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Preliminary TLC Screening for Paclitaxel-Like Compounds in Endophytic Fungi Isolated from Plants in Basrah, Iraq

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ABSTRACT
Endophytic fungi are microorganisms that colonize plant tissues during all or part of their life cycle without causing obvious disease symptoms. In this study, endophytic fungi were isolated from wild and cultivated plant samples collected from different areas of Basrah Governorate, Iraq, and 50 isolates were screened for paclitaxel-like metabolites. The isolates were putatively identified by morphological examination and fermented in potato dextrose broth (PDB), potato carrot broth (PCB), and yeast peptone dextrose (YPD) media at 25-28 °C and 120 rpm for 14-21 days. Crude fungal extracts were examined by thin-layer chromatography (TLC) and compared with a paclitaxel reference standard. Several isolates produced TLC bands with retention factor (Rf) values comparable to the standard, suggesting the possible presence of paclitaxel-like or taxane-like compounds. Because TLC is a preliminary screening method, these findings should be considered presumptive and require confirmation by more specific analytical methods such as HPLC, LC-MS, or NMR. The results indicate that endophytic fungi associated with plants in Basrah may be useful candidates for further chemical investigation of paclitaxel-like secondary metabolites.

INTRODUCTION

Endophytic fungi colonize plant tissues during all or part of their life cycles without causing visible damage or disease symptoms **(Pimentel *et al.*, 2011)**. These fungi may establish long-term symbiotic relationships with host plants and can produce biologically active secondary metabolites that are similar to, or derived from, compounds produced by the host **(Priti *et al.*, 2009; Chandra, 2012; Subramaniyam & Vimala, 2012; Wang *et al.*, 2014; Uzor *et al.*, 2015; Ebada *et al.*, 2016)**.

Fungal secondary metabolites are commonly produced by filamentous fungi, particularly members of the subphylum Pezizomycotina within Ascomycota, as well as some Basidiomycota. These metabolites include polyketides, non-ribosomal peptides, terpenes, indole terpenes, indole alkaloids, cytochalasins, flavonoids, and steroids **(Keller *et al.*, 2005; Fox & Howlett, 2008; Guo *et al.*, 2008; Bayram & Braus, 2012; Voss & Riley, 2013; Krause *et al.*, 2018; Keller, 2019)**.

Natural products remain an important source of anticancer agents, and considerable research has focused on discovering new bioactive compounds from living organisms **(Ferreira *et al.*, 2011; Amaral *et al.*, 2019)**.

Related environmental biotechnology studies have also explored antimicrobial nanomaterials, photocatalytic wastewater treatment, and plant-biomass-derived sorbents as applied approaches for biological and environmental problems **(Al-Salih & Samsudin, 2019; Al-Salih *et al.*, 2021a, b; Al-Salih & Samsudin, 2025)**. The present study remains focused specifically on plant-associated endophytic fungi and preliminary TLC screening of paclitaxel-like secondary metabolites.

Paclitaxel is one of the most important anticancer compounds reported from fungi. It was first reported from *Taxomyces andreanae*, an endophytic fungus associated with Pacific yew **(Stierle *et al.*, 1993)**. Cancer remains a major health problem because of its high incidence and mortality worldwide **(Kharwar *et al.*, 2011; World Health Organization, 2013; Jinu & Jayabaskaran, 2015; Uzma *et al.*, 2018)**.

Paclitaxel, commercially known as Taxol, is a well-known natural anticancer compound originally isolated from the bark of yew trees **(Wani *et al.*, 1971; Pandi *et al.*, 2011)**. It has been used alone or in combination with other chemotherapeutic agents for several cancers, including breast, ovarian, lung, colon, stomach, and pancreatic cancers, and has also been investigated for non-cancer conditions such as polycystic kidney disease and neurodegenerative disorders **(Ross *et al.*, 2004; Wang *et al.*, 2007; Demain & Vaishnav, 2011)**.

MATERIALS AND METHODS

Collection of Plant Samples

Sixty wild and cultivated plant samples were collected from different areas of Basrah Governorate, Iraq, during the period from 10 March 2025 to 11 January 2026. Samples were taken from different plant parts, including twigs, leaves, flowers, and fruits. No fungal isolates used in this study were obtained from marine water, estuarine sediment, or coastal-water samples.

Isolation of Fungi from Plants

Endophytic fungi were isolated from plant tissues following the method of **Arnold *et al.* (2000)**. Plant parts were cut into 5 x 5 cm pieces and washed twice with tap water to remove dust and debris. The pieces were surface-sterilized by immersion in 5% sodium hypochlorite (NaOCl), transferred to 70% ethanol for 3-4 min, rinsed twice with sterile distilled water to remove sterilant residues, and dried between sterile Whatman filter papers. The sterilized segments were placed on potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA), with 5-7 pieces per plate and 4-5 replicates per plant. Plates were incubated at 25 +/- 2 °C for 7 days and monitored periodically for fungal growth.

Morphological Identification of Fungal Isolates

Fungal isolates were putatively identified to genus or species level based on colony morphology and microscopic characteristics. Because molecular identification was not performed in this study, all species-level names should be considered provisional and are presented as morphologically identified or putatively identified taxa.

Fermentation of Fungi

Crude fungal extracts were produced according to the method of **Ismaiel *et al.* (2017)**, with modifications. Pure fungal isolates were activated on PDA and SDA and incubated at 25 +/- 2 °C for 7-14 days. Two to three discs from actively growing colonies were transferred into 250 mL flasks containing 125 mL of PDB, PCB, or YPD broth, and the pH was adjusted to 6.0. The flasks were incubated in a shaking incubator at 25-28 °C and 120 rpm for 14-21 days. After incubation, cultures were centrifuged at 10,000 rpm for 10 min to separate the mycelial biomass from the filtrate. The filtrate containing extracellular organic metabolites was kept under refrigeration until extraction.

Extraction of Putative Paclitaxel-Like Metabolites

Putative paclitaxel-like metabolites were extracted according to **Strobel *et al.* (1996b)**. The filtrate was passed through filter paper to remove fermentation residues. An equal volume of dichloromethane (DCM) was added as an organic solvent, followed by 0.25% sodium carbonate (Na2CO3) to remove fatty acids. The mixture was shaken in a separating funnel for 2 min and left for 10 min until two layers were formed. The aqueous layer was discarded, whereas the organic layer was collected, treated with 0.5 g anhydrous sodium sulfate (Na2SO4) to remove residual water, and filtered. The samples were air-dried for 2 days, dissolved in 1 mL methanol, transferred to sterile 2 mL Eppendorf tubes, and stored under refrigeration until TLC analysis.

Preliminary TLC Screening for Paclitaxel-Like Compounds

Putative paclitaxel-like compounds were preliminarily screened by TLC following **Strobel *et al.* (1996b)** and were compared with a paclitaxel reference standard. Silica gel 60 F254 TLC plates were used. Methanolic extracts were applied to marked points on the plate using a capillary tube, allowed to dry, and reapplied to concentrate the spots. Plates were developed in chloroform:methanol (7:1, v/v) for approximately 10-15 min. After development, plates were air-dried and visualized using vanillin reagent **(Cardellina, 1991)** and iodine vapor **(Sherman & Fried, 1986)**. The retention factor (Rf) values of the detected bands were calculated and compared with the paclitaxel standard. TLC was used only as a preliminary screening technique; therefore, the possible presence of paclitaxel-like compounds cannot be considered confirmed without HPLC, LC-MS, NMR, or another confirmatory analytical method.

RESULTS

Isolation of Endophytic Fungi from Plant Tissues

Endophytic fungi were isolated from leaves, branches, flowers, and fruits of 60 plant samples collected from different areas of Basrah Governorate. Fungal growth appeared from plant tissues as white mycelia after approximately 3 days of incubation. All plant samples

yielded fungal isolates, and each plant hosted one or more endophytic fungal taxa. The isolates reported below are morphologically identified and should be regarded as putative taxa pending molecular confirmation.

Extraction of Putative Paclitaxel-Like Metabolites

Fermentation of Selected Endophytic Isolates

Fifty endophytic fungal isolates were fermented and screened for TLC evidence of paclitaxel-like metabolites (Table 1).

Table 1. Putatively identified endophytic fungal taxa used in the fermentation process, number of isolates, and host plant/source of isolation.

No.	Putatively identified fungal taxon	No. of isolates	Host plant/source	Fermentation time (days)	Colony age (days)
1	*Alternaria alternate*	1	*Nerium oleander*	14	8
2	*Alternaria* sp.	4	*Vitis vinifera*, *Lawsonia inermis*, *Allium sativum*,	21	10
3	*A. tenuissima*	3	*Tamarix aphylla*, *Trigonella foenum-graecum*	21	7
4	*A. ulocladia*	1	*Allium sativum*	21	8
5	*Aspergillus fumigatus*	3	*Tamarix aphylla*	21	7-10
6	*A. fumigatus* isolate 1	2	*Allium sativum*	14	12
7	*A. fumigatus* isolate 2	1	*Nerium oleander*	21	8
8	*A. fumigatus* isolate 3	2	*Conocarpus* sp.	21	7
9	*A. fumigatus* isolate 4	1	*Zea mays*	14	8
10	*A. terreus*	1	*Eucalyptus camaldulensis*	21	8
11	*Bipolaris* sp.	1	*Jasminum sambac*	21	8
12	*Chaetomium globosum*	4	*Tamarix aphylla*, *Lawsonia inermis*, *Allium sativum*	14-21	7-12
13	*Chaetomium* sp.	1	*Tamarix aphylla*	21	8
14	*Cladosporium phlei*	1	*Allium sativum*	14	12
15	*Cladosporium* sp.	1	*Sesbania sesban*	14	12
16	*Colletotrichum gloeosporioides*	1	*Jasminum sambac*	21	8
17	*Corynespora smithii*	1	*Jasminum sambac*	21	8
18	*Curvularia hominis*	1	*Jasminum sambac*	21	8-10
19	*Eurotium chevalieri*	3	*Allium sativum*	14	12
20	*Fusarium* sp.	4	*Rhanterium epapposum*, *Hordeum* sp., *Jasminum sambac*	21	10
21	*Fusarium brachygibbosum*	1	*Cyperus aucheri*	21	10
22	*Fusarium longipes*	1	*Moltkiopsis ciliata*	21	10
23	*Fusarium oxysporum*	1	*Jasminum sambac*	21	8-10
24	*Nigrospora zimmermanii*	1	*Nerium oleander*	21	12
25	*Paecilomyces* sp.	1	*Ziziphus* sp.	21	8
26	*Penicillium* sp.	2	*Conocarpus* sp.,	21	8

			Nerium oleander		
27	*Persiciospora Africana*	1	*Moltkiopsis ciliata*	21	8-10
28	*Phoma* sp.	2	*Punica granatum*, *Astragalus* sp.	21	8-10
29	*Scopulariopsis brevicaulis*	1	*Savignya parviflora*	21	8-10
30	*Scopulariopsis candida*	1	*Savignya parviflora*	21	8-10
31	*Sepedonium* sp.	1	*Rhanterium epapposum*	21	8-10

During periodic monitoring of the fermentation process, fungal isolates began to grow and form spores after 3-5 days of incubation under shaking conditions.

Fermentation results showed clear variation among isolates in growth pattern, biomass formation, and medium color. Isolates belonging to *Aspergillus, Alternaria, Chaetomium, Cladosporium*, and *Phoma* showed the most pronounced biomass pigmentation and sporulation, indicating active fungal growth and secondary metabolism (Fig. 1).

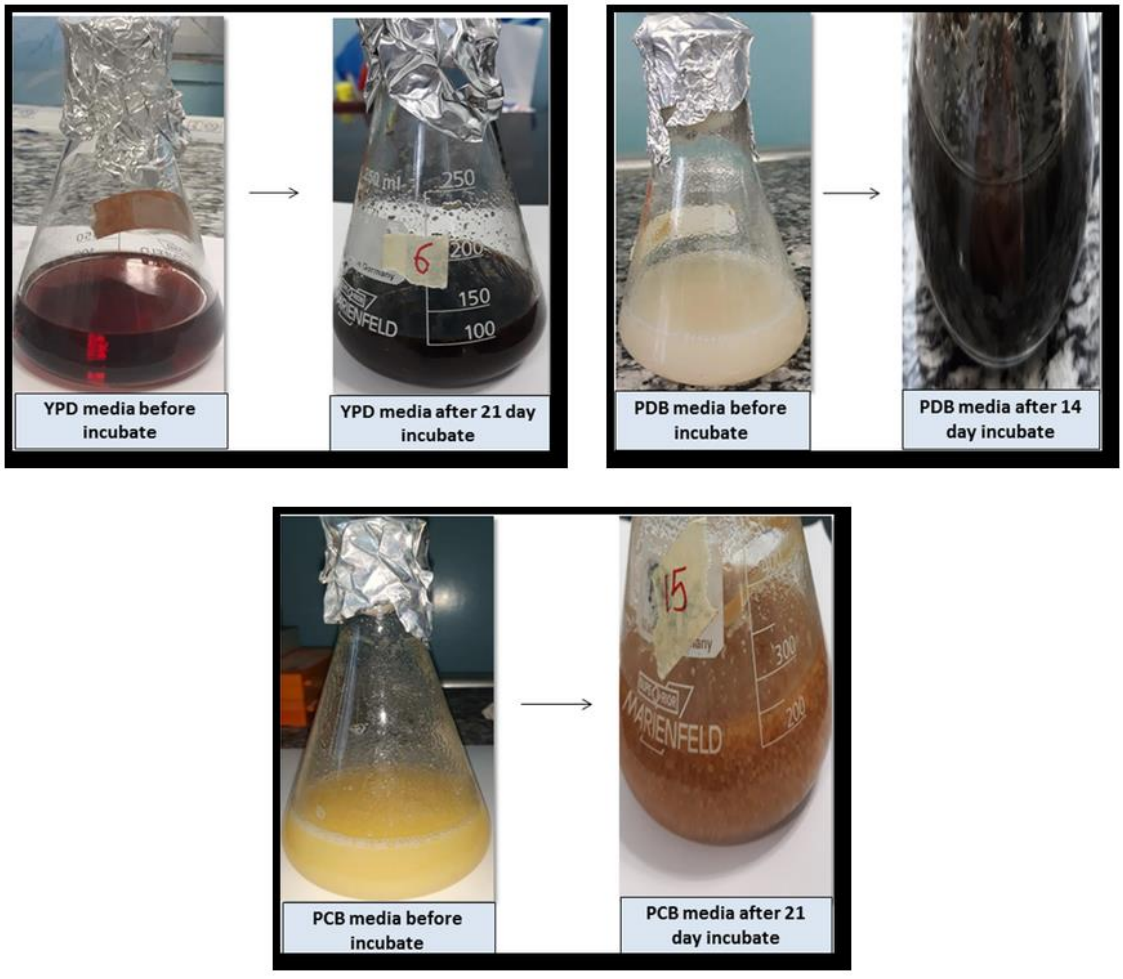

Fig. 1. Fermentation media used in this study before and after the incubation period.

Fungal Filtrate Extraction

The filtrates of all pure fungal isolates screened for paclitaxel-like metabolites were extracted using dichloromethane as the organic solvent (Fig. 2).

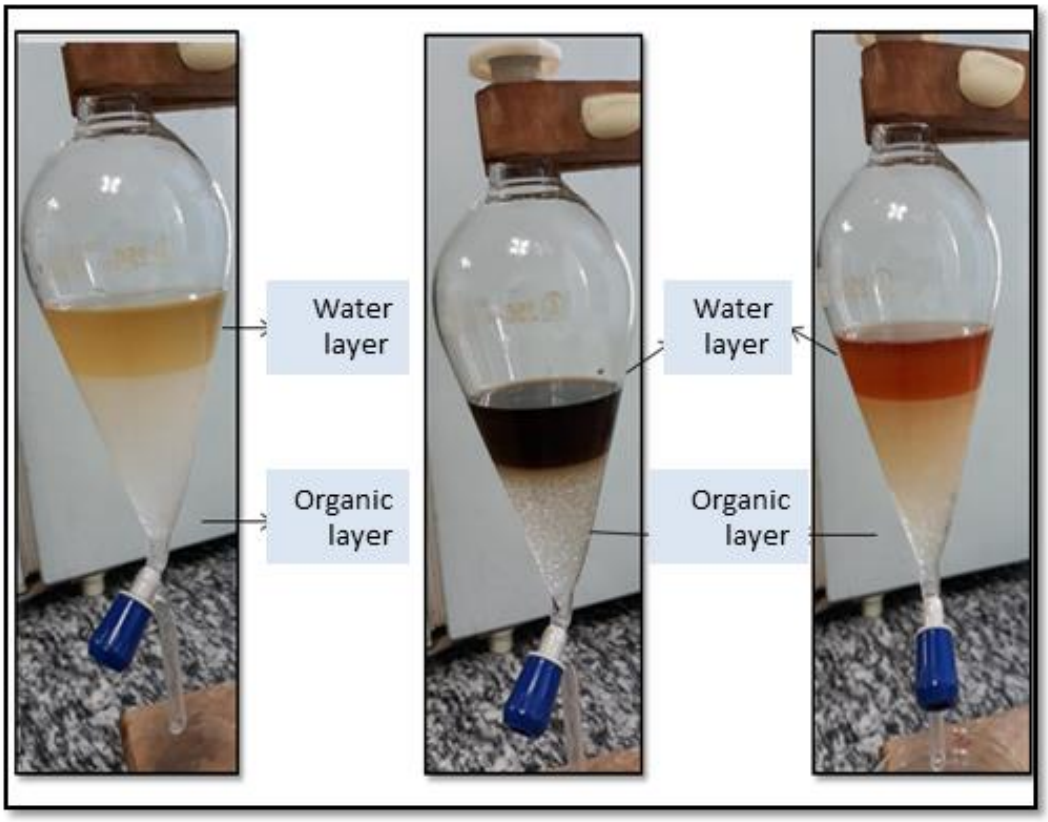

Fig. 2. Filtrate separation using dichloromethane: A. *Chaetomium* sp. filtrate in PCB medium; B. *Alternaria* sp. filtrate in YPD medium; C. *Alternaria tenuissima* filtrate in PDB medium.

Filtrate separation produced an upper aqueous layer and a lower organic layer containing the crude extract and diverse metabolites. Some isolates, particularly *Aspergillus, Alternaria, Chaetomium,* and *Phoma*, produced relatively large amounts of extract, whereas some isolates of *Fusarium, Eurotium chevalieri*, and *Chaetomium* produced smaller amounts.

Preliminary TLC Screening for Paclitaxel-Like Compounds

TLC was used only as a preliminary screening method to detect bands consistent with paclitaxel-like compounds in crude extracts of the tested fungal isolates after fermentation (Table 2).

Table 2. Preliminary TLC screening of paclitaxel-like compounds in tested fungal isolates, including the paclitaxel standard used for comparison.

No.	Putatively identified fungal taxon / standard	No. of reported TLC bands	Rf values
Std.	Paclitaxel reference standard	4	0.50, 0.60, 0.67-0.70, 0.90
1	*Alternaria alternate*	6	0.90, 0.80, 0.70, 0.67, 0.60, 0.50

No.	Putatively identified fungal taxon / standard	No. of reported TLC bands	Rf values
2	*Alternaria* sp. isolate 2	3	0.80, 0.70, 0.60
3	*Alternaria tenuissima* isolate 3	4	0.80, 0.70, 0.60, 0.50
4	*Alternaria ulocladia*	5	0.90, 0.80, 0.70, 0.60, 0.50
5	*Aspergillus fumigatus* (3 isolates)	3	0.70, 0.60, 0.50
6	*Aspergillus fumigatus* isolate 1	2	0.70, 0.40
7	*Aspergillus fumigatus* isolate 2	4	0.80, 0.76, 0.60, 0.50
8	*Aspergillus fumigatus* isolate 3	3	0.88, 0.80, 0.68
9	*Aspergillus fumigatus* isolate 4	4	0.80, 0.76, 0.60, 0.50
10	*Aspergillus terreus*	4	0.80, 0.70, 0.60, 0.50
11	*Bipolaris* sp.	4	0.80, 0.70, 0.60, 0.50
12	*Chaetomium globosum* isolate 4	5	0.90, 0.80, 0.70, 0.60, 0.50
13	*Chaetomium* sp.	2	0.70, 0.50
14	*Cladosporium phlei*	3	0.90, 0.50, 0.30
15	*Cladosporium* sp.	3	0.90, 0.50, 0.30
16	*Colletotrichum gloeosporioides*	2	0.70, 0.60
17	*Corynespora smithii*	2	0.90, 0.70
18	*Curvularia hominis*	3	0.80, 0.70, 0.50
19	*Eurotium chevalieri* isolate 3	4	0.30, 0.70, 0.80, 0.60
20	*Fusarium* sp. isolate 3	3	0.80, 0.67, 0.50
21	*Fusarium brachygibbosum*	3	0.80, 0.70, 0.60
22	*Fusarium longipes*	4	0.70, 0.60, 0.50, 0.40
23	*Fusarium oxysporum*	3	0.70, 0.60, 0.40
24	*Nigrospora zimmermanii*	3	0.80, 0.70, 0.50
25	*Paecilomyces* sp.	4	0.80, 0.70, 0.60, 0.40
26	*Penicillium* sp.	3	0.80, 0.60, 0.50
27	*Persiciospora Africana*	4	0.76, 0.70, 0.60, 0.50
28	*Phoma* sp. isolate 2	3	0.80, 0.70, 0.60
29	*Scopulariopsis candida*	3	0.67, 0.50, 0.40
30	*Sepedonium* sp.	5	0.90, 0.70, 0.69, 0.64, 0.40

Note: TLC evidence is preliminary only. Bands with Rf values comparable to the paclitaxel standard indicate putative paclitaxel-like or taxane-like compounds and require confirmation by HPLC, LC-MS, NMR, or another confirmatory method.

Preliminary TLC screening revealed multiple bands with different Rf values among the tested isolates, indicating variation in their secondary metabolite profiles. Several putatively identified isolates of *Aspergillus*, *Alternaria*, *Chaetomium*, *Fusarium*, *Phoma*, and other genera showed bands with Rf values close to those of the paclitaxel standard. These findings suggest the possible presence of paclitaxel-like or taxane-like metabolites, but they do not confirm paclitaxel identity. Rf = retention factor

The TLC results showed different metabolite profiles in the crude fungal extracts. Some isolates produced several TLC bands, including *Aspergillus fumigatus* isolates 2 and 3, *Fusarium*

brachygibbosum, and *Alternaria alternata*. *Chaetomium* sp., *Nigrospora zimmermanii*, and *Eurotium chevalieri* produced fewer bands. Based on this preliminary screening, several endophytic fungal isolates may produce paclitaxel-like compounds and should be prioritized for confirmatory HPLC, LC-MS, or NMR analysis (Fig. 3).

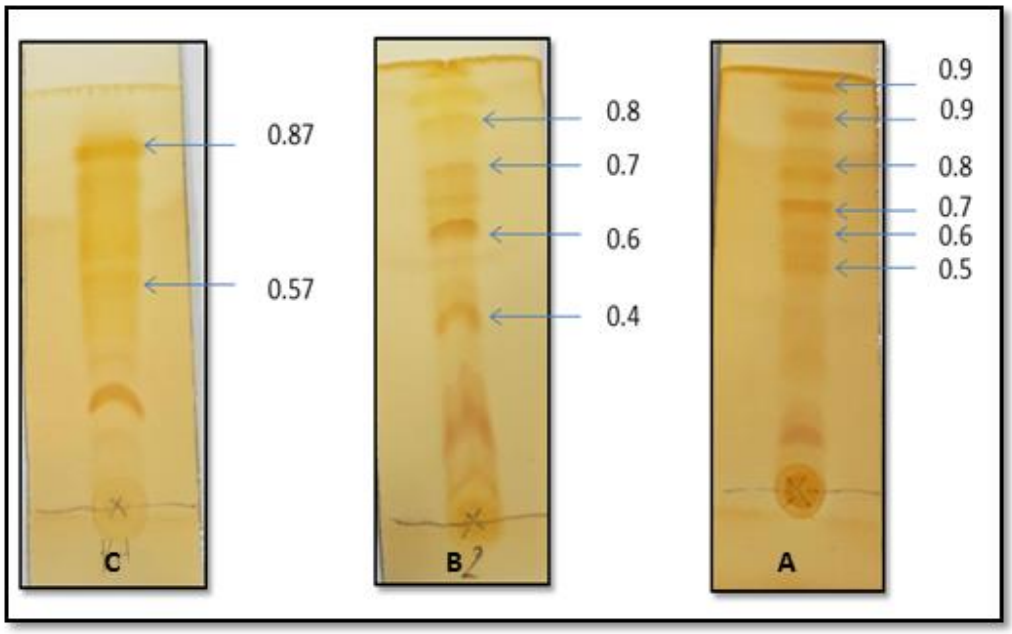

Fig. 3. Thin-layer chromatography of selected isolates: A. *Aspergillus fumigatus* isolate 2 extract; B. *Alternaria* sp. extract; C. *Alternaria tenuissima* extract. TLC provides preliminary evidence only and does not confirm paclitaxel identity.

DISCUSSION

Paclitaxel has been widely used as an anticancer agent since the 1970s **(Zhao *et al.*, 2009)**, and fungal paclitaxel or taxane production was first reported from *Taxomyces andreanae* in the 1990s **(Stierle *et al.*, 1993)**.

Fermentation conditions strongly affect the biosynthesis of fungal secondary metabolites, including paclitaxel and related taxanes **(Dai & Tao, 2008)**. Important factors include temperature, pH, inoculum size, liquid-medium volume, fermentation period, and shaking speed. **Stierle *et al.* (1995)** reported that the highest paclitaxel yield was obtained after 12 days of fermentation at 26 °C, and that the addition of sugars as glucose sources may stimulate compound production.

In the present study, 50 endophytic fungal isolates from plant tissues were fermented in PDB, YPD, and PCB media at pH 6.0-6.5. The cultures were incubated for 14 or 21 days at 25-28 °C and 120 rpm. These conditions are consistent with **Ismaiel *et al.* (2017)**, who used several media to test paclitaxel-like metabolite production from endophytic fungi, including 14 days of incubation at 25 °C and 120 rpm. The use of PDB for 21 days at 25-28 °C is also consistent with earlier studies **(Wang *et al.*, 2000; Guo *et al.*, 2006; Chakravarthi *et al.*, 2008; Li *et al.*, 2008; Elavarasi *et al.*, 2012; Das *et al.*, 2017)**.

Several previous studies used modified liquid medium (MID) for fungal paclitaxel-like metabolite production **(Gangadevi & Muthumary, 2009; Pandi *et al.*, 2011; Kathiravan *et al.*, 2012; Merlin *et al.*, 2012; Michalczyk *et al.*, 2014)**. **Deng *et al.* (2009)** reported that MID supported high paclitaxel-like metabolite production because it contains components such as sodium acetate, sodium benzoate, histidine, and phenylalanine, which may improve taxane biosynthesis during fermentation.

The fermentation pH was adjusted to 6.0 to support fungal growth and secondary metabolite production, a condition consistent with previous reports **(Sonaimuthu *et al.*, 2010; Zhao *et al.*, 2011)**. By contrast, **Garyali *et al.* (2014)** reported that pH 6.5 was optimal for paclitaxel-like metabolite production by *Fusarium redolens*. Other studies found that pH 7.0 or 8.0 supported higher yields under specific fermentation conditions **(Dai & Tao, 2008; Rui *et al.*, 2011)**.

All media used in the present study supported fungal growth and metabolite production. PDB appeared to be more suitable than YPD and PCB for the tested isolates, although MID has been reported as a highly productive medium in previous studies. The choice of media in the present work was influenced by availability and cost, whereas MID was not used because some of its components were difficult to obtain.

Temperature and fermentation time are important determinants of fungal metabolite production. The incubation temperature of 25 °C used in this study is consistent with reports of paclitaxel-like metabolite production at 25 °C **(Xu *et al.*, 2006; Li *et al.*, 2008; Zhang *et al.*, 2009)**. The use of 28 °C is also consistent with other studies **(Lakshmi & Selvi, 2013; Ismaiel *et al.*, 2017)**. Whereas, **Rui *et al.* (2011)** used 30 °C for paclitaxel-like metabolite production.

A 14-day incubation period was used for some isolates, which agrees with fermentation durations used by **Xu *et al.* (2006)** and **Ismaiel *et al.* (2017)**. A 21-day incubation period was used for most isolates, consistent with reports by **Li *et al.* (2008)** and **Sreekanth *et al.* (2011)**.

Fermentation was carried out at 120 rpm. During fermentation, fungal growth appeared after approximately 3 days, depending on the physiology of each isolate and its ability to sporulate. This finding agrees with **Zhang *et al.* (2009)** and **Ismaiel *et al.* (2017)**. Other studies used higher shaking speeds, such as 160 or 180 rpm, for paclitaxel-like metabolite production by *Fusarium solani* Tax-3 and *Fusarium maire* Y117 **(Xu *et al.*, 2006; Deng *et al.*, 2009)**. Static fermentation can produce lower yields; for example, **Zhao *et al.* (2009)** reported paclitaxel-like metabolite production by *Aspergillus niger* HD86-9 at 273.46 µg/L under static conditions.

In the present study, extraction of putative paclitaxel-like metabolites involved centrifugation to separate fungal biomass from the filtrate, followed by solvent extraction. The organic layer was collected, and anhydrous sodium sulfate was used to remove residual water. This procedure is consistent with **Strobel *et al.* (1996b)**. Some studies obtained crude extracts by drying without anhydrous sodium sulfate **(Kumaran *et al.*, 2009; Kumaran *et al.*, 2011; Kathiravan *et al.*, 2012; Rajendran *et al.*, 2013)**. Both approaches can yield crude extracts suitable for preliminary TLC analysis.

TLC was used as a preliminary screening method for paclitaxel-like compounds in the tested fungal extracts. Iodine vapor **(Sherman & Fried, 1986)** and vanillin reagent **(Cardellina, 1991)** were used to visualize organic compounds, and Rf values were compared with those of the paclitaxel standard. Because TLC depends on co-migration and color reaction, it provides presumptive evidence only and cannot confirm compound identity without additional analytical methods.

The paclitaxel standard showed Rf values of approximately 0.50, 0.60, 0.67-0.70, and 0.90 across different TLC runs, and these values were added to Table (2) for comparison. Comparable values have been reported previously. **Chakravarthi *et al.* (2008)** reported paclitaxel-like metabolite production by *Fusarium solani* isolated from *Taxus celebica*, with an Rf value of 0.58 and a low yield of 1.6 µg/L. **Kumar *et al.* (2019)** confirmed paclitaxel in *Aspergillus fumigatus* TPF-06 isolated from *Taxus* sp., with an Rf value of 0.9. **Ismaiel *et al.* (2017)** reported an Rf value of 0.67 for paclitaxel detection by TLC.

Several fungal isolates tested in this study showed TLC bands suggesting the possible presence of paclitaxel-like compounds. For example, *Alternaria alternata* showed Rf values of 0.5, 0.6, 0.67-0.7, and 0.9, and paclitaxel production from this species has previously been reported **(Renpeng *et al.*, 2006)**. However, in the present study, this assignment remains preliminary because confirmatory HPLC, LC-MS, or NMR analysis was not performed.

Alternaria tenuissima and *Aspergillus fumigatus* isolate 2 showed Rf values of 0.5, 0.6, and 0.7, consistent with the TLC range reported by **Ismaiel *et al.* (2017)**, who confirmed paclitaxel-like metabolite production by *Alternaria tenuissima* and *Aspergillus fumigatus* using additional analyses.

Chaetomium globosum showed Rf values of 0.5, 0.6, 0.7, 0.8, and 0.9. Paclitaxel production by *Chaetomium* sp. has been confirmed in previous work using TLC, HPLC, UV, and other analytical methods **(Rebecca *et al.*, 2012)**, but the present evidence for *Chaetomium* remains limited to TLC screening.

For *Cladosporium* sp., TLC showed Rf values of 0.5 and 0.9. **Gohar *et al.* (2015)** confirmed paclitaxel in extracts of *Cladosporium cladosporioides* isolated from several plants, including *Taxus* sp., *Ficus* sp., *Hibiscus rosa-sinensis*, *Terminalia arjuna*, and *Citrus medica*, and reported that *Cladosporium cladosporioides* UH-10 isolated from *Taxus* sp. produced 140 µg/L in liquid fermentation. The current *Cladosporium* result should be interpreted only as preliminary TLC evidence.

In the current study, *Colletotrichum gloeosporioides* showed Rf values of 0.6 and 0.7. This species has previously been reported to produce paclitaxel-like compounds. **Gangadevi and Muthumary (2008)** isolated *Colletotrichum gloeosporioides* JGC-9 from the medicinal plant *Justicia gendarussa* and quantified paclitaxel-like metabolite production by HPLC at 163.4 µg/L. **Andrade *et al.* (2018)** also demonstrated paclitaxel-like metabolite production by *C. gloeosporioides* isolated from *Polantag* major leaves and confirmed productivity using several analytical methods. **Xiong *et al.* (2013)** reported paclitaxel-like metabolite production by *C. gloeosporioides* TA67 isolated from *Taxus* x media, with a yield of 120 ng/L. Similarly,

Senthilkumar *et al.* (2013) reported paclitaxel-like metabolite production by *Colletotrichum gloeosporioides* isolated from *Tectona grandis* leaves and confirmed the compound using TLC, UV, and HPLC analyses.

Fusarium spp. showed Rf values of 0.5, 0.67, and 0.8. Several studies have confirmed paclitaxel-like metabolite production in this genus. **Merlin *et al.* (2012)** reported paclitaxel-like metabolite production by *Fusarium solani* isolated from *Tylophora indica* roots and characterized the compound using several analytical methods. **Deng *et al.* (2009)** isolated *Fusarium solani* Tax-3 from the bark of *Taxus chinensis* and reported paclitaxel-like metabolite production of 163.35 µg/L. **Li *et al.* (2008)** reported that *Fusarium arthrosporioides* isolated from *Taxus cuspidata* produced paclitaxel in PDB, with confirmation by TLC, HPLC, LC-MS, and NMR and a yield of 131 µg/L. In the present study, *Fusarium* results remain preliminary because only TLC screening was conducted.

Fusarium oxysporum showed Rf values of 0.6 and 0.7. This species may produce paclitaxel-like compounds, as **Elavarasi *et al.* (2012)** isolated it from *Rhizophora annamalayana* and confirmed paclitaxel-like metabolite production in PDB using UV, IR, and HPLC analyses, with a yield of 172 µg/L. The current evidence is limited to TLC similarity.

Nigrospora zimmermanii showed Rf values of 0.5 and 0.7. **Ruiz-Sanchez *et al.* (2010)** reported paclitaxel-like metabolite production by *Nigrospora* sp. isolated from *Taxus globosa*, with an estimated yield of 142 ng/L. The current finding should be interpreted as putative until confirmed analytically.

Paecilomyces sp. showed Rf values of 0.6, 0.7, and 0.8. **Huang *et al.* (2001)** evaluated endophytic fungi including *Nigrospora, Trichoderma, Fusarium,* and *Paecilomyces* species and reported antitumor and antifungal activities, with *Paecilomyces* extracts showing strong activity against HL-60 cancer cells. Further chemical analysis is needed to confirm whether the bands observed here represent paclitaxel-like compounds.

Phoma sp. extracts showed Rf values of 0.6 and 0.7. **Hemamalini *et al.* (2015)** reported paclitaxel-like metabolite production by *Phoma* sp. isolated from *Calotropis gigantea*, with a yield of 76.13 µg/L, and confirmed the compound using several analytical methods. The current result is therefore reported only as preliminary TLC evidence for a paclitaxel-like metabolite.

Overall, TLC screening identified several candidate isolates for further study, but the results should not be interpreted as confirmed paclitaxel production. Future work should include molecular identification of promising fungal isolates and confirmatory chemical characterization by HPLC, LC-MS, and/or NMR.

REFERENCES

Al-Salih, M. and Samsudin, S. (2019). Synthesis and characterizations of titanium dioxide nanocomposite by laser ablation for antimicrobial applications. Journal of Bacteriology & Mycology: Open Access, 7(4): 81-84.

Al-Salih, M. and Samsudin, S. (2025). Phytoremediation and green synthesis: a circular economy model for valorizing water hyacinth (*Eichhornia crassipes*) biomass into nano-sorbents. Egyptian Journal of Aquatic Biology & Fisheries, 29(6): 3065-3081.

Al-Salih, M.; Samsudin, S. and Al-Salih, S. W. (2021a). Adjustment and application of the band gap of nano titanium dioxide: its usefulness on photo degradation of wastewater (phenol). Egyptian Journal of Aquatic Biology and Fisheries, 25(3): 815-830.

Al-Salih, M.; Samsudin, S. and Arshad, S. S. (2021b). Synthesis and characterizations iron oxide carbon nanotubes nanocomposite by laser ablation for anti-microbial applications. Journal of Genetic Engineering and Biotechnology, 19(1): 76.

Amaral, R. G.; dos Santos, S. A.; Andrade, L. N.; Severino, P. and Carvalho, A. A. (2019). Natural products as treatment against cancer: a historical and current vision. Clin. Oncol., 4(5): 1562.

Andrade, H. F. D.; Araújo, L. C. A. D.; Santos, B. S. D.; Paiva, P. M. G.; Napoleão, T. H.; Correia, M. T. D. S. and Silva, M. V. D. (2018). Screening of endophytic fungi stored in a culture collection for taxol production. Brazilian Journal of Microbiology, 49: 59-63.

Arnold, A. E.; Maynard, Z.; Gilbert, G. S.; Coley, P. D. and Kursar, T. A. (2000). Are tropical fungal endophytes hyperdiverse? Ecology Letters, 3(4): 267-274.

Bayram, Ö. and Braus, G. H. (2012). Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiology Reviews, 36(1): 1-24.

Cardellina, J. H. (1991). HPLC separation of taxol and cephalomannine. Journal of Liquid Chromatography, 14(4): 659-665.

Chakravarthi, B. V. S. K.; Das, P.; Surendranath, K.; Karande, A. A. and Jayabaskaran, C. (2008). Production of paclitaxel by *Fusarium solani* isolated from *Taxus celebica*. Journal of Biosciences, 33(2): 259-267.

Chandra, S. (2012). Endophytic fungi: novel sources of anticancer lead molecules. Applied Microbiology and Biotechnology, 95(1): 47-59.

Dai, W. and Tao, W. (2008). Preliminary study on fermentation conditions of taxol-producing endophytic fungus. Chemical Industry and Engineering Progress, 27(6): 883-886.

Das, A.; Rahman, M. I.; Ferdous, A. S.; Amin, A.; Rahman, M. M.; Nahar, N. and Khan, H. (2017). An endophytic basidiomycete, *Grammothele lineata*, isolated from *Corchorus olitorius*, produces paclitaxel that shows cytotoxicity. PLoS One, 12(6): e0178612.

Demain, A. L. and Vaishnav, P. (2011). Natural products for cancer chemotherapy. Microbial Biotechnology, 4(6): 687-699.

Deng, B. W.; Liu, K. H.; Chen, W. Q.; Ding, X. W. and Xie, X. C. (2009). *Fusarium solani* Tax-3, a new endophytic taxol-producing fungus from *Taxus chinensis*. World Journal of Microbiology and Biotechnology, 25(1): 139-143.

Ebada, S. S.; Eze, P.; Okoye, F. B.; Esimone, C. O. and Proksch, P. (2016). The fungal endophyte *Nigrospora oryzae* produces quercetin monoglycosides previously known only from plants. ChemistrySelect, 1(11): 2767-2771.

Elavarasi, A.; Rathna, G. S. and Kalaiselvam, M. (2012). Taxol-producing mangrove endophytic fungi *Fusarium oxysporum* from *Rhizophora annamalayana*. Asian Pacific Journal of Tropical Biomedicine, 2(2): S1081-S1085.

Ferreira, P. M. P.; Farias, D. F.; Viana, M. P.; Souza, T. M.; Vasconcelos, I. M.; Soares, B. M. and Carvalho, A. F. (2011). Study of the antiproliferative potential of seed extracts from Northeastern Brazilian plants. Anais da Academia Brasileira de Ciências, 83(3): 1045-1058.

Fox, E. M. and Howlett, B. J. (2008). Secondary metabolism: regulation and role in fungal biology. Current Opinion in Microbiology, 11(6): 481-487.

Gangadevi, V. and Muthumary, J. (2008). Isolation of Colletotrichum gloeosporioides, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant, *Justicia gendarussa*. Mycologia Balcanica, 5: 1-4.

Gangadevi, V. and Muthumary, J. (2009). Taxol production by Pestalotiopsis terminaliae, an endophytic fungus of *Terminalia arjuna* (arjun tree). Biotechnology and Applied Biochemistry, 52(1): 9-15.

Garyali, S.; Kumar, A. and Reddy, M. S. (2014). Enhancement of taxol production from endophytic fungus *Fusarium redolens*. Biotechnology and Bioprocess Engineering, 19: 908-915.

Gohar, U. F.; Mukhtar, H. and Haq, I. U. (2015). Isolation and screening of endophytic fungi for the reduction of taxol. Pakistan Journal of Botany, 47(SI): 355-358.

Guo, B. H.; Wang, Y. C.; Zhou, X. W.; Hu, K.; Tan, F.; Miao, Z. Q. and Tang, K. X. (2006). An endophytic taxol-producing fungus BT2 isolated from *Taxus chinensis* var. *mairei*. African Journal of Biotechnology, 5(10).

Guo, B.; Wang, Y.; Sun, X. and Tang, K. (2008). Bioactive natural products from endophytes: a review. Applied Biochemistry and Microbiology, 44(2): 136-142.

Hemamalini, V.; Kumar, D. M.; Rebecca, A. I. N.; Srimathi, S.; Muthumary, J. and Kalaichelvan, P. (2015). Isolation and characterization of taxol-producing endophytic *Phoma* sp. from Calotropis gigantea and its anti-proliferative studies. J. Acad. Ind. Res., 3: 645-649.

Huang, Y.; Wang, J.; Li, G.; Zheng, Z. and Su, W. (2001). Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxus mairei*, *Cephalataxus fortunei* and *Torreya grandis*. FEMS Immunology and Medical Microbiology, 31(2): 163-167.

Ismaiel, A. A.; Ahmed, A. S.; Hassan, I. A.; El-Sayed, E. S. R. and El-Din, A. Z. A. K. (2017). Production of paclitaxel with anticancer activity by two local fungal endophytes, *Aspergillus fumigatus* and *Alternaria tenuissima*. Applied Microbiology and Biotechnology, 101(14): 5831-5846.

Jinu, M. V. and Jayabaskaran, C. (2015). Diversity and anticancer activity of endophytic fungi associated with the medicinal plant Saraca asoca. Current Research in Environmental and Applied Mycology, 5: 169-179.

Kathiravan, G.; Sureban, S. M.; Sree, H. N.; Bhuvaneshwari, V. and Kramony, E. (2012). Isolation of anticancer drug Taxol from *Pestalotiopsis breviseta* with apoptosis and B-cell lymphoma protein docking studies. Journal of Basic and Clinical Pharmacy, 4(1): 14.

Keller, N. P. (2019). Fungal secondary metabolism: regulation, function and drug discovery. Nature Reviews Microbiology, 17(3): 167-180.

Keller, N. P.; Turner, G. and Bennett, J. W. (2005). Fungal secondary metabolism - from biochemistry to genomics. Nature Reviews Microbiology, 3(12): 937-947.

Kharwar, R. N.; Mishra, A.; Gond, S. K.; Stierle, A. and Stierle, D. (2011). Anticancer compounds derived from fungal endophytes: their importance and future challenges. Natural Product Reports, 28(7): 1208-1228.

Krause, D. J.; Kominek, J.; Opulente, D. A.; Shen, X. X.; Zhou, X.; Langdon, Q. K. and Hittinger, C. T. (2018). Functional and evolutionary characterization of a secondary metabolite gene cluster in budding yeasts. Proceedings of the National Academy of Sciences, 115(43): 11030-11035.

Kumar, P.; Singh, B.; Thakur, V.; Thakur, A.; Thakur, N.; Pandey, D. and Chand, D. (2019). Hyper-production of taxol from *Aspergillus fumigatus*, an endophytic fungus isolated from *Taxus* sp. of the Northern Himalayan region. Biotechnology Reports, 24: e00395.

Kumaran, R. S.; Jung, H. and Kim, H. J. (2011). *In vitro* screening of taxol, an anticancer drug produced by the fungus, *Colletotrichum capsici*. Engineering in Life Sciences, 11(3): 264-271.

Kumaran, R. S.; Muthumary, J.; Kim, E. K. and Hur, B. K. (2009). Production of taxol from *Phyllosticta dioscoreae*, a leaf spot fungus isolated from *Hibiscus rosa-sinensis*. Biotechnology and Bioprocess Engineering, 14(1): 76-83.

Lakshmi, P. J. and Selvi, K. V. (2013). Anticancer potentials of secondary metabolites from endophytes of *Barringtonia acutangula* and its molecular characterization. International Journal of Current Microbiology and Applied Sciences, 2(2): 44-45.

Li, C. T.; Li, Y.; Wang, Q. J. and Sung, C. K. (2008). Taxol production by *Fusarium arthrosporioides* isolated from yew, Taxus cuspidata. Journal of Medical Biochemistry, 27(4): 454-458.

Merlin, J. N.; Christhudas, I. N.; Kumar, P. P.; Kumar, M. and Agastian, P. (2012). Taxol production by endophytic Fusarium solani LCPANCF01 from *Tylophora indica*. J. Acad. Indus. Res., 1(5): 281.

Michalczyk, A.; Cieniecka-Roslonkiewicz, A. and Cholewinska, M. (2014). Plant endophytic fungi as a source of paclitaxel. Herba Polonica, 60(4).

Pandi, M.; Kumaran, R. S.; Choi, Y. K.; Kim, H. J. and Muthumary, J. (2011). Isolation and detection of taxol, an anticancer drug produced from *Lasiodiplodia theobromae*, an endophytic fungus of the medicinal plant *Morinda citrifolia*. African Journal of Biotechnology, 10(8): 1428-1435.

Pimentel, M. R.; Molina, G.; Dionísio, A. P.; Maróstica Junior, M. R. and Pastore, G. M. (2011). The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnology Research International, 2011.

Priti, V.; Ramesha, B. T.; Singh, S.; Ravikanth, G.; Ganeshaiah, K. N.; Suryanarayanan, T. S. and Uma Shaanker, R. (2009). How promising are endophytic fungi as alternative sources of plant secondary metabolites? Current Science, 97(4): 477-478.

Rajendran, L.; Rajagopal, K.; Subbarayan, K.; Ulagappan, K.; Sampath, A. and Karthik, G. (2013). Efficiency of fungal taxol on human liver carcinoma cell lines. American Journal of Research Communication, 1: 112-121.

Rebecca, A. I. N.; Hemamalini, V.; Kumar, D. M.; Srimathi, S.; Muthumary, J. and Kalaichelvan, P. T. (2012). Endophytic *Chaetomium* sp. from *Michelia champaca* L. and its taxol production. J. Acad. Ind. Res., 1(68): e72.

Renpeng, T.; Qiao, Y.; Guoling, Z.; Jingquan, T.; Luozhen, Z. and Chengxiang, F. (2006). Taxonomic study on a taxol-producing fungus isolated from bark of *Taxus chinensis* var. *mairei*. Wuhan Zhi Wu Xue Yan Jiu, 24(6): 541-545.

Ross, J. L.; Santangelo, C. D.; Makrides, V. and Fygenson, D. K. (2004). Tau induces cooperative Taxol binding to microtubules. Proceedings of the National Academy of Sciences, 101(35): 12910-12915.

Rui, J.; Ji-Chuan, K.; Ting-Chi, W.; Jing, H. and Bang-Xing, L. (2011). A study on optimal fermentation of an endophytic fungus producing taxol. Mycosystema, 30: 235-241.

Ruiz-Sanchez, J.; Flores-Bustamante, Z. R.; Dendooven, L.; Favela-Torres, E.; Soca-Chafre, G.; Galindez-Mayer, J. and Flores-Cotera, L. B. (2010). A comparative study of taxol production in liquid and solid-state fermentation with *Nigrospora* sp., a fungus isolated from *Taxus globosa*. Journal of Applied Microbiology, 109(6): 2144-2150.

Senthilkumar, N.; Murugesan, S.; Mohan, V. and Muthumary, J. (2013). Taxol-producing fungal endophyte, *Colletotrichum gloeosporioides* (Penz.) from *Tectona grandis* L. Current Biotica, 7: 8-15.

Sherman, J. and Fried, B. (1986). Thin layer chromatography, Vol. 35. Marcel Dekker, Inc., New York, USA, pp. 121-127.

Sonaimuthu, V.; Krishnamoorthy, S. and Johnpaul, M. (2010). Optimization of process parameters for improved production of taxol by a novel endophytic fungus

Pestalotiopsis oxyanthi SVJM060 isolated from *Taxus baccata*. Journal of Biotechnology, 150S: S1-S576.

Sreekanth, D.; Sushim, G. K.; Syed, A.; Khan, B. M. and Ahmad, A. (2011). Molecular and morphological characterization of a taxol-producing endophytic fungus, Gliocladium sp., from *Taxus baccata*. Mycobiology, 39: 151-157.

Stierle, A.; Strobel, G. and Stierle, D. (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science, 260(5105): 214-216.

Stierle, A.; Strobel, G.; Stierle, D.; Grothaus, P. and Bignami, G. (1995). The search for a taxol-producing microorganism among the endophytic fungi of the Pacific yew, *Taxus brevifolia*. Journal of Natural Products, 58(9): 1315-1324.

Strobel, G.; Yang, X.; Sears, J.; Kramer, R.; Sidhu, R. S. and Hess, W. M. (1996b). Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*. Microbiology, 142(2): 435-440.

Subramaniyam, R. and Vimala, R. (2012). Solid state and submerged fermentation for the production of bioactive substances: a comparative study. International Journal of Science and Nature, 3(3): 480-486.

Uzma, F.; Mohan, C. D.; Hashem, A.; Konappa, N. M.; Rangappa, S.; Kamath, P. V. and Abd_Allah, E. F. (2018). Endophytic fungi - alternative sources of cytotoxic compounds: a review. Frontiers in Pharmacology, 9: 309.

Uzor, P. F.; Ebrahim, W.; Osadebe, P. O.; Nwodo, J. N.; Okoye, F. B.; Müller, W. E. G. and Proksch, P. (2015). Metabolites from *Combretum dolichopetalum* and its associated endophytic fungus *Nigrospora oryzae* - evidence for a metabolic partnership. Fitoterapia, 105: 147-150.

Voss, K. A. and Riley, R. T. (2013). Fumonisin toxicity and mechanism of action: overview and current perspectives. Food Safety, 1(1): 2013006.

Wang, J.; Li, G.; Lu, H.; Zheng, Z.; Huang, Y. and Su, W. (2000). Taxol from Tubercularia sp. strain TF5, an endophytic fungus of *Taxus mairei*. FEMS Microbiology Letters, 193(2): 249-253.

Wang, K. W.; Wang, S. W.; Wu, B. and Wei, J. G. (2014). Bioactive natural compounds from the mangrove endophytic fungi. Mini Reviews in Medicinal Chemistry, 14(4): 370-391.

Wang, Y. D.; Wu, J. C. and Yuan, Y. J. (2007). Salicylic acid-induced taxol production and isopentenyl pyrophosphate biosynthesis in suspension cultures of *Taxus chinensis* var. mairei. Cell Biology International, 31(10): 1179-1183.

Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P. and McPhail, A. T. (1971). Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. Journal of the American Chemical Society, 93(9): 2325-2327.

World Health Organization. (2013). WHO report on the global tobacco epidemic, 2013: enforcing bans on tobacco advertising, promotion and sponsorship. World Health Organization.

Xiong, Z. Q.; Yang, Y. Y.; Zhao, N. and Wang, Y. (2013). Diversity of endophytic fungi and screening of fungal paclitaxel producer from Anglojap yew, *Taxus* x media. BMC Microbiology, 13(1): 1-10.

Xu, F.; Tao, W.; Cheng, L. and Guo, L. (2006). Strain improvement and optimization of the media of taxol-producing fungus *Fusarium maire*. Biochemical Engineering Journal, 31: 67-73.

Zhang, P.; Zhou, P. P. and Yu, L. J. (2009). An endophytic taxol-producing fungus from Taxus x media, *Aspergillus candidus* MD3. FEMS Microbiology Letters, 293(2): 155-159.

Zhao, K.; Ping, W.; Li, Q.; Hao, S.; Zhao, L.; Gao, T. and Zhou, D. (2009). *Aspergillus niger* var. *taxi*, a new species variant of taxol-producing fungus isolated from *Taxus cuspidata* in China. Journal of Applied Microbiology, 107(4): 1202-1207.

Zhao, K.; Li, Z.; Ge, N.; Li, X.; Wang, X. and Zhou, D. (2011). Investigation of fermentation conditions and optimization of medium for taxol production from taxol-producing fungi. Journal of Medicinal Plants Research, 5: 6528-6535.