the widespread of cancer and the lack of studies on the production of anti-cancer drugs by fungi, researchers need to intensify their efforts to conduct molecular studies on fungal endophytes and their secondary metabolism, which may be a source for discovering new medically important compounds.

The discovery of new species of fungal endophytes within plants as producing sources of paclitaxel enhances the possibility of genetically engineering the organism to enhance paclitaxel production and the search for a gene in the organism encodes for the compound. New fungal strains producing paclitaxel at high concentrations should be searched for and isolated from different plants, most of which were not used as host plants.

Conclusions

The Paclitaxel compound produced from the fungi in this study has efficacy and cytotoxicity against the MCF-7 breast cancer cell line and may have an effect on all types of human cancer cells.

References

1. Garcia D, Ramos AJ, Sanchis V, Marín S. Predicting mycotoxins in foods: a review. Food microbiology. 2009 Dec 1;26 (8):757-69.
2. Dreyfuss MM, Chapela IH. Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. Discovery of Novel Natural Products with Therapeutic Potential. 1994 Jan 1:49-80.
3. Zhou X, Zhu H, Liu L, Lin J, Tang K. A review: recent advances and future prospects of taxol-producing endophytic fungi. Applied microbiology and biotechnology. 2010 May;86(6):1707-17.
4. Herdeg C, Oberhoff M, Baumbach A, Blattner A, Axel DI, Schröder S, Heinle H, Karsch KR. Local paclitaxel delivery for the prevention of restenosis: biological effects and efficacy in vivo. Journal of the American College of Cardiology. 2000 Jun;35(7):1969-76.
5. Zhang B, Maiti A, Shively S, Lakhani F, McDonald-Jones G, Bruce J, Lee EB, Xie SX, Joyce S, Li C, Toleikis PM. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. Proceedings of the National Academy of

Sciences. 2005 Jan 4;102(1):227-31.
6. Somjaipeng S, Medina A, Kwaśna H, Ortiz JO, Magan N. Isolation, identification, and ecology of growth and taxol production by an endophytic strain of Paraconiothyrium variabile from English yew trees (Taxus baccata). Fungal biology. 2015 Nov 1;119(11):1022-31.
7. Sonaimuthu V, Johnpaul M. Taxol (anticancer drug) producing endophytic fungi: an overview. International Journal of Pharma and Bio Sciences. 2010;1(3).
8. Patel RN. Tour de paclitaxel: biocatalysis for semisynthesis. Annual review of microbiology. 1998 Oct;52(1):361-95.
9. Nicolaou KC, Dai WM, Guy RK. Chemistry and biology of taxol. Angewandte Chemie International Edition in English. 1994 Jan 17;33(1):15-44.
10. Yukimune Y, Tabata H, Higashi Y, Hara Y. Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in Taxus cell suspension cultures. Nature biotechnology. 1996 Sep;14(9):1129-32.
11. Stierle A, Strobel G, Stierle D. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science. 1993 Apr 9;260(5105):214-6.
12. Flores-Bustamante ZR, Rivera-Orduna FN, Martínez-Cárdenas A, Flores-Cotera LB. Microbial paclitaxel: advances and perspectives. The Journal of antibiotics. 2010 Aug;63(8):460-7.
13. Mirjalili MH, Farzaneh M, Bonfill M, Rezadoost H, Ghassempour A. Isolation and characterization of Stemphylium sedicola SBU-16 as a new endophytic taxol-producing fungus from Taxus baccata grown in Iran. FEMS microbiology letters. 2012 Mar 1;328(2):122-9.
14. Hao X, Pan J, Zhu X. Taxol producing fungi. Natural products. 2013:2797-812.
15. Wang Y, Guo B, Miao Z, Tang K. Transformation of taxol-producing endophytic fungi by restriction enzyme-mediated integration (REMI). FEMS microbiology letters. 2007 Aug 1;273(2):253-9.
16. Zhao K, Ping W, Zhang L, Liu J, Lin Y, Jin T, Zhou D. Screening and breeding of high taxol producing fungi by genome shuffling. Science in China Series C: Life Sciences. 2008 Mar;51(3):222-31.
17. Li JY, Sidhu RS, Bollon AR, Strobel GA. Stimulation of taxol production in liquid cultures of Pestalotiopsis microspora. Mycological Research. 1998 Apr 1;102(4):461-4.
18. Ajikumar PK, Xiao WH, Tyo KE, Wang Y, Simeon F, Leonard E, Mucha O, Phon TH, Pfeifer B, Stephanopoulos G. Isoprenoid pathway optimization for Taxol precursor overproduction in Escherichia coli. Science. 2010 Oct 1;330(6000):70-4.
19. Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. Are tropical fungal endophytes hyperdiverse?. Ecology letters. 2000 Jul;3(4):267-74.
20. Watanabe T. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC press; 2010 May 21.
21. Pitt JI, Hocking AD. Fungi and food spoilage. New York: Springer; 2009 Jul 25.
22. Seifert K, Morgan-Jones G, Gams W, Kendrick B. The genera of Hyphomycetes. CBS-KNAW Fungal Biodiversity Centre. Utrecht: The Netherlands. 2011.
23. Guarro J, Gene J, Stachigel AM, Figueras J. Atlas of soil Ascomycetes, CBS-KNAW fungal biodiversity center Utrecht. Netherland. 997pp. 2012.
24. De Gruyter J, Woudenberg JH, Aveskamp MM, Verkley GJ, Groenewald JZ, Crous PW. Redisposition of Phoma-like anamorphs in Pleosporales. Studies in Mycology. 2013 Jun 1;75:1-36.
25. Crous PW, Wingfield MJ, Lombard L, Roets F, Swart WJ, Alvarado P, Carnegie AJ, Moreno G, Luangsaard J, Thangavel R, Alexandrova AV. Fungal Planet description sheets: 951–1041. Persoonia: Molecular Phylogeny and Evolution of Fungi. 2019;43:223.
26. Wanasinghe DN, Hyde KD, Jeewon R, Crous PW, Wijayawardene NN, Jones EB, Bhat DJ, Phillips AJ, Groenewald JZ, Dayarathne MC, Phukhamsakda C. Phylogenetic revision of Camarosporium (Pleosporineae, Dothideomycetes) and allied genera. Studies in Mycology. 2017 Jun 1;87:207-56.
27. Strobel G, Yang X, Sears J, Kramer R, Sidhu RS, Hess WM. Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. Microbiology. 1996 Feb 1;142(2):435-40.

28. Zhang P, Zhou PP, Yu LJ. An endophytic taxol-producing fungus from Taxus media, Cladosporium cladosporioides MD2. Current microbiology. 2009 Sep;59(3):227-32.
29. Cardellina JH. HPLC separation of taxol and cephalomannine. Journal of liquid chromatography. 1991 Feb 1;14(4):659-65.
30. White TJ, Bruns T, Lee SJ, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990 Jan 1;18(1):315-22.
31. Pavia DL, Lampman GM, Kriz GS, Vyvyan JA. Introduction to spectroscopy. Nelson Education; 2014.
32. Al-Shammari AM, Alshami MA, Umran MA, Almukhtar AA, Yaseen NY, Raad K, Hussien AA. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an Iraqi patient. Breast Cancer: Targets and Therapy. 2015;7:223.
33. Al-Shammari AM, Al-Esmaeel WN, Al Ali AA, Hassan AA, Ahmed AA. Enhancement of Oncolytic Activity of Newcastle Disease virus Through Combination with Retinoic Acid Against Digestive System Malignancies. InMOLECULAR THERAPY 2019 Apr 22 (Vol. 27, No. 4, pp. 126-127). 50 HAMPSHIRE ST, FLOOR 5, CAMBRIDGE, MA 02139 USA: CELL PRESS.
34. Freshney RI. Culture of animal cells: a manual of basic technique and specialized applications. John Wiley & Sons; 2015 Dec 23.
35. Kumaran RS, Hur BK. Screening of species of the endophytic fungus Phomopsis for the production of the anticancer drug taxol. Biotechnology and Applied Biochemistry. 2009 Sep;54(1):21-30.
36. Huang Y, Wang J, Li G, Zheng Z, Su W. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants Taxus mairei, Cephalataxus fortunei and Torreya grandis. FEMS Immunology & Medical Microbiology. 2001 Aug 1;31(2):163-7.
37. Lakshmi PJ, Selvi KV. Anticancer potentials of secondary metabolites from endophytes of Barringtonia acutangula and its molecular characterization. Int J Curr Microbiol App Sci. 2013;2(2):44-5.
38. Goveas SW, Madtha R, Nivas SK, D'Souza L. Isolation of endophytic fungi from Coscinium fenestratum-a red listed endangered medicinal plant. EurAsian journal of BioSciences. 2011 Dec 1;5.
39. Miller KI, Qing C, Sze DM, Neilan BA. Investigation of the biosynthetic potential of endophytes in traditional Chinese anticancer herbs. PLoS One. 2012 May 22;7(5):e35953.
40. Rather RA, Srinivasan V, Anwar M. Seasonal deviation effects foliar endophyte assemblage and diversity in Asparagus racemosus and Hemidesmus indicus. BMC ecology. 2018 Dec;18(1):1-1.
41. Hazalin NA, Ramasamy K, Lim SS, Wahab IA, Cole AL, Majeed AB. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. BMC complementary and alternative medicine. 2009 Dec;9(1):1-5.
42. Katoch M, Phull S, Vaid S, Singh S. Diversity, Phylogeny, anticancer and antimicrobial potential of fungal endophytes associated with Monarda citriodora L. BMC microbiology. 2017 Dec;17(1):1-3.
43. Luo ZL, Hyde KD, Liu JK, Bhat DJ, Bao DF, Li WL, Su HY. Lignicolous freshwater fungi from China II: Novel Distoseptispora (Distoseptisporaceae) species from northwestern Yunnan Province and a suggested unified method for studying lignicolous freshwater fungi. Mycosphere. 2018 Jan 1;9(3):444-61.
44. Hyde KD, Fryar S, Tian Q, Bahkali AH, Xu J. Lignicolous freshwater fungi along a north–south latitudinal gradient in the Asian/Australian region; can we predict the impact of global warming on biodiversity and function?. Fungal ecology. 2016 Feb 1;19:190-200.
45. Dix NJ, Webster J. Fungi of extreme environments. InFungal ecology 1995 (pp. 322-340). Springer, Dordrecht.
46. Rebecca AI, Hemamalini V, Kumar DM, Srimathi S, Muthumary J, Kalaichelvan PT. Endophytic Chaetomium sp. from Michelia champaca L. and its taxol production. J. Acad. Ind. Res. 2012;1(68):e72.
47. Miller KI, Qing C, Sze DM, Neilan BA. Investigation of the biosynthetic potential of endophytes in traditional Chinese anticancer herbs. PLoS One. 2012 May 22;7(5):e35953.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 5209 - 5223
Received 25 April 2021; Accepted 08 May 2021.

48. Ismaiel AA, Ahmed AS, Hassan IA, El-Sayed ES, El-Din AZ. Production of paclitaxel with anticancer activity by two local fungal endophytes, Aspergillus fumigatus and Alternaria tenuissima. Applied microbiology and biotechnology. 2017 Jul;101(14):5831-46.
49. Yeung TK, Germond C, Chen X, Wang Z. The mode of action of taxol: apoptosis at low concentration and necrosis at high concentration. Biochemical and biophysical research communications. 1999 Sep 24;263(2):398-404.
50. Renpeng T, Qiao Y, Guoling Z, Jingquan T, Luozhen Z, Chengxiang F. Taxonomic study on a taxol producing fungus isolated from bark of Taxus chinensis var. mairei. Wuhan zhi wu xue yan jiu= Wuhan Botanical Research. 2006 Jan 1;24(6):541-5.