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Taxol Activity of Endophytic Fusarium Solani as Antibacterial. Antioxidant and Anticancer

A dissertation

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
(وَقُلْ رَبِّ زِدْنِي عِلْمًا)
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Dedication

To those who planted in my heart a love for knowledge and instilled in my soul patience and determination.....

To those who, after the grace of Allah, paved the way for this achievement.....

To my beloved family, the heartbeat that never fades and the unwavering pillar of support.

And to everyone who encouraged me and lifted me forward with sincere words or silent prayers from afar

I dedicate this humble work, in gratitude, appreciation, and endless love.

Abdullah

Summary

Endophytic fungi, including *Fusarium solani* have emerged as promising candidates for Taxol production, a vital anticancer compound. Thus This study aimed to isolate endophytic *Fusarium solani* Sal- 1 that isolated from *Solanum lycopersicum* plant. And tested it as anticancer, antioxidant and antibacterial against bacterial isolates were coinfection in cancer patients. Clinical specimens from suspected infections (various cancers, period from June to October 2024) from the Cancer Center at Marjan Hospital in Hillah city \Babylon were cultured onto different media.

The study looked at the prevalence of bacteria in 100 clinical specimens that were collected and isolated. According to the results, 83 (83%) of the specimens had no bacterial growth and (17%) had it. And, the most common cancer diagnosed is breast cancer (33%), followed by uterine cancer (29%). Also prostate cancer had (13%) and lung cancer had (23%). Lastly, (2%) of people have skin cancer. The results of isolation and Identification by VITEK-2 System showed, *Staphylococcus aureus*. with isolates , (5%) followed by *S. haemolyticus* (4%), *Escherichia coli* (3%), *S. equorum*, *S.*(1%) *hyicus*, (1%) *Enterococcus* spp with (1%) and *Klebsiella* spp(2%) , for each.

Results of isolated and identification of endophytic fungus from *Solanum lycopersicum* and according of morphological and molecular assay confirmed that isolate was *Fusarium solani*. Additionally, the high performance liquid chromatography (HPLC) assay confirmed that the endophytic fungus *F. solani* produced taxol with a higher yield of 66.48 mg/mL in the fermented medium type1, which was further supported by UV IR spectra, XRD, and SEM tests.

.....Summary

According to the results of the biological activities of the extracted taxol, it has anticancer, anti-oxidant, and antibacterial properties. The extracted taxol has anticancer properties. The IC₅₀ value of HdFn is 118.0, which indicates that cell viability is 50% decreased while, MCF7 is more sensitive to the tested taxol than HdFn, as evidenced by its lower IC₅₀ value of 91.75.

The following are the cytotoxic effects of taxol extract on human breast cancer MCF-7 cells: as concentration rose, cell viability declined. As the dosage increased, so did the effect on MCF-7 cells. 400 mg/mlµg/mL was the ideal concentration for getting the best results, and the study showed a notable effect on MCF-7 cancer cells.

In this study, the taxol extracted from *Fusarium* demonstrated antioxidant activity by decreasing free radicals; as concentration increased, so did antioxidant activity.

The agar well diffusion method was used to assess the antibacterial activity of pure fungal taxol against bacterial pathogens. Results indicate that extracted crude taxol has a significant impact on *S. aureus* (G+) and two other bacterial types (Po+, Ne-). greater than G-*E. coli*.

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List of Abbreviation

Abbreviation	Meanings
SMs	Secondary metabolites
MRSA	Methicillin-resistant Staphylococcus aureus
MEK	Mitogen-activated protein kinase
ERK	Extracellular signal- regulated kinase
BMS	Bristol-Myers Squibb
IAA	like indole acetic acid
TLC	Thin Layer Chromatography
MS	Mass Spectrometry
RT	Retention Time
MDR	multidrug-resistant
CoNS	Coagulase-negative Staphylococci
HPLC	High-Performance Liquid Chromatography
FTIR	Fourier Transform Infrared spectroscopy
SEM	scanning electron microscopy
PBS	Phosphate Buffer Saline
EDTA	ethylene-diamine-tetra acetic acid
RPMI	Roswell Park Memorial Institute
HEPES	hydroxyethyl)--2)-4 1piperazine ethane sulfonic acid
MCF-7	human breast cancer cell
HdFn	Primary Dermal Fibroblast Normal
HGT	horizontal gene transfer
NCBI	National Center for Biotechnology Information
PTX	Paclitaxel
TLR4	Toll-like receptor 4

..... **List of content**

LC50	Lethal Concentration 50
IC50	half-maximal inhibitory concentration
NCBI	National Center for Biotechnology Information

Chapter one

Introduction

1- Introduction

Recently, natural substances have demonstrated an intriguing potential for use as therapies that can treat cancer or inhibit bacteria that are resistant to multiple drugs. (Abdel-Shafi,*et.al.*,2020; El-Gazzar, and Enan,2020). Being safe agents is another appealing quality they have. Regarding this, the oxygenated diterpenoid taxol (paclitaxel) was initially isolated from the bark of the Pacific yew tree (*Taxus brevifolia*) and demonstrated anticancer properties. (Senthil Kumaran,*et.al.*,2008; Pimentel,*et.al.*,2011).

According to earlier reports, taxol demonstrated encouraging activity against Kaposi's sarcoma, ovarian cancer, breast cancer, non-small cell lung cancer, head and neck carcinoma, and other cancer types linked to AIDS. (Suresh,*et.al.*,2020; Sinha, 2020; Gallego-Jara,*et.al.*,2020).

Taxus species are the natural source of taxol; however, it has been shown that natural production is not both economically and environmentally viable. Because of this, scientists from all over the world have been focused on finding novel ways to obtain paclitaxel. Currently, additional techniques such as microbial fermentation and artificial *Taxus* plant cultivation. (Yan-Hua, *et.al.*,2020). Also, its cost has increased, and the need to find alternative anticancer treatments has increased due to the limited supply of mature yew trees, the slower growth of cultivated plants, and the lower yield of the taxol substance. For many patients, this means that taking taxol is an investment burden. (Sabzehzari,*et.al.*,2020).

According to reports, there is a significant relationship between fungal endophytes from various *Taxus* species and secondary metabolites, growth developers, and taxol productivity (Yan-Hua, *et.al.*,2020). There are a number of benefits to producing metabolites in fungi, including short cultivation periods (15–20 days), cheaper culture media compared to plant tissue cultures, and, most importantly, a stable and reliable production

system with minimal genetic variability (Guevara-Sánchez,*et.al.*,2025).

It was known that taxus-associated fungi, a significant group of microorganisms, produced secondary metabolites that were useful against infections and tumors. (Han, *et.al.* 2020). promising potential as an inexpensive industrial fermentation production method by using fungi. Numerous endophytic fungal species have been shown to be capable of producing taxol. (Véléz,*et.al.*,2022). Therefore, the ultimate goal for biotechnologists is to find new fungal saprophytes with a stable molecular machinery system for the potential independent production of Taxol. (Stadler. and Kolarik, 2024).

Unquestionably, patients in oncology the districts are especially vulnerable to infections. These patients are more susceptible to infection due to cancer and chemotherapy. (Zembower, 2014). Cancer patients frequently contract infections, which can disrupt their treatment plan, result in longer hospital stays, raise medical expenses, and lower their chances of survival. Infection is still a major or related cause of death, despite the fact that mortality rates have declined recently. Bacteria are the most common cause of infection-associated mortality, followed by fungi. (Ray,*et.al.*,2023;Zheng,*et.al.*,2021;Sepich-Poore,*et.al.*,2021). Finding new bioactive products is crucial right now because the medications used to treat various illnesses are expensive and have negative side effects (Pandy, *et.al.*,2023)

Numerous bacteria are connected in various ways to the development of cancer. Furthermore, the exact roles of various virulence factors and the complex interactions between particular bacteria and the target cells of our body are largely unknown. On the other hand, several microbial products are being investigated for potential use as therapeutic agents that fight cancer.

Clinicians should always be careful to get patients to take part in screening tests while research is ongoing, especially if they are at risk for high-virulence pathogens. Cancer treatment can be enhanced by getting rid of these pathogens early in the course of the illness. The overall prognosis and survival of cancer patients are significantly impacted by secondary bacterial infections because of the immunosuppressive nature of cancer and the associated chemotherapy and radiation treatments. (Zheng,*et.al.*,2021; Kanjanapan and Yip,2020).

Owing to its mandatory use as an anticancer agent, the possibility of producing it inexpensively through fungal fermentation, and the recent effort to identify alternative sources of the expensive taxol, the purpose of this work was to find additional fungi that produce taxol. Taxol production and characterization from a single *F. solani* strain.

1-1. Aim of study

this study is the first to isolate Taxol in Iraq. The aim of this study are to extraction and purification of the metabolic products Taxol from one of endophytic fungus (*Fusarium solani*) that isolated from tomato plant as natural source and ecofriendly. And evaluation of it the anticancer activity, antioxidant and antimicrobial, to achieve this aim, take the following steps:

- 1- Isolation and Identification of endophytic fungus
- 2- extraction and purification of fungal Taxol
- 3- Isolation and identification of bacterial species from specimen's blood from cancer patients
- 4- Determination of anticancer activity of fungal extract taxol
- 5- Determination of antioxidant activity of fungal extract taxol
- 6- Determination of antibacterial activity of fungal extract taxol

Chapter Two

Literature Review

2- Literature review

2-1- Secondary Metabolites

Secondary metabolites (SMs), also known as natural products, are biomolecules with a low molecular weight that, although not essential for the development of its producer, can increase their tolerance to different types of environmental stresses and hostile conditions, and consequently, their survival rate (Koza *et al.*, 2022). Bioactive SMs are produced mainly by microorganisms, which can be isolated from soil microbiota, marine environments, or extreme environments. The most promising are those obtained from underexplored niches, including plant-associated microbes called entophytes, which live in symbiotic relationships with host plants in their internal tissues and structures. Endophytes can be isolated from herbaceous plants, grasses, trees, shrubs, mosses, ferns, and aquatic plants (Mishra *et al.*2021).

Bioactive substances are abundantly produced by soil microorganism (Basit *et al.*2021). Due to bacteria's capacity to produce a variety of useful products, such as antibiotics, fungicides, herbicides, hydrolytic enzymes, antitumor, antivirals, and immune suppressants, there is increased interest in employing them for medical and agricultural applications (Keswani *et al.*2020). Recently, pathogen resistance demands the discovery of novel antimicrobial substances that are effective against serious phytopathogens; thus, there is significant interest in screening new microbes with antimicrobial activity from various environments in order to explore novel potential medications against infections that are resistant to drugs (Pancu *et al.*2021).

The exploration of secondary metabolites, particularly Taxol (paclitaxel), in fungi has garnered significant interest due to its potent

anticancer properties. Taxol, originally derived from the Pacific yew tree, has been identified in various fungal species, particularly endophytic fungi, which live symbiotically within plants. Despite the initial excitement about fungi as a potential source for Taxol production, challenges remain in achieving commercially viable yields (Swamy *et al.*, 2022).

2-2- Secondary metabolic products of fungi

The secondary metabolic products of fungi are characterized by the production of a multitude of diverse compounds with a wide range and variety of biological activities. Their occurrence in nature indicates that they have various functions that are important for fungal life (Devi, *et al.*2020). Secondary metabolites typically accumulate during the latter stage of fermentation, referred to as the idiophase, which succeeds the vigorous growth phase known as the trophophase. Compounds generated during the idiophase are not directly related to the synthesis of cellular material and the regular growth of microorganisms. Secondary metabolites are produced in a fermentation medium following the completion of microbial growth (Nigam, *et al.*2014).

Numerous fungi possess a sophisticated secondary metabolism. The variety of fungal species and the diversification of biosynthetic gene clusters highlight an almost boundless potential for metabolic variation and an unexploited resource for drug development and synthetic biology and Many species produce various types of secondary metabolites, with their expression coordinated by both the life cycle and environmental factors. A substantial portion of their genomes is dedicated to encoding and regulating the synthesis of these compounds. (Bills, and Gloer, 2016).

The ecological success of filamentous fungus in populating the earth is largely attributed to their capacity to utilize secondary metabolites with

their penetrative and absorptive lifestyle. Fungal secondary metabolites demonstrate biological activities that have been transformed into life- saving pharmaceuticals and agrochemicals (Keller, 2019). Fungal secondary metabolites exhibit biological activities that have been developed into life-saving medicines and agrochemicals. Toxic metabolites, known as mycotoxins, contaminate human and livestock food and indoor environments. Secondary metabolites are determinants of fungal diseases of humans, animals, and plants. Secondary metabolites exhibit a staggering variation in chemical structures and biological activities, yet their biosynthetic pathways share a number of key characteristics. (Calvo, *et at.*2016).

2-3 Taxol

Taxol is a remarkable natural product that has gained widespread recognition and is commonly employed as an extremely effective anticancer agent, with a specific focus on targeting proliferating cells. This compound showcases an impressive and broad spectrum of antitumor activities, which makes it especially impactful on cancers such as ovarian, breast, and lung cancers. Moreover (Gallego-Jara,*et.al.*,2020)

Based on a tetracyclic diterpenoid core known as baccatin III, taxol possesses a complex chemical structure that includes multiple functional groups and a distinctive oxetane ring. Among them are two acetyl groups, one benzoyl group, and two hydroxyl groups. Along with the core structure, the molecule has an amide tail, specifically (2R,3S)-N-benzoyl-3-phenylisoserine. The formula for the molecule is C₄₇H₅₁NO₁₄. Figure (2-1) (Wangn and and Tang, 2012)

Taxol demonstrates substantial therapeutic efficacy against various tumors, particularly in difficult cases such as multiple myeloma, human

pancreatic carcinoma, and head and neck malignancies. (Nawara, *et al.*2021). Taxol operates by exhibiting potent cytotoxic effects on neoplastic cells. It accomplishes this by inducing mitotic arrest at the pivotal G2-M phase of the cell cycle, an essential stage in cellular preparation for division. This time is crucial for facilitating healthy cell division and function. Besides its function in inhibiting mitosis, Taxol also triggers apoptosis, a mechanism of planned cell death. This activity transpires via the modulation of many cellular signaling pathways, thereby preventing the cancer cells from further proliferation. This ultimately results in the obliteration of both malignant and benign tumor tissues. (Weaver, 2014).

Generally, these tumor tissues demonstrate a greater proliferation rate than other normal tissues in the body. Currently, Taxol is utilized in the treatment of several malignant tumors. It has justifiably attained acknowledgement as one of the most potent agents in anticancer therapy, demonstrating a wide range of therapeutic potential and applications in medicine. Nonetheless, despite its numerous advantages, the natural availability of the sources from which Taxol is extracted is quite restricted. (Mosca, *et al.* 2022).

Increasing apprehensions are emerging about the possible ecological consequences linked to the extraction of these natural resources. As a result, a growing number of individuals are rigorously committing their efforts to the investigation and advancement of alternative strains that can produce Taxol. In recent years, considerable efforts have been dedicated to exploring the production and mechanisms of action of Taxol, which is derived from endophytic fungi within the plant family Taxaceae, sourced from diverse environments. It is important to highlight that various studies have demonstrated that the fungal species *Sp. subsporaceus* possesses the

ability to produce Taxol, maintaining the identical chemical structure, production methodology, and action mechanism as the natural Taxol sourced from the *Taxus brevifolia* plant. This breakthrough achievement in successfully obtaining Taxol through innovative fungal fermentation processes opens up a novel and innovative avenue for acquiring this highly valuable natural product. (Shankar Naik, 2019).

Fungal secondary metabolites, particularly Taxol, have shown promising antibacterial properties, making them a potential solution to combat antimicrobial resistance. Taxol, a well-known anticancer agent, is also produced by endophytic fungi and has demonstrated significant antibacterial activity. This potential is particularly relevant in the context of increasing resistance to conventional antibiotics, such as those seen in Methicillin-resistant *Staphylococcus aureus* (MRSA). The exploration of fungal sources for Taxol production not only offers a sustainable alternative to plant-derived Taxol but also opens new avenues for antibacterial drug development. (Johari,*et.al.*2021).

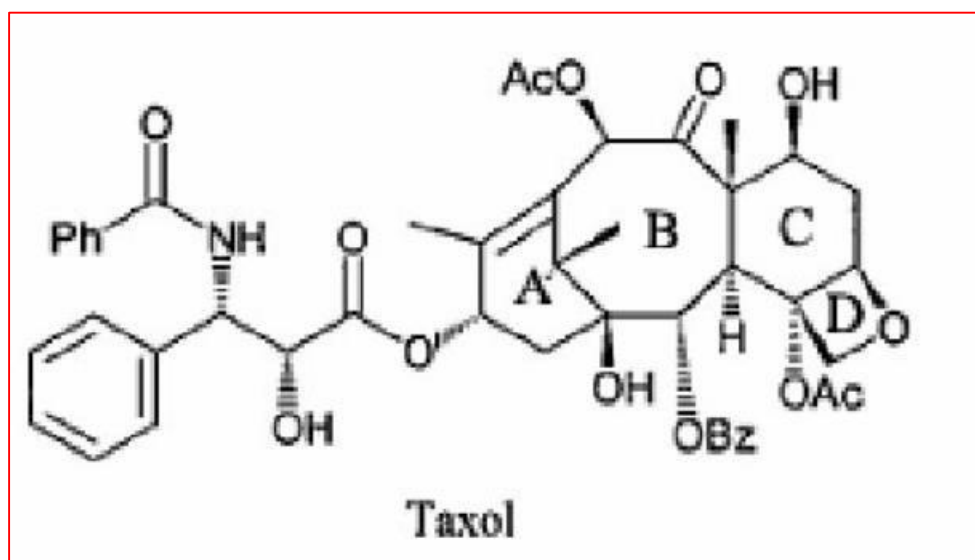


Figure (2-1) Structurally Taxol. (Wang and Tang, 2012)

2-4- Fungal Taxol

2-4.1-Endophytic Fungi as Taxol Producers

Microorganisms are natural products that have a variety of uses in the creation of potent drugs. and 80% of the population of the globe is treated with traditional medicine (Abdel-Razek,*et.al.*,2020).

Endophytic fungi, such as *Aspergillus niger* and *Pseudodidymocyrtis lobariellae*, have been identified as potential Taxol producers. These fungi can produce Taxol in small quantities, with *Aspergillus niger* achieving a yield of 173.9 µg/L, which can be increased through optimization techniques (Cao *et al.*, 2022).

Endophytic fungi, including *Aspergillus niger* and *Pseudodidymocyrtis lobariellae*, have emerged as promising candidates for Taxol production, a vital anticancer compound. Research indicates that *Aspergillus fumigatiaffinis*, another endophytic fungus, demonstrated significant paclitaxel production, yielding 26.373 µg/L, highlighting the potential of these fungi in biotechnological applications (Obiedallah *et al.*, 2024). Furthermore, *Pseudodidymocyrtis lobariellae* KL27 was shown to enhance Taxol accumulation in *Taxus chinensis* by 3.26-fold through the regulation of key biosynthetic genes and phytohormone signaling pathways (Cao *et al.*, 2022). Despite the low yields historically associated with fungal Taxol production, advancements in molecular biology and fermentation techniques are paving the way for improved yields and large-scale production. Thus, endophytic fungi represent a valuable resource for sustainable Taxol production. (Subban & Kempken, 2023) new extraction methods of taxol from endophytic fungus have demonstrated high potential in increasing the efficiency of taxol extraction for more efficient and sustainable production of taxol and cancer treatment products. isolation and identification of endophytic fungi types, extraction and detection

methods of endophytic taxol in plants, and improved efficiency of the extraction process. With the advancement of science and technology, new techniques in biotechnology, such as fungal strain improvement and recombining technique and microbial fermentation engineering, have increased the extraction yield from taxol-producing fungi, thereby improved the overall efficiency of taxol production. (Zhou, X.*et al.* 2010)

2-4.2-Biosynthetic Pathways

Three steps create a typical Taxol biosynthesis pathway: Figure (2-2) the biosynthesis of baccatin III using more hydroxylase and acetylase enzymes, the formation of Taxol side chains through phenylation, and the diterpene precursor biosynthesis of taxadiene using more carbocycles. For endophytic fungi that produce Taxol, these stages are explained in greater detail. Genomic and transcriptomic analyses are being used to identify key genes and enzymes involved in the process (Subban & Kempken, 2023).

Although there have been more than 200 reports of endophytic fungi producing Taxol, the yield of this product is frequently quite low. Little is known about the molecular signatures of Taxol biosynthesis and its regulation, despite significant efforts to increase the production of Taxol/taxanes in fungi through coculture manipulation, mutagenesis, genome shuffles, and gene overexpression(El-Sayed,*et.al.*,2021).

Since the general structure of the Taxol biosynthesis pathway is unknown, it is known that certain fungi have orthologs of the pathway. A few genes for metabolite residues may be found on dispensable chromosomes, according to a biosynthetic putative gene homology approach that was combined with transcriptomics and genomics analysis(Xiong,*et.al.*,2018).

[illegible]

Efforts to enhance Taxol production include genetic manipulation, such as overexpressing key biosynthetic genes and using genome mining to identify rate-limiting enzymes. Metabolic manipulation and co-cultivation strategies are also being explored to boost yields (El- Sayed *et al.*, 2021). The use of elicitors, such as plant-derived signals, can trigger the expression of cryptic Taxol-encoding genes, potentially increasing production. Optimization of culture conditions and the use of response surface methodology have shown promise in enhancing yields. While the potential of fungi as a source of Taxol is promising, the challenges of low yield and reproducibility must be addressed to realize its commercial viability. The exploration of fungal secondary metabolites continues to be a vibrant field, with ongoing research focused on understanding and optimizing the biosynthetic pathways involved. Despite the hurdles, the potential for fungi to contribute to sustainable Taxol production remains an exciting prospect for the future of pharmaceutical development. (Gamal *et al.*, 2024).

2-4.4-Production and Optimization of Fungal Taxol

Endophytic Fungi as a Source: Endophytic fungi, such as those from the *Taxus* spp., have been identified as prolific producers of Taxol. These fungi offer advantages over traditional plant sources, including faster growth rates and the ability to be genetically manipulated for enhanced production (El-Sayed *et al.*, 2020). Various strategies have been employed to increase Taxol yield from fungi, such as co-cultivation with other fungi, which can stimulate Taxol biosynthesis. For example, co-culturing with *Alternaria* and *Phomopsis* fungi resulted in a significant increase in Taxol production from *Paraconiothyrium* SSM001. One of the challenges in utilizing fungal Taxol is maintaining its production stability over time. Metabolic reprogramming and gene downregulation during sub culturing

can lead to decreased yields. Research is ongoing to address these issues through genetic and cultural optimization (Gamal *et al.*, 2024).

The exploration of fungal secondary metabolites, including Taxol, is crucial for developing new antibacterial agents. The diversity of these compounds and their multi-target effects make them promising candidates for overcoming bacterial resistance (Conrado *et al.*, 2022).

While the antibacterial potential of fungal Taxol is promising, it is important to consider the broader context of secondary metabolites in drug development. The diversity of fungal metabolites offers a vast resource for discovering new antimicrobial agents. However, challenges such as production stability and the need for efficient extraction and purification methods must be addressed to fully realize their potential in clinical applications. Additionally, the ecological and evolutionary dynamics of endophytic fungi and their interactions with host plants and other microbes present both opportunities and challenges for harnessing these natural products for therapeutic use. Optimization of culture conditions, including media composition and cultivation parameters, has been shown to enhance taxol production. For instance, the use of modified flask basal broth under shaking conditions resulted in higher yields of taxol and baccatin III (Chakravarthi *et al.*, 2020).

2-5- Biological activities of Taxol

2-5-1- Taxol as an Antibacterial Agent

Taxol produced by endophytic fungi has been shown to effectively inhibit a range of bacterial pathogens. For instance, fungal Taxol from *Cochliobolus hawaiiensis* demonstrated significant antibacterial activity against human pathogens, suggesting its potential as a therapeutic agent (Pandy *et al.*, 2023).

The antibacterial properties of fungal secondary metabolites,

including Taxol, are attributed to their ability to interfere with bacterial cell processes. These compounds can disrupt cell wall synthesis, inhibit quorum sensing, and penetrate biofilms, which are protective layers that bacteria form to resist antibiotics (Estrela & Abraham, 2016).

Numerous studies have demonstrated the antibacterial qualities of taxol, which has been isolated from a variety of fungal species, especially endophytic fungi. For example, Pandey *et al.* (2023) found that the fungal taxol has antibiotic-like effects on *S. aureus*, *E. coli*, and *S. typhi*.

2-5-2- Anticancer activity of Taxol

Taxol, also known as paclitaxel, is a widely used chemotherapeutic agent with significant anticancer activity. It primarily functions by stabilizing microtubules, thereby preventing their depolymerization, which leads to cell cycle arrest at the G2/M phase and subsequent apoptosis of cancer cells. This mechanism is particularly effective against various cancers, including breast, ovarian, and lung cancers. However, the efficacy of Taxol can be influenced by several factors, including genetic mutations and cellular pathways that contribute to drug resistance. (Lim *et al.*, 2022; Gallego-Jara *et al.*, 2020).

Taxol binds to tubulin, stabilizing microtubules and preventing their normal breakdown during cell division, which results in mitotic arrest and apoptosis. Taxol modulates apoptotic-regulating proteins, such as the Bcl-2 family, and affects signaling pathways like JNK/SAPK and NFκB, promoting cancer cell death. Taxol can influence immune cells, such as regulatory T cells and macrophages, and has been shown to reprogram macrophages from a pro-tumor to an anti-tumor phenotype, enhancing immune responses against cancer cells (Lim *et al.*, 2022; Swamy & Vasamsetti, 2022).

Resistance to Taxol can arise from mutations in tubulin, which affect

the drug's binding affinity. For instance, mutations like T274I and R320P significantly impair Taxol's antitumor activity (Zhou *et al.*, 2023). Taxol can activate the MEK/ERK (Mitogen-activated protein kinase kinase\ Extracellular signal-regulated kinase) pathway, contributing to intrinsic chemo resistance. This pathway's activation can protect cancer cells from the cytotoxic effects of Taxol (Adam, 2023; Formby, 2023). Taxol has been shown to have suppressive effects on a variety of breast, skin and ovarian cancers by preventing the de-polymerization of tubulins. A generic drug named “paclitaxel” has produced based on Taxol which has a registered trade name as Taxol(R) BMS (Bristol-Myers Squibb). (Sarli,2020).

Combining Taxol with MEK inhibitors, such as CI-1040, has shown enhanced therapeutic efficacy in preclinical models. This combination inhibits tumor growth more effectively than Taxol alone by suppressing MEK and associated pathways .Recent studies suggest that non-mitotic mechanisms, such as the formation of multiple micronuclei and the triggering of innate immunity, may also play a crucial role in Taxol's anticancer activity (Adam, 2023;Formby, 2023).

Paclitaxel (taxol) is the most well-known taxanes, and it is employed in the treatment of a wide range of human cancers, including ovarian, breast, lung, prostate, and head and neck cancers . The drug can be obtained from yew (*Taxus sp.*) but leads to the destruction of trees, resulting in deforestation. Alternative paclitaxel production methods, such as chemical synthesis and *Taxus sp.* tissue and cell cultures, are more expensive and offer lower yields. Hence, several recent investigations have shown considerable production of plant secondary metabolites by fungal endophytes, raising the prospect of exploiting them as bioactive metabolite sources. (Pandy, *et al.* 2023).

2-6- *Fusarium solani*

An endophytic fungus is one of the many ecological roles that the extremely varied and common fungal species *Fusarium solani* can play. Research highlighted its capacity to produce valuable bioactive compounds and its function in plant-microbe interactions when discussing its endophyte characteristics, particularly around 2020. (Akram,*et.al.*,2023).

In a variety of habitats and environments, including mangroves, medicinal plants, and agricultural crops like sugarcane and tomatoes, *Fusarium solani* can be found as an endophyte in a broad range of host plants. This suggests that it is versatile and can associate with a wide variety of plant species. (Dos Reis,*et.al.*,2022).

It usually displays white, cottony mycelia when it is young, as it is an endophyte in culture. The mycelia have branches, resemble threads, and are septate, or separated by cross walls. It produces distinctive conidia, or asexual spores, which are usually septa-containing and cylindrical to oval (micro conidia) or sickle-shaped or fusiform (macro conidia) Figure (2-3). However, because of its variability within the species and the difficulty of differentiating it from closely related *Fusarium* species, morphological identification alone can be difficult. (Hassan, and Chang, 2022)

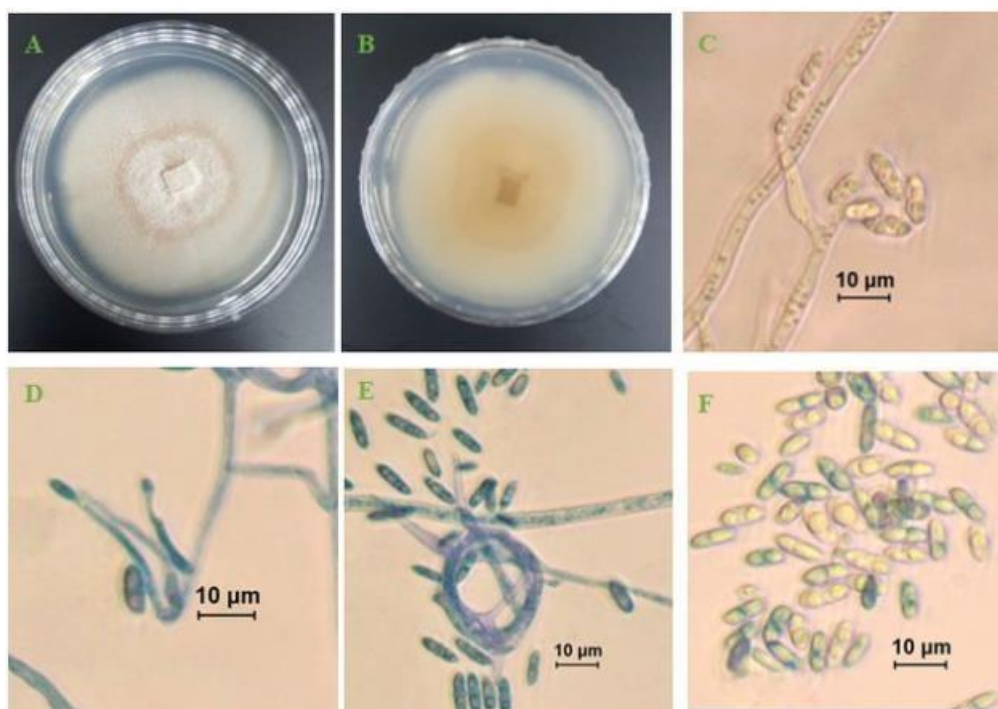


Figure (2-3) Morphological characteristics of *Fusarium solani*; A-B: Colony on PDA; C: Micro conidia; D: Macro conidia; E-F: Chlamydospores. (Hassan, and Chang, 2022)

From a biotechnological standpoint, *Fusarium solani* ability to produce bioactive metabolites is arguably its most important endophyte trait as of 2020. By generating antimicrobial metabolites, competing for nutrients, or causing systemic resistance in the host plant, *F. solani* endophytes can function as biocontrol agents against plant pathogens. Some strains produce phytohormone (like indole acetic acid, or IAA), solubilize phosphates, fix nitrogen, or improve nutrient absorption, among other characteristics that promote plant growth. They are therefore desirable for sustainable farming. Its capacity to generate a diverse range of secondary metabolites with a range of biological activities piqued the interest of researchers. (Akram,*et.al.*,2023). Numerous studies have demonstrated its capacity to generate compounds with antifungal and antibacterial (e.g., against *S. aureus* and *E. coli*) qualities. Its study in the context of drug discovery, especially for new antibiotics to fight resistance, was greatly influenced by this. (Xu,*et.al.*,2023).

The discovery that certain strains of the endophytic *F. solani* produce anticancer compounds, such as Taxol (paclitaxel), was noteworthy because it provided a more sustainable source for this important medication. There have also been reports of other anti-cancer substances, such as mitoxantrone and aloe-emodin. (Subban, and Kempken, 2023). The capacity of *F. solani* isolates to generate paclitaxel and its precursors (such as baccatin III and 10-deacetylbaccatin III) in liquid cultures has been validated by a number of investigations. A *Fusarium solani* strain that was isolated from *Taxus celebica* and produced 1.6 µg/L of paclitaxel is specifically mentioned in one report. (Chakravarthi,*et.al.*,2018).

Genetic manipulation, including mutagenesis and genetic engineering of fungal strains to improve their biosynthetic pathways for taxol, and the optimization of culture conditions, which included determining the optimal growth media, temperature, and other environmental factors to maximize taxol production. Additionally, Co-cultivation: It has been demonstrated that growing *F. solani* alongside other microorganisms or plant extracts increases the production of Taxol (for example, co-culturing with *Taxus* suspension cells increased yield by up to 38-fold for *Fusarium solani*.). (Subban, and Kempken, 2023; Kousar,*et.al.*,2022).

2-7- Extraction and purification of taxol from fungi

A multi-step procedure is used to extract and purify taxol from fungi, especially endophytic fungi, in order to separate this valuable substance from the intricate fungal culture. The standard procedure is to cultivate the fungi, extract the metabolites, and then purify the crude extract using different methods. (Surapaneni,*et.al.*,2012; Turner,*et.al.*,2017)

- I. Fungal Culture and Fermentation
- II. Growth and Fermentation

III. Extraction of Taxol (Mycelial Extraction, Culture Filtrate Extraction)

IV. Purification of Taxol –

- Column Chromatography
- High-Performance Liquid Chromatography (HPLC)

V. Identification and Characterization

1- Thin Layer Chromatography (TLC): Quick and simple for initial screening and monitoring fractions during column chromatography. Uses specific solvent systems (e.g., methylene chloride/methanol/dimethyl form amide) and detection reagents (e.g., vanillin/sulfuric acid spray).

2- Ultraviolet (UV) Spectroscopy: Taxol has characteristic UV absorption peaks (e.g., at 227-230 nm).

3- Infrared (IR) Spectroscopy: Provides information about functional groups present in the molecule.

4- Mass Spectrometry (MS), especially LC-MS/MS: Provides molecular weight information and characteristic fragmentation patterns, which are crucial for definitive identification. The (M+H)⁺ peak at 854 and (M+Na)⁺ peak at 855 are characteristic for Taxol.

2-8- HPLC

High-Performance Liquid Chromatography is referred to as HPLC. Individual components within a complex liquid mixture can be identified, separated, and quantified using this potent analytical chemistry technique. It is a more advanced and effective version of conventional column chromatography. (Lozano-Sánchez,*et.al.*,2018)

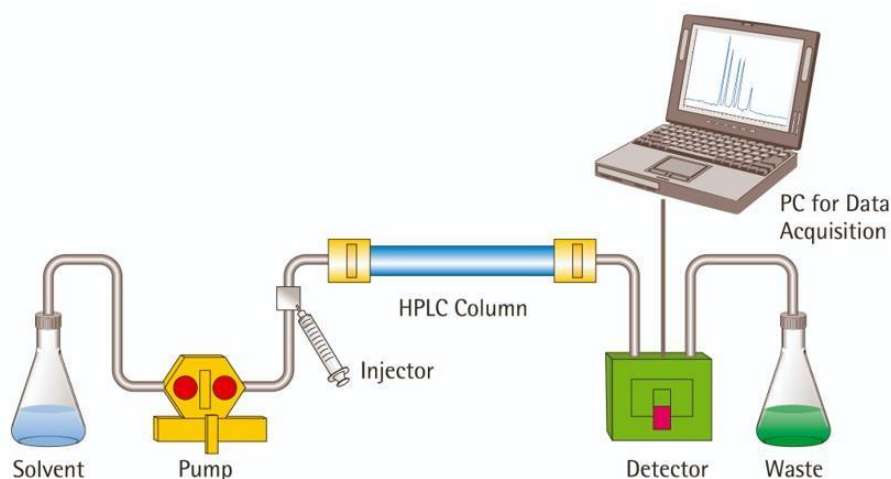


**Figure (2-4). Practical high-performance liquid chromatography
(Meyer, 2010)**

The fundamental principle of HPLC is differential partitioning or differential migration between a stationary phase and a mobile phase. Mobile Phase (Solvent): A liquid solvent or a mixture of solvents (e.g., water, methanol, acetonitrile) is continuously pumped at high pressure through the system. This is the "mobile" part because it moves and carries the sample. Stationary Phase (Column): The heart of the HPLC system is the column, which is a tube packed with very small, uniformly sized particles (the "stationary" part). These particles are typically made of silica, often chemically modified (e.g., C18 for reverse-phase HPLC).

Sample Injection, a small, precise amount of the sample mixture is injected into the flowing mobile phase. while Separation: As the mobile phase carries the sample through the stationary phase in the column, the different components (analytes) in the mixture interact with the stationary phase to varying degrees (Jandera, P., 2000).

Detection: As the separated components (now called "eluent") exit the column, they pass through a detector. The detector measures a specific physical property of each eluting compound (e.g., UV absorption, fluorescence, refractive index, or mass-to-charge ratio if coupled with

Mass Spectrometry). The detector generates an electrical signal proportional to the concentration of the compound.

Chromatogram: The signal from the detector is sent to a computer, which plots it as a chromatogram. This is a graph where the x-axis represents time (retention time) and the y-axis represents the detector signal intensity. Each peak on the chromatogram corresponds to a separated component in the sample (Ismail, B.P., 2024).

Retention Time (tR): The time it takes for a specific component to travel from injection to detection. This is a characteristic property for a given compound under specific HPLC conditions and helps in identification. **Peak Area/Height:** The area or height of a peak is proportional to the concentration of that component in the sample, allowing for quantification. (Siddique, 2021:Siddique,2023).

2-9- Bacterial coinfection in cancer patients

Some human-endemic microorganisms are connected to his cancer, but their function has so far been underestimated due to the lack of understanding of the cancer mechanism. Alternatively, the tumor microenvironment may provide a specific niche in which those organisms may remain in a manner that is difficult for normal tissue. In any case, discovering distinct microbial signatures associated with different cancers is critical for understanding the interplay and diagnosis of microbiome-cancer (Banerjee *et al.*, 2015).

The initiation and development of microbiota and chronic inflammation with human cancer has received little attention (Urbaniak *et al.*, 2014). Bacteria can have an oncogenic effect on the human cells in three ways: causing chronic inflammation, acting as an anti-apoptotic and producing cancerous substances (Gardete, S. and Tomasz,2014).

Cancer patients are at a significantly higher risk of bacterial infections

due to several factors:

1- Immunosuppression from Cancer Itself: Many cancers, especially hematological malignancies (blood cancers like leukemia and lymphoma), directly impair the immune system. (Norris, 2020)

2- Cancer Treatments (Krensky,*et.al.*,2011):

3- Chemotherapy: A major cause of neutropenia, a condition where there's a dangerously low count of neutrophils (a type of white blood cell crucial for fighting bacterial infections). This is the most common reason for bacterial infections during treatment.

-Radiotherapy: Can damage mucosal barriers (e.g., in the mouth, gut), creating entry points for bacteria.

-Surgery: Introduces breaks in the skin barrier, increasing the risk of surgical site infections.

-Immunosuppressive Drugs: Used in some cancer treatments or after stem cell transplants further suppress the immune response.

4- Medical Devices (Jarding, and Makic, 2021; Anderson, and Saunders, 2025):

-Central Venous Catheters (CVCs): Commonly used for chemotherapy and other infusions, CVCs are a major portal for bloodstream infections.

-Urinary Catheters: Increase the risk of urinary tract infections.

-Breakdown of Natural Barriers: Tumors can obstruct organs (e.g., bile ducts, urinary tract, bowel), leading to stasis and increased risk of infection. Mucositis (inflammation of mucous membranes) caused by chemotherapy or radiation also creates open sores.

-Poor Nutritional State and Hygiene: Can further weaken the immune system and increase exposure to pathogens.

-Prolonged Hospitalization: Increases exposure to healthcare-associated infections, often involving multidrug-resistant organisms.

2-10- Typical Bacterial Infections in Cancer Patients

During cancer treatment, a set of six bacterial microorganisms known as "ESKAPE" pathogens are at the forefront of bacterial infection and antibiotic resistance. ESKAPE are one of the biggest challenges in public health because of their high resistance to antibiotics, which makes the infections they cause difficult to treat. The bacteria in the "ESKAPE" group :((Miller, and Arias, 2024)

E: *Enterococcus faecium* S: *Staphylococcus aureus* K: *Klebsiella pneumonia*

A: *Acinetobacter baumannii* P: *Pseudomonas aeruginosa* E: *Enterobacteriaceae*

2-10-1- *Enterococcus faecalis*:

Frequently causes urinary tract infections and bloodstream infections, especially in patients who have had long-term neutropenia and previous antibiotic exposure. *E. faecalis* is a gram-positive Figure (2-5) facultative anaerobe that primarily colonizes human and animal reproductive organs, the gut, the urinary tract, and the oral cavity. It is a member of the *Enterococcus* genus within the *Enterococcaceae* family. Chemical preparations cannot reach root canal bifurcations and irregular areas where

E. faecalis thrives. (Xiang,*et.al.*,2020).

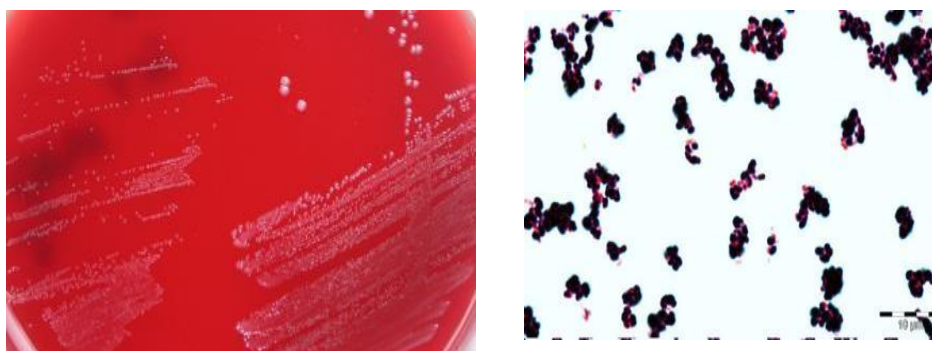


Figure (2-5). *Enterococcus faecalis* (Wellinghausen,*et.al.*,2019

2-10-2- *Staphylococcus aureus*

Staphylococcus aureus: Often responsible for bloodstream infections, especially those associated with catheter use, pneumonia, and infections of the skin and soft tissues. The antibiotic resistance of methicillin-resistant *S. aureus* (MRSA) is a serious concern. (Hogan,*et.al.*,2016). *S. aureus* has a tendency to group together in clusters that resemble grapes, just like other bacteria in its genus. Gram-positive, nonmotile, non-spore-forming, and facultative anaerobic (not oxygen-dependent) are the characteristics of *S. aureus*. It has a diameter of 0.5 to 1.5 µm Figure (2-6). and a high degree of variability in its growth parameters; for instance, different strains can grow at pH values between 4.2 and 9.3 and temperatures between 4 and 48 °C. (Chaturvedi, 2022).

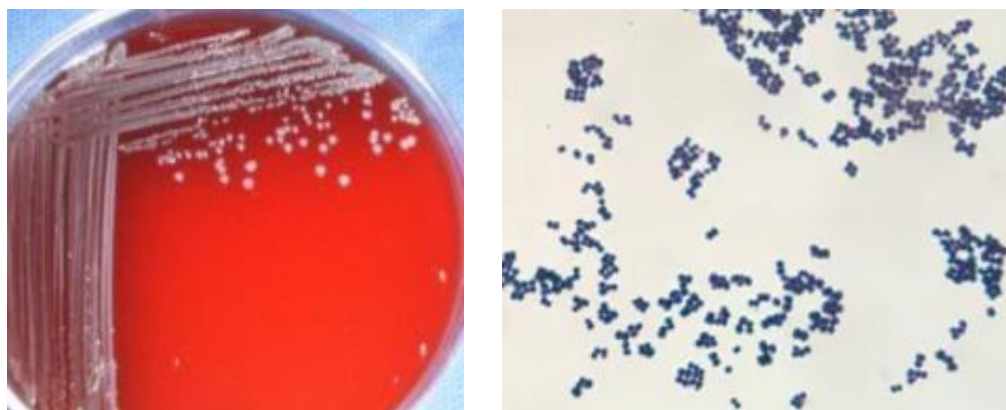


Figure (2-6). *Staphylococcus aureus* (Cervantes-García,*et.al.*,2015)

2-10-3- *Klebsiella pneumonia*

Among these clinically significant organisms that have raised significant concerns for public health is *Klebsiella pneumoniae*. One of the opportunistic pathogens that causes a wide range of illnesses and is increasingly acquiring antibiotic resistance is *Klebsiella pneumoniae*, a significant member of the Enterobacteriaceae. (Navon-Venezia,*et.al.*,2017). About one-third of all Gram-negative Figure (2-7). infections, including septicemia, endocarditis, pneumonia, surgical wound infections, cystitis, and urinary tract infections, are caused by this organism. Additionally, it results in endogenous endophthalmitis, pyogenic liver abscesses, and necrotizing pneumonia. (Effah,*et.al.*,2020).



Figure (2-7). *Klebsiella pneumonia* (Sharma,*et.al.*,2015)

2-10-4- *Acinetobacter baumannii*

Gram-negative, aerobic, nonmotile, and a coccobacillus, *Acinetobacter baumannii* is a member of the Moraxellaceae family of bacteria. Figure (2-8). It is catalase-positive, oxidase-negative, and usually non-fermentative, though it may be oxidative. It is well-known for its capacity to cause nosocomial infections, especially in patients with weakened immune systems, and for developing a growing resistance to several antibiotics. (Ahuatzin-Flores,*et.al.*,2024).



Figure (2-8). *Acinetobacter baumannii*(Al-Ahmer,*et.al.*,2021)

2-10-5: *Pseudomonas aeruginosa*

Pseudomonas aeruginosa: Frequently causes bloodstream infections, urinary tract infections, and pneumonia in immunocompromised patients. It frequently exhibits resistance to several antibiotics. Gram-negative aerobic bacilli with dimensions of 0.5 to 0.8 μm by 1.5 to 3.0 μm . Figure (2-9). There is only one polar flagellum used for motility. Biochemical and DNA hybridization tests are used to distinguish between species. Serovars exhibit cross-reactivity with lipopolysaccharide and outer membrane protein antisera. (Qin,*et.al.*,2022)

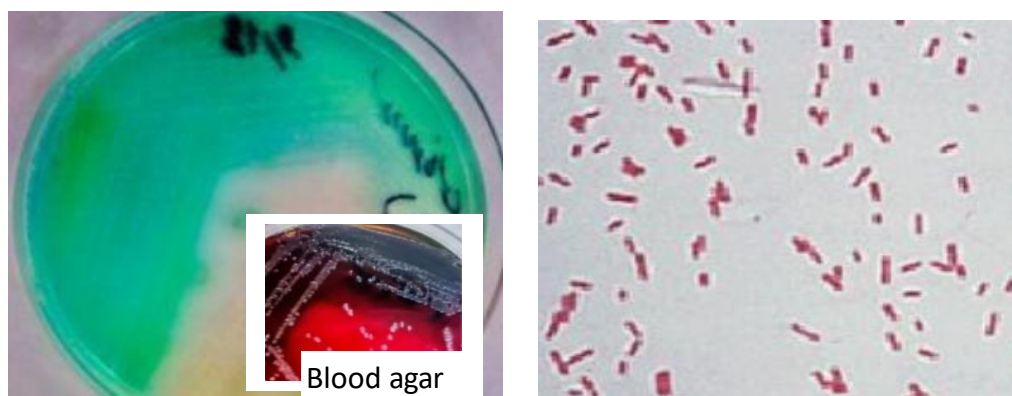


Figure (2-9). *Pseudomonas aeruginosa* (Jyothi,*et.al.*,2011)

2-10-6- *Enterobacteriaceae*

One of the most significant groups of bacteria is the Enterobacteriaceae. Figure (2-10). The typhoid bacillus *Salmonella* serotype Typhi (*Salmonella typhi*) and the plague bacillus *Yersinia pestis* are among the most significant bacterial pathogens in human history. Additionally, according to the Centers for Disease Control and Prevention, it contains two genera of intrinsic enteric pathogens, *Shigella* and *Salmonella*, nearly all of which have the ability to cause intestinal infections or diarrhea. Only a small percentage of the numerous serotypes (strains) of two other genera, *Escherichia* and *Yersinia*, possess the virulence factors necessary for intestinal tract infection or colonization and enterotoxin production. Several other Diarrhea is linked to species. (Octavia, and Lan, 2014; Baldelli,*et.al.*,2021).

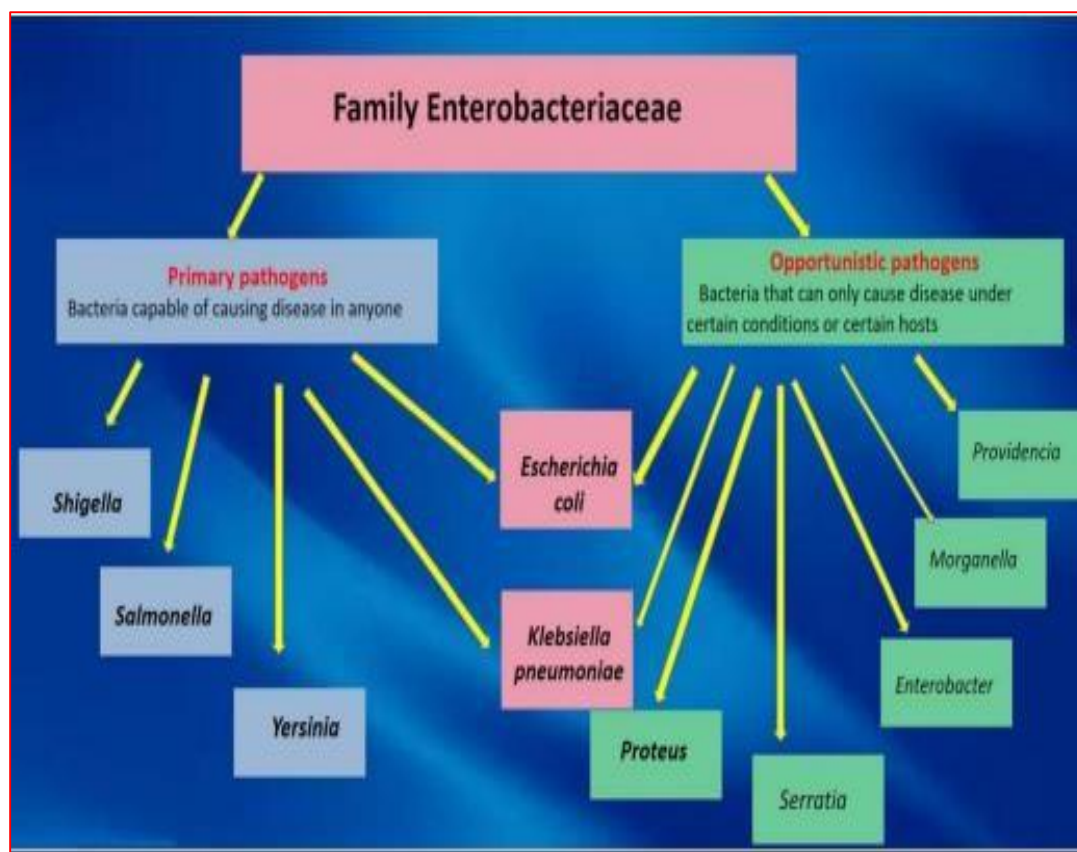


Figure (2-9). Classification of family Enterobacteriaceae

<https://faculty.uobasrah.edu.iq/uploads/teaching/1721541093.pdf>

In patients with cancer, Enterobacteriaceae bacteremia is a frequent consequence. This condition is predisposed by the cancer itself, immunosuppression brought on by chemotherapy, and other cancer-related procedures. (Çeken,*et.al.*,2018).

2-10-7- Other typical bacterial infections in cancer patients 1-*Escherichia coli*

Commonly responsible for bloodstream, gastrointestinal, and urinary tract infections. Cancer patients are especially susceptible to infections because of their weakened immune systems, which are frequently brought on by radiation, chemotherapy, or the disease itself. Bloodstream, urinary tract, and other opportunistic infections are among the many infections that

E. coli continues to cause in these patients. (Zhou,*et.al.*,2023). Studies conducted in 2020 showed that cancer patients were more likely to contract multidrug-resistant (MDR) *E. coli* infections, which could seriously jeopardize treatment results. Finding risk factors for *E. coli* bloodstream infections in cancer patients was another area of research, with notable distinctions from the general population, including greater rates of hospital-onset infections and antibiotic resistance. (Basu,*et.al.*,2022).

2-Coagulase-negative Staphylococci (e.g., *Staphylococcus epidermidis*) Coagulase-negative Staphylococci (CoNS), particularly *Staphylococcus epidermidis*, play a significant and complex role in cancer patients. While often harmless commensals of the skin, they emerge as prominent opportunistic pathogens in immunocompromised individuals, which frequently includes cancer patients due to their underlying disease and/orcancertreatments (chemotherapy, radiation, surgery) (França,*et.al.*,2021).

The most frequent reason for bloodstream infections (BSIs) is: Among the most commonly isolated organisms from blood cultures in cancer patients, especially those suffering from febrile neutropenia (a common complication of chemotherapy where white blood cell counts are low), are CoNS, and in particular, *S. epidermidis*. (Allen-Taylor,*et.al.*,2024).

2- Streptococcus species (e.g., Viridans group streptococci, *Streptococcus pneumoniae*)

The dual nature of *Streptococcus* species in cancer patients is demonstrated by their roles as important opportunistic pathogens and, in certain situations, their intricate interactions that may impact the development of cancer or even be investigated for potential therapeutic uses. Due to weakened immune systems (caused by chemotherapy,

radiation, or the cancer itself), compromised mucosal barriers (such as chemotherapy-induced Mucositis), and frequent use of indwelling medical devices (such as central venous catheters), cancer patients are particularly vulnerable to infections. One of the main causes of infections in this population is *Streptococcus* species. (Dulanto Chiang,*et.al.*,2018).

3- *Clostridioides difficile* (formerly *Clostridium difficile*) infection (CDI)

A significant worry for patients with cancer because of their weakened immune systems, frequent hospital stays, and exposure to chemotherapy and antibiotics . This group of patients has a much higher chance of getting CDI and frequently has worse results. (Delgado,*et.al.*,2017).

Globally, gram-negative bacteria (such as *Klebsiella*, *Pseudomonas*, *E. coli*, and *Acinetobacter*) are more common causes of cancer, though gram-positive bacteria (such as *Staphylococcus* and *Enterococcus*) also play a major role. Cancer patients who have bacterial coinfections may experience serious side effects, (Yusuf,*et.al.*,2023) such as:

Septic Shock and Sepsis

Higher Mortality and Morbidity Rates: Cancer Treatment Delays or Disruptions: Extended Hospital Stay:

Resistance to Antimicrobials

Chapter Three

Material& Method

3- Materials and Methods

A group of laboratory instruments were used in this study, shown in Table (3-1), mentioning their manufacturing companies

Table (3-1): Manufacturing companies for the laboratory instruments

Instrument	Manufacturers / Brands
Cabinet hood	(BioLAB/ Korea)
Centrifuge	Eppendorf, Thermo Fisher Scientific, Sigma
Centrifuge	(DLAB /Ghain)
Electrophoreses	Clarivate /UK
FTIR	Perkin Elmer TWO, USA
HPLC system (e.g., Knauer)	Knauer (Germany), Agilent, Shimadzu, Waters, Thermo Scientific
Rotary Evaporator	Buchi, Büchi Labortechnik, Heidolph
Scanning Electron Microscope (SEM)	JEOL, FEI, Hitachi, Zeiss
Sensitive balance	(Sartorius /Germany)
Spectrophotometer / UV- Vis	Shimadzu, PerkinElmer, Agilent, Thermo Fisher
Thermostatic Incubator	Zxinstrument/Chain
Vacuum Pump	Edwards, Welch, KNF, Thomas Scientific
Vortex	Fisher Scientific/ USA
Water Distillatory	(GFL/ Germany)
X-ray Diffract meter (XRD)	PHILIPS, PW1730, The Netherland

3.1.2. Equipment

A group of laboratory equipment's were used in this study, shown in Table (3-2), mentioning their manufacturing companies.

Table (3-2): Laboratory equipment's and their manufacturing companies

Materials	Manufacturers / Brands
6X Loading dye	Intron / Korea
Acetonitrile (HPLC mobile phase)	Merck, Sigma-Aldrich, Honeywell, Fisher
Agarose	Carl Roth/Germany
Buffer solutions (if used)	Suppliers vary (ready-made buffers from Thermo Scientific, Sigma)
Chloroform	Sigma-Aldrich, Merck, Alfa Aesar
Dilute acids (HCl)	Sigma-Aldrich, Merck
Distilled/Milli-Q Water	Merck, Millipore (MilliporeSigma), Thermo Scientific
Ethanol or Methanol	Merck, Sigma-Aldrich, Honeywell, Fisher
Ethyl Acetate	Sigma-Aldrich, Merck, Alfa Aesar
Extraction solvents (e.g., petroleum ether)	Sigma-Aldrich, Merck
FavorPrep Fungi/ Yeast Genomic DNA Extraction Mini Kit	Korea
KBr (Potassium Bromide)	Sigma-Aldrich, Alfa Aesar, Merck
Ladder100 bp	Kapa /USA
Master Mix or GoTaq® Green Master Mix	Promega/USA
Organic solvents (methanol, ethanol, chloroform, ethyl acetate, hexane)	Sigma-Aldrich, Merck, Fisher Scientific, Alfa Aesar
primer	Macrogen/ Korea
Red safe staining souluion	Intron / Korea
Silica gel or alumina (for chromatography)	Sigma-Aldrich, Merck, VWR
Sodium hydroxide (NaOH)	Sigma-Aldrich, Merck
Standard Taxol	Sigma-Aldrich, Cayman Chemical, Tocris
TBE buffer10 X	Intron / Korea

3.1.3. Culture media

The culture media used in this study are listed in Table (3-3) along with the companies that manufacture them.

Table (3-3). Culture media used in the study and their companies

No	Medium	manufacturer company
5	Blood agar base	Himedia\India
4	Brain heart infusion Agar	Himedia\India
3	MacConkey agar	Himedia\India
1	Nutrient agar	Himedia\India
2	Nutrient broth	Rasmi\India
7	Potato Dextrose Agar (PDA)	Himedia\India
6	Sabouraud's Dextrose Agar	Himedia\India

3.1.4. Primers used in the study

Table (3-4). The sequence of primers that used this study

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of Product (bp)
ITS	F=ITS1	5'-TCCGTAGGTGAACCTGC	61.6	63.1	567 bp
		GG -3'	4	6	
	R=ITS4	5'-TCCTCCGCTTATTGATA	55.0	45.0	
		TGC-3'	9	0	

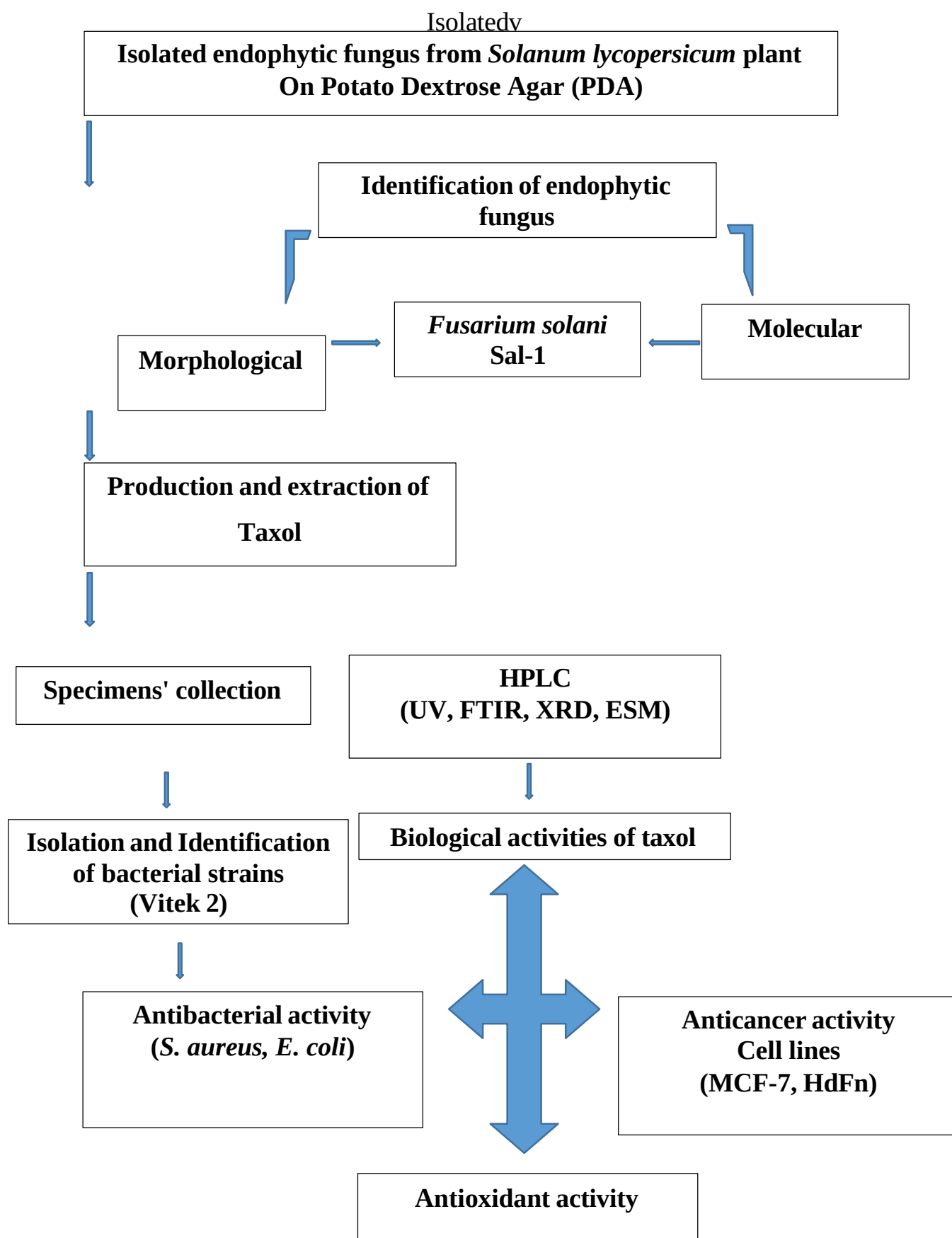


Figure (3-1) Design study

3.2. Methods

3.2.1. Sterilization

1- wet heat sterilization

Culture media were autoclave sterilized for 15 minutes at 121°C and 15 pounds per square inch of pressure.

2- Sterilization with dry heat

An electric oven set to 180°C for two hours was used to sterilize the glass tools used in this study

3- Filtration-based sterilization

In this technique, extracts and solutions that are affected by heat were sterilized using membrane filters with a 0.22 micrometer diameter.

3.2.2. Preparation of culture media

All media prepared according of according to manufactured company directions.

3.2.3. Specimens' collection

Specimens were collected From the Cancer Center at Marjan Hospital in Hillah during the period June to October, 2024. The clinical isolates were collected from cancer patients, the total specimens collected were 100 blood specimens from patients, Specimens were transferred to the bacteriology lab in the hospital after put it in a suitable container, all Specimens are cultivated within less than two hours by hart- brain broth media in 37°C for 24 hours.

3.2.3. 1.Isolation and identification of bacterial strains

According to McFadden (2000), all isolates are grown on blood agar and MacConkey agar media to examine the bacteria present. They are then incubated for 24 hours at 37°C and then re-cultivated on selective and differential media. The following procedures were used to identify the bacterial strains:

A. Morphological characteristics

This identification is considered primary identification it depends on characteristics of growing bacterial colony on culture media like colony color, colony, colony altitude, colony diameter, and forming pigments.as well as Gram stain.

B-Vitek 2 Compact automated system

The Vitek 2 device has been used to validate biochemical and antibiotic Tests Using the Programme version 7.01 and the AST-N201, AST-P632, ASTP586 and AST-ST01 cards for Gram negative bacteria, staphylococci, enterococci and streptococci respectively, we performed AST with the VITEK 2 system according to the manufacturer's instructions.

3.2.4. Fungal strain

Fusarium solani Sal-1, an endophytic fungus. It was isolated from *Solanum lycopersicum* plant, with its optimal growth occurring at PH 7 at 25 °C. At 28°C, this fungus was kept on potato dextrose agar (PDA) slants.

3.2.4.1. Isolation and Identification A-Isolation

The fungal strain *Fusarium solani* Sal-1 was isolated as an endophytic fungus from the internal tissues of *Solanum lycopersicum* (tomato) plants. The plant tissues were carefully collected from and transported to the laboratory under sterile conditions., plant tissues underwent surface sterilization, typically involving sequential rinses with sodium hypochlorite, and sterile distilled water, to eliminate surface microbes while preserving internal endophytes. The sterilized tissues were sectioned into small pieces and placed on suitable nutrient media, such as Potato Dextrose Agar (PDA), and incubated at 28°C. After a few days, fungal hyphae emerging from the tissue fragments were transferred to fresh PDA plates to obtain pure cultures.

B- Identification

1- Morphological Identification

Identification of strain was depended on micro and macro features, such as characterized based on colony morphology, conidial shape, and microscopic features (Krasnow *et.al.*, 2024; abd-Elsalam *et al.*, 2015, Sukarno *et al.*, 2023; Patel *et al.*, 2020).

2- Molecular Identification

A. General Protocol:

1. 1~ 5 x10 of cultures (fungal cells) was Transferred to a 1.5 ml micro centrifuge tube.

2.1 ml of FA Buffer was Added to the cells and resuspending the cells by pipetting. the cells the cells were descended by centrifuging at 5,000 rpm for 2 min and discard the supernatant completely.

3. the cells were Resuspending in 550 µl of FB buffer and added 50 µl of lyticase solution, mix well by vortexing. Incubate the sample at 37 °C for 30 min. the cells were descended by centrifuging at 5,000 rpm for 10 min. the supernatant was Removed completely.

4. 350 µl TG1 Buffer was added and mixed well by pipetting. the sample mixture was Transferred to a bead tube. then was mix ed well by Plus- vortexing for 5 minutes.

5. 20 µl of Proteinase K (10 mg/ml) was added and mixed well by vortexing. Incubated at 55 °C for 15 min, vortex 30 seconds for every 5 minutes' incubation.

6. the cells were Descended by centrifuging at 5,000 rpm for 1 min and transferred 200 µl of supernatant to a new 1.5 ml micro centrifuge tube.

7. 200 µl of TG2 Buffer was Added and mixed well by pipetting.

8. 200 µl of ethanol (96-100%) was Added and mixed well by pulse- vortexing for 10 seconds.

9. A TG Mini Column was Placed in Collection Tube. Transferred the sample mixture (including any precipitate) carefully to TG Mini Column. Centrifuge at 14,000 rpm for 30 second then place the TG Mini Column to a new Collection Tube.

10. 400 μ l of W1 Buffer was Added to the TG Mini Column. Centrifuge at 14,000 rpm for 30 seconds and was discard the flow-through. Placed the TG Mini Column back to the Collection Tube. and was sure the ethanol has been added into W1 Buffer when first use.

11. 750 μ l of Wash Buffer was Added to the TG Mini Column. Centrifuge at 14,000 rpm for 30 seconds and discard the flow-through. Placed the TG Mini Column back to the Collection Tube. --and was make sure ethanol has been added into Wash Buffer when first use.

12. Centrifuged at full speed 14,000 rpm for an additional 3 min to dry the column.

13. the TG Mini Column was Placed to a Elution Tube.

14. 50 ~100 μ l of Elution Buffer or ddH₂O was Added to the membrane center of the TG Mini Column. Stand TG Mini Column for 3 min.

15. Centrifuge at full speed 14,000 rpm for 1 min to eluted total DNA. Stored total DNA at 4°C or -20°C.

B-. Determination of DNA Concentration:

Gel Electrophoresis to Analyze DNA Quality: -

1- An agarose solution was prepared by dissolve 1g of agarose powder in 100 ml of 1x TBE in the (100) ml flask, agarose was melted in hot block until the solution became clear.

2- The agarose solution was made cool to about (50- 55°C), swirling the flask occasionally to cool evenly.

3- Red stain (3 μ l) was added to the warm gel then sealed the ends of the casting tray with two layer of tape.

- 4- The combs were placed in the gel-casting tray.
- 5- Melted agarose solution was poured into the casting tray.
- 6- The agarose was allowed to solidify at room temperature, the comb pulled out carefully and the tape was removed. The gel was placed onto the electrophoresis chamber that was filled with TBE (1x) buffer.
- 7- DNA samples (5µl) were mixed with (3µl) DNA loading buffer and loaded in agarose gel wells.
- 8- The agarose gel electrophoresis was completed at 70V, 65Amp for 1hour. The DNA was observed by viewed under UV trans illuminator

C-PCR preparation

As indicated in table (3.5) below, PCR reactions were conducted using the Mix kit and in compliance with the manufacturer's instructions.

Table (3-5) Reaction components of PCR.

Component	25 µl (Final volume)
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl

D-Conditions for PCR Thermo cyclers

The requirements for performing a successful touch-down PCR with the conventional thermo cycler and the web application Optimize Protocol Writer TM are displayed in the following table (3-6).

Table (3-6): The optimum condition of detection

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1 cycle
2-	Denaturation -2	95°C	45 sec	35 cycle
3-	Annealing	72°C	1 min	
4-	Extension-1	72°C	1 min	
5-	Extension -2	72°C	5 min.	1 cycle

E-DNA Sequence Analysis

ITS gene primers from fungal isolates were used for DNA sequencing in order to investigate the genetics of fungal species identification. The ITS PCR results were sent to the MacroGen Company in Korea for an AB DNA sequencing study after being cold-packed by DHL. Mega 6.0, the most recent version of the program used for Molecular Evolutionary Genetics Analysis, was used to analyze the brief ITS gene fragment. Using multiple sequence alignments with ClustalW and the Maximum Composite Likelihood approach based on a phylogenetic tree built using the UPGMA method, the evolutionary distance between species was then determined. Sequences with similar properties are subjected to the NCBI BLAST analysis in order to detect mutations and other alterations. Finally, the fungal isolates received GenBank accession numbers after being added to the NCBI-GenBank database.

3.2.5. Production and extraction of Taxol

3.2.5.1. Production

The different liquid media used in this experiment were as follows (g/L):

Type (1) consist from (yeast extracts:(0.5), sucrose (6), fructose (3), sodium acetate (1), Cu(NO₃)₂(1), Ca(NO₃)₂4H₂O (0.65), MgSO₄7H₂O (0.36), KCl (0.06), MnSO₄ 4H₂O (0.0005), ZnSO₄7H₂O (0.0025), sodium

benzoate (0.1), FeCl₂(0.002), KH₂PO₄(0.136), phenylalanine (0.005).

Type (2) consist from (glucose (20), potato (200), MgSO₄7H₂O (3), KH₂PO₄(5), vitamin B1(0.01), leucine(0.0131), acid ammonia(0.0077), phenylalanine(0.00825).

Frist in 500 ml of the above media, *Fusarium solani* was cultured and incubated at 28°C for 7 days using a shaking incubator (160-cycle). For the second stage, this culture was used as the germinating culture., the mycelium mixture was filtered, separated, dried at 50°C, and ground. It was extracted once using an ultrasonic device and by adding ethyl acetate for one hour, while the fermented medium was extracted three times using equal volumes of ethyl acetate. Every extract was dried under low pressure in a revolving evaporator. Following a 25 mL solution of 100% methanol (v/v) dissolution of the residues, (Wu *et al.*, 2003)

3.2.5.2. Extraction

1- The remaining culture medium was extracted three times using equal quantities of ethyl acetate to ensure maximum Taxol recovery.

2- Each extract was concentrated under low pressure in a rotary evaporator, removing excess solvents.

3.2.5.3. Purification & Detection of Taxol by HPLC

1 - The dried extract was dissolved in 25 mL of 100% methanol (v/v) to ensure complete solubility.

2- High-Performance Liquid Chromatography (HPLC) Screening was performed to confirm Taxol presence and purity.

5. Final Product Analysis

3- Taxol fractions obtained from HPLC were analyzed for chemical structure and bioactivity.

3.2.5.4. Identifying Taxol

The following were the HPLC detection conditions:

The C18 column (Knauer, Germany) was used for the separation (250 × 4.6 mm i.d., 5 µm particle size, 80 Å pore size). The separation and detection were conducted in accordance with reference [Schmitt, K 2018]. The mobile phase included mobile phase A (water) and mobile phase B (acetonitrile) at a flow rate of 1.2 ml/min. All samples were examined at 227 nm and a temperature of 40°C. The injection volume was set at 20 µl, and the chromatograph was constructed as outlined in Table (3-7).

Table (3-7). Optimization of Gradient Programs

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	60	40
25	60	40
41	40	60
41.1	0	100
50	0	100
50.1	60	40
55	60	40

By comparing the retention time and absorbance spectrum of each standard, each compound was found. The concentration was then determined by serially concentrating external standard materials to create a calibration curve fig (3-2) that connected the concentration to its equivalent peak area.

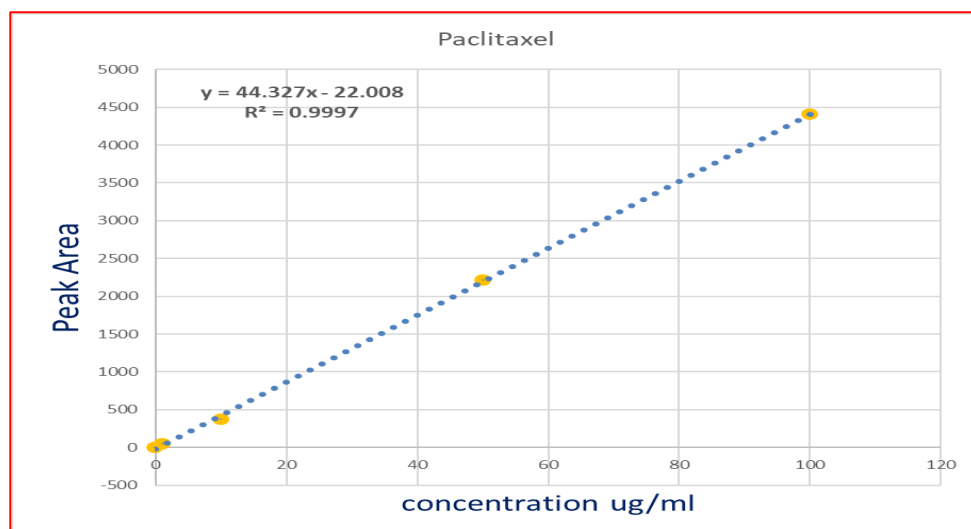


fig (3-2) calibration curve

3.2.5.5. Characterize of extracted taxol 1- FTIR analysis

By Using an ATR-FTIR Perkin-Elmer 100S spectrophotometer, the taxol powder was analyzed by FTIR. The samples were collected in a KBr pellet and scanned between 500 and 4000 cm^{-1} in the infrared spectrum. **A- Sample Preparation:**

- The taxol powder was carefully prepared for FTIR analysis by blending a small amount of the sample with potassium bromide (KBr) powder.

- The mixture was ground into a fine, homogeneous pellet using a pellet press under hydraulic pressure (typically around 10 tons) to ensure a clear, uniform pellet with good contact.

B-Measurement Process:

- The KBr pellet containing the taxol sample was mounted directly onto the ATR crystal of the spectrophotometer.

- The spectrophotometer was set to scan in the infrared region, specifically from 500 cm^{-1} to 4000 cm^{-1} .

- Scans were performed in multiple accumulations (usually 16 or more scans) to improve the signal-to-noise ratio.

- The instrument accumulated and averaged the spectra to produce a

clear, reproducible FTIR spectrum of the sample.

C- Data Collection and Analysis:

- The generated spectrum displayed absorbance (or transmittance) versus wavenumber.

- Key peaks corresponding to functional groups present in taxol were identified and labeled, such as carbonyl stretches, aromatic rings, hydroxyl groups, etc.

- The spectrum was compared to reference spectra for confirmation of Taxol's characteristic functional groups.

2- UV spectroscopy

The extracted crude sample was subjected to UV spectroscopic examination. The spectrum of the crude taxol samples was recorded using a Beckman DU-40 spectrophotometer (USA), and quantification was achieved by comparison with the standard Taxol.

A- Preparation of Sample:

- A suitable aliquot of the extracted crude sample was dissolved in a solvent compatible with UV analysis, typically ethanol, methanol, or water depending on the solubility of taxol.

- The sample was filtered to remove any particulate matter that could interfere with the spectroscopic measurement.

B- Spectral Recording:

- The spectrophotometer was calibrated with a blank solvent sample to zero the readings.

- The crude taxol solution was scanned across a wavelength range, typically from 200 nm to 400 nm.

- The resulting spectrum displayed characteristic absorption peaks corresponding to the chromophoric groups in taxol, often near 227 nm, which is indicative of its aromatic and conjugated structures.

C- Quantitative Analysis:

- The absorbance at the characteristic wavelength (e.g., 227 nm) was recorded.

- A calibration curve was prepared using standard taxol solutions of known concentrations measured under identical conditions.

- The concentration of the crude sample was calculated by comparing its absorbance to the standard curve, applying Beer-Lambert Law:

$A = \epsilon lc$, where A is absorbance, ϵ is molar absorptivity, l is path length, and c is concentration.

3- SEM analysis

The morphology of these microspheres for taxol was studied by scanning electron microscopy (SEM)

A-Sample Preparation:

- The purified fungal taxol was first dried to remove any residual solvents or moisture.

- Since taxol is a crystalline compound and a non-conductive material, small amounts of the isolated sample were mounted onto SEM stubs using conductive adhesive.

- To enhance electrical conductivity and improve image quality, the sample was sputter-coated with a thin layer (~10 nm) of gold or platinum.

B- SEM Imaging:

- The samples were examined under the SEM at various accelerating voltages (usually 5-20 kV).

- The electron beam scanned the sample surface, producing secondary electrons that generated detailed surface topography images.

4- XRD analysis

X-ray diffraction analysis was conducted to ascertain whether the

entrapped taxol existed or no.

A- Sample Preparation:

- The encapsulated Taxol sample was finely ground and prepared on an XRD sample holder for uniform diffraction measurements.

- A thin film or powder form was used to maximize surface interaction with X-rays.

B-XRD Measurement Parameters:

- The analysis was performed using a Cu K α radiation source ($\lambda = 1.5406 \text{ \AA}$) in a θ - 2θ scanning mode.

- Scanning range: Typically, 5° to 50° (2θ) to capture major diffraction peaks.

- Step size: $\sim 0.02^\circ$ for high-resolution data acquisition.

C-Data Interpretation:

- Presence of characteristic Taxol peaks would indicate that the drug maintains its crystalline nature within the encapsulation system.

- Peak broadening or absence of Taxol-specific peaks could suggest amorphous dispersion, molecular interactions, or structural transformation due to entrapment in a polymeric matrix or nanoparticle system.

- Comparative analysis with unencapsulated pure Taxol standard confirms its stability or possible changes in crystallinity.

3.2.5.6. Biological activities of extracted Taxol.

3.2.5.6.1. Anticancer activity of Taxol

Solutions and Media Used in Tissue Culture Technique were prepared according to Freshney (2010).

A- Solutions

1--antibiotic solution

a- Streptomycin (1g/vial):

It was prepared by dissolving vial contents in 5 ml of sterile distilled water to prepare a stock solution (200,000 μ g/ml). The stock was stored at -18°C. And 0.5 ml of it was added to 1 litter of culture media.

b- Benzyl Penicillin:

It was prepared by dissolving the contents of one vial which has 106 IU in 5ml of sterile distilled water to prepare a stock solution (200,000 IU/ml). The stock was stored at -18°C. And 1 ml of it was added to 1 litter of culture media.

2- Sodium Bicarbonate Solution

The solution was prepared by dissolving 2.2 g of NaHCO₃ in 1000ml distilled water. The solution was sterilized by autoclaving and kept at 4°C until use.

3- Phosphate Buffer Saline (PBS)

This buffer was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.15 g NaH₂PO₄ and 0.2g Na₂HPO₄ in 900ml of distilled water, pH was adjusted to 7.2. The solution was sterilized by autoclaving and stored at 4°C until use.

4- Trypsin Solution

It was prepared by dissolving 1 g of trypsin powder in 100ml PBS and sterilized by filtration using Millipore's filter (0.22 μ m). The solution was dispensed into 10ml aliquots and stored at -20°C.

5- EDTA Solution

It was prepared by dissolving 1 g of ethylene-diamine-tetra acetic acid (EDTA) in 100ml of PBS and sterilized in autoclave for 10 minutes. The solution was dispensed in 10 ml aliquots and stored at 4°C.

6- Trypsin-EDTA Solution

It was prepared by mixing 20ml of trypsin solution, 10 ml EDTA solution and 370 ml PBS. The mixture was stored at 4°C.

B- Media

1- Roswell Park Memorial Institute – 1640 Medium (RPMI)

A ready to use package (100 ml) RPMI was used throughout this study. The medium was already supplied with 4-(2-hydroxyethyl)-1piperazine- ethane sulfonic acid (HEPES) and L-glutamine as illustrated by manufacturer.

The medium was completed by adding the following ingredients:
Penicillin G 103 IU

Streptomycin 0.001 g

Sodium Bicarbonate 1%

Fetal Bovine Serum 10 %

2- Serum Free Medium

Serum free medium is RPMI-1460 excluded from fetal calf serum.

C- Cell lines 1-MCF-7

Is a human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors.

2-HdFn cell line

The HdFn cell line, also known as Primary Dermal Fibroblast Normal; Human, Neonatal (HdFn), was obtained from the foreskin of newborns.

These cell lines were acquired from the Medicine College's Pharmacology Department at Malaya University in Kuala Lumpur, Malaysia. Passage number: 15.

D-Cytotoxic Effect of Taxol Cell Line Maintenance:

When the cells in the vessel formed confluent monolayer, the following protocol was performed:

1- The growth medium was aspirated and the cell sheet washed with PBS.

2- Two to three ml trypsin/EDTA solution was added to the cell. The vessel was turned over to cover the monolayer completely with gentle rocking. The vessel allowed incubation at 37°C for 1 to 2 minutes, until the cells were detached from the vessel.

3- Fresh complete RPMI medium (15-20 ml) was added and cells were dispersed from the wedding surface into growth medium by pipetting.

4- Cells were redistributed at required concentration into culture vessels, flasks or plates whatever needed and incubated at 37°C in 5% CO₂ incubator. Cell concentration was achieved by counting the cells using the hemocytometer and applying the formula:

Total Cell Count/ml: cell count x dilution factor (sample volume) x 10⁴.

C- MTT assay

The cytotoxic effect of F3,OX3,and T2 compounds with presence different concentrations was performed by using MTT ready to use kit (Intron Biotech):

1- Kit contents:

- MTT solution 1 ml x 10 vials.
- Solubilization solution 50 ml x 2 bottle.

2- **Protocol:**

•Tumor cells (1x10⁴ – 1x10⁶ cells/ml) were grown in 96 flat well micro- titer plates, in a final volume of 200ml complete culture medium per each well. The microplate was covered by sterilized parafilm and shacked gently.

- The plates were incubated at 37°C, 5% CO₂ for 24 hrs.
- After incubation, the medium was removed and two fold serial dilutions of the compounds (400, 200, 100, 50 and 25 µg/ml) were added to the wells.
- Triplicates were used per each concentration as well as the controls (cells treated with serum free medium). Plates were incubated at 37°C, 5% CO₂ for selected exposure time (4 hrs).
- 20ml of each compounds was added to each well for 24 H.
- After exposure, 10 ml of the MTT solution was added to each well. Plates were further incubated at 37°C, 5% CO₂ for 4 hrs.
- The media were carefully removed and 100µl of solubilization solution was added per each well for 5 min.
- The absorbance was determined by using an ELISA reader at a wavelength of 575 nm. The data of optical density was subjected to statistical analysis in order to calculate the concentration of compounds required to cause 50% reduction in cell viability for each cell line, through the following equation:

$$Y = D + \frac{A - D}{1 + 10^{(x - \log C)B}}$$

3.2.5.6.2. Antioxidant Activity

Antioxidant activities of Taxol were detected by using DPPH radical scavenging assay according to the procedure described by Kumar et al., (2008), as the following:

1. Aliquots (0.5mL) of two-fold serial dilution from Taxol and ascorbic acid (12.5, 25, 50 and 100µg/mL) were added in the test tubes reaction.
2. Simultaneously, 3ml of methanol-DMSO mixture and 0.3ml of DPPH solution were added to each concentration.
3. Samples were incubated at 37°C for one hour.
4. Radical scavenging activity of samples against the stable DPPH radical was determined spectrophotometrically using ELISA. The

colorimetric changed (from deep- violet to light- yellow) when DPPH reduction was measured at 517 nm.

5. The % inhibition of radical by the samples was calculated according to the following formula:

$$\% \text{ inhibition} = 100 \times \frac{\text{Absorbance of - ve control} - \text{Absorbance of sample}}{\text{Absorbance of - ve control}}$$

The negative control consisted of methanol-DMSO mixture and DPPH solution, while ascorbic acid was used as a reference.

3.2.5.6.3. Antibacterial activity of extracted Taxol

The antimicrobial activity of Taxol was estimated using the well diffusion susceptibility method. Bacterial incola calibrated according to McFarland standards was used to cultivate the bacterial cultures. On petri dishes, 8 mm-diameter wells were drilled into sterile media. Thirty microliters of crude taxol were put into wells. The inhibition zones, which were measured in millimeters, were used to determine the results after the petri dishes were incubated for 24 hours at 37°C. Each bacterium was replicated three times

(Pinho, 2011)

3.2.6. Statistical Analysis

A one-way analysis of variance ANOVA (Duncan) was performed to test whether group variance was significant or not, statistical significance was defined as $p \leq 0.05$. Data were expressed as mean $\pm$ standard deviation and statistical significances were carried out using Graph Pad Prism version 9.4(Graph Pad Software Inc., La Jolla, CA).

Chapter Four

Results & Discussion

4- Results and Discussion

4.1. Collection and Isolations

The present study comprised 100 clinical specimens and examined the prevalence of microorganisms in the specimens., were collected from patients who were admitted to the Cancer Center at Marjan Hospital in Hillah between June and October of 2024. Results show,17(17%) specimens with bacterial growth and 83(83%) no growth. (Table 4-1).

Table (4-1) specimen's distribution

No. of specimens		%
growth	17	17%
No growth	83	83%
total	100	100%

In order to start the disease processes, pathogenic bacteria have a wide range of molecules and mechanisms that function as virulence factors and evade the host defenses. Bacteria secrete a variety of substances, including enzymes, toxins, and exopolysaccharides, in addition to their cell surface structures and metabolic arrangements. Many of these biomolecules can enter host cells and have the ability to alter or destroy intracellular signaling and structures. (Leitão,2020), In the end, each of these bacterial biomolecules increases virulence and could make clinical treatment more difficult. Autopsy studies show that about 60% of deaths in cancer patients with underlying hematological malignancies are caused by infectious complications, which are a major cause of morbidity and mortality in these patients. (Zheng,*et.al.*,2021).

Table (4-2), Figure (4-1) show number of specimens and type of cancer. Its appear among all cancer types, breast cancer is the one that is diagnosed the most frequently with (33, 33%) Uterus cancer with (29,

29%).And lung cancer with (23, 23%), Prostate cancer with (14,14%).

Finally, Skin cancer with (2, 2%).In study by (Giaquinto,*et.al*,2022), reported that The most common cancer diagnosed in women in the United States is breast cancer. It is the primary cause of cancer death for Black and Hispanic women, but it is the second most common cause of cancer death for women in general, after lung cancer. Breast cancer is one of the most common cancers for several reasons. Genetic Factors can play a role in increasing the risk of breast cancer, especially if there is a family history of the disease, Hormonal changes in the body, such as changes in estrogen and progesterone levels, can affect the risk of breast cancer, some lifestyle factors, such as an unhealthy diet and lack of physical activity, can contribute to an increased risk of breast cancer. Some environmental factors, such as exposure to radiation, can affect the risk of breast cancer.(Giaquinto,*et.al*,2022, Lei,*et.al*,2021, Ali Salman,2023)

Table (4- 2) No. of specimens and type of cancer

Type of cancer	No. of specimens	%
Breast cancer	33	33%
lung cancer	23	23%
<i>Prostate cancer</i>	13	13%
Skin cancer	2	2%
Total no. (%)	100	100%
<i>Uterus cancer</i>	29	29%

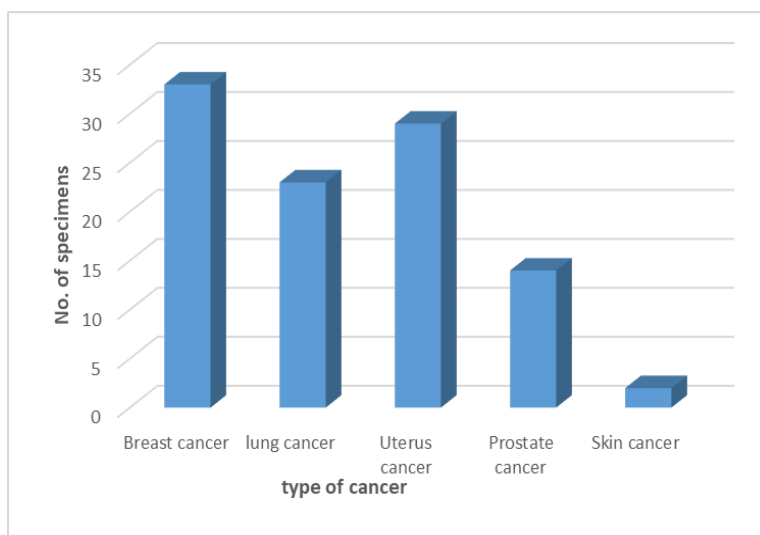


Figure (4-1) show number of specimens and type of cancer.

The results of isolation and Identification by VITEK-2 System showed, *Staphylococcus aureus*. with isolates (29.4%) followed by *S. haemolyticus* (23.5%), *Escherichia coli* (17.6%), *S. equorum*, (5.9%) *S. hyicus* (5.9%), *Klebsiella spp* (11.8%) and *Enterococcus spp* with (5.9%) for each. Table (4- 3)

According to a study by Kumar *et al.* in Mumbai, the most prevalent pathogens were *Pseudomonas* species (26.2%), *Enterococcus* species (11.66%), *S. aureus* species (11.44%), *E. coli* species (11.34%), *Klebsiella* species (10.59%), and *Acinetobacter* species (9.95%) In our study, this was not the case, the cause may be in size of samples and environmental conditions.

Table (4- 3) No. of isolates and percentage

genus	No. of isolates	%
<i>Enterococcus spp</i>	1	5.9
<i>Escherichia coli</i>	3	17.6
<i>Klebsiella spp</i>	2	11.8
<i>S. equorum</i>	1	5.9
<i>S. haemolyticus</i>	4	23.5
<i>S. hyicus</i>	1	5.9
<i>Staphylococcus aureus</i>	5	29.4
Total no. (%)	17	100

Similar to the results of our investigation, another study reported that 68.18% of the isolates from bloodstream infections were gram negative. (Garg,*et.al.*,2019). It was unclear whether cancer patients were at risk for bacterial infections. Immune-related side effects that necessitate immunosuppressive treatment may influence the risk of infection. (Kanjapan and Yip, 2020).

The connection between some bacterial virulence factors and carcinogenesis is an interesting feature. It is believed that *E. coli*-induced chronic inflammation in inflammatory bowel disease may increase the risk of colon cancer development. (Khan, *et.al.*,2017).

4.2. Fungal strain

4.2.1. Morphological Identification

Figure (4-2) illustrates the cotton-like texture of the colony and the yellow-to-tan mycelium encircling the center of the colony on the medium. Microscopic culture morphologies. The septate hyphae were distinguished from the tips of the mycelia by their erect, simple or branched, sickle-shaped conidial (Domsch, 1993). Furthermore, the aerial mycelia contained Chlamydospores (Ajmal,*et.al.*,2022). The isolate's taxonomy was identified by the aforementioned features as *Fusarium solani*.



2. Molecular Identification

The polymerase chain reaction (PCR) was used to confirm the phenotypic diagnosis and identify the fungus. The results indicated that the DNA outputs could be doubled with Purity 1.75 (Table 4-4). circulating

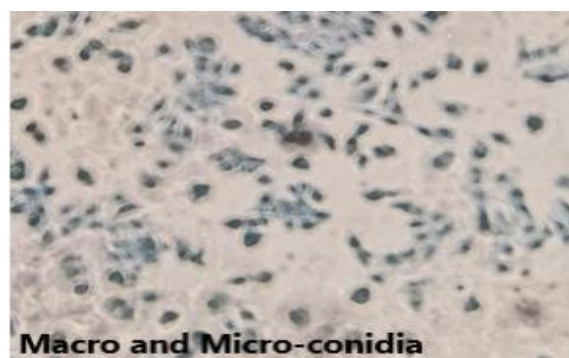


Figure (4-2). *Fusarium solani*

polymerase chain reaction (PCR) with a size of 567 base pairs (bp) and the presence of the primer pair F & R, Fig (4-3). As a first-line method, ITS sequencing is now advised. As stated by (Lücking,*et.al.*,2020).



Figure (4-3) PCR product the band size . The product was electrophoresis on 1.5% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. M: DNA ladder (1000).

Table 4-4- Purity and Nucleic acid concentration.

ID Sample	Nucleic acid conc. (ng/ML)	260/280 Purity
1.	192	1.75

Following comparison with information from the US National Center for Biotechnology Information (NCBI), the nitrogenous base sequences (PCR product) of DNA multiplexed from a fungal isolate indicate that the isolates are members of the *Fusarium solani*, the isolates from this study have been submitted to the World Gene Bank, and Fig. (4-4) illustrates how much they converged with isolated species from other nations. By

comparing the nitrogenous base sequence of the fungus *F. solani*, which was isolated in this study (PQ555253.1), it was discovered that many isolates shared similarities with the fungus, which was previously registered with the National Center for Biotechnology Information (NCBI). Table (4-5)

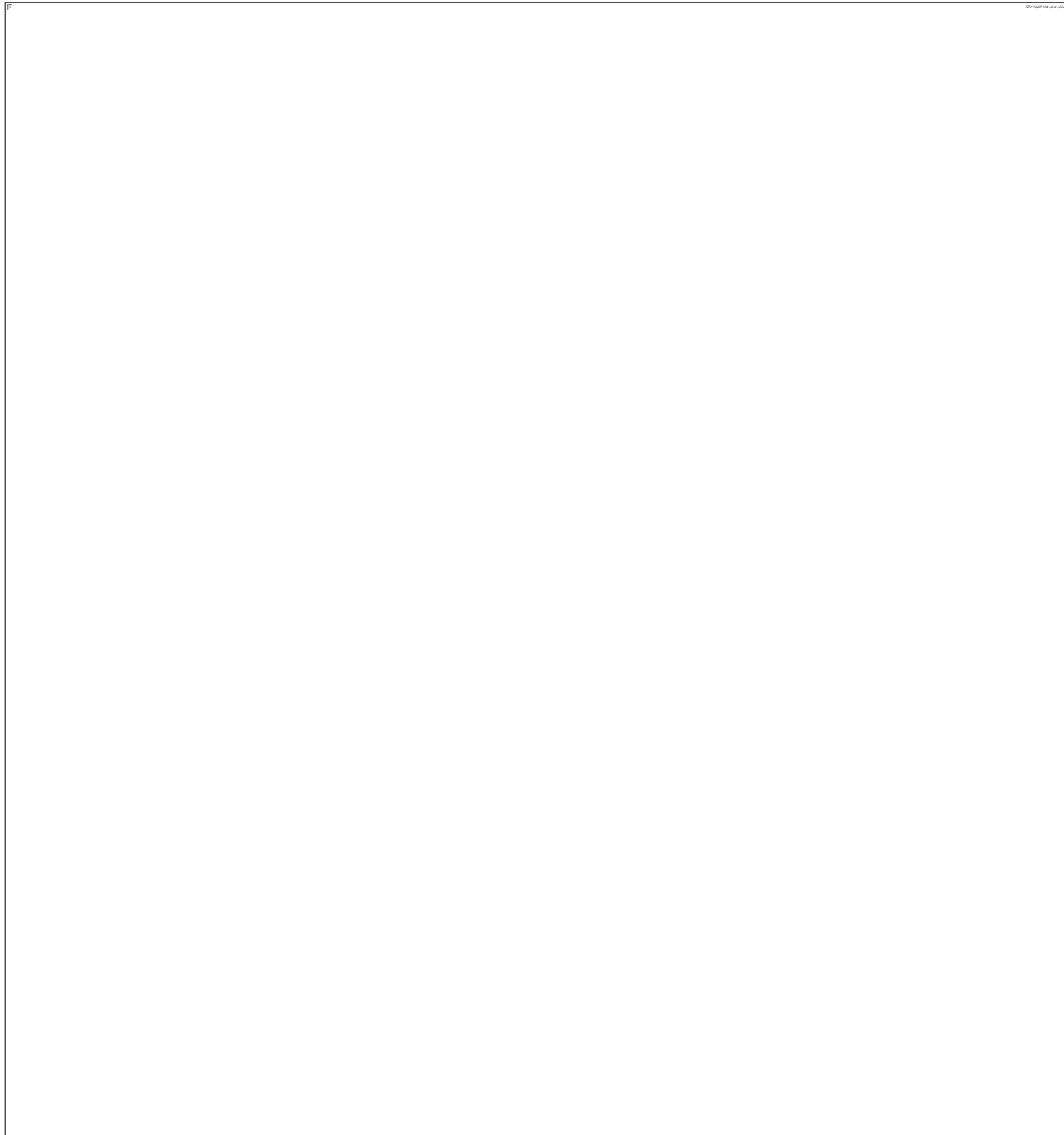


Figure. (4-4) The genetic relationships between the fungal isolates in this study and other isolates that have already been registered with the National Center for Biotechnology Information (NCBI) are displayed by the neighbor-joining tree. the isolate in this study

Table (4- 5) Compatibility the isolate with other isolates previously registered in the National Center for Biotechnology Information (NCBI)

Source : <i>Fusarium solani</i> ; 18S ribosomal RNA gene					
	Accession	Country	Isolation Source	date of registration	Compatibility
	ID: PQ555253.1	IRAQ	plant	2024	99%
1.	ID: PQ432857.1	Egypt	Chickpea	2024	99%
2.	ID: FJ545374.1	Ghana	Theobroma cacao	2008	99%
3.	ID: MW073508.1	Greece	Fruit mesocarp	2020	99%
4.	ID: OR137122.1	Mexico	-----	2023	99%
5.	ID: KX379155.1	South Africa	-----	2016	99%
6.	ID: KT192216.1	China	leaf	2015	99%
7.	ID: OP758117.1	Viet Nam	-----	2022	99%
8.	ID: DQ094431.1	USA	human cornea	2005	99%
9.	ID: OL630915.1	Brazil	-----	2012	99%
10.	ID: LN714546.1	Netherlands	-----	2014	99%
11.	ID: PQ357319.1	South Korea	Seaweed	2024	99%
12.	ID: HQ651162.1	Kenya	Grevillea robusta	2010	99%
13.	ID: MT002830.1	Turkey	Juglans regia	2020	99%
14.	ID: KJ125735.1	Belgium	-----	2014	99%
15.	ID: JN589806.1	Ecuador	Sapium glandulosum	2011	99%
16.	ID: KU377518.1	Venezuela	Theobroma cacao	2015	99%
17.	ID: OM662233.1	Russia	potato tuber	2022	99%

The phylogenetic tree Fig. (4-4) conclude from the ribosomal DNA ITS sequences confirmed that the isolate, was classified into the clade closely related to *Fusarium solani* with the ITS sequence similarity of 100%. So the isolate was identified as *F. solani*.

Previous studies have demonstrated that using morphological features in the classification of fungi can provide correct results; nonetheless, many researchers do not used these characters because they require sufficient experience in the taxonomy, especially in the fungal

groups. They are fairly similar in that they both take a lot of time and attempt. and are inconsistent. because environmental variables affect the size, shape, and color of spores and fungal colonies This was pointed out by (Murugan *et al*,2020). The use of molecular techniques like as DNA sequencing of the ITS region has been extensively acknowledged and recommended as a first line diagnostic test for *F. solani* as mentioned by (Villarino *et al* ,2021).

4.3-Production and extraction of Taxol

Figure. (4-5) show the scheme of Taxol production by the endophytic fungus (*F. solani*) used two types of fermentation media (type1, type 2). Usually, the fungus *Fusarium*, an endophyte that grows on yew trees (*Taxus* sp) and produces taxol, has specific biological ties to its host that enable it to produce taxol. This species of yew grows mostly in damp, moist conditions and is found throughout the temperate Himalayan region, as well as in the hills of Meghalaya, Nagaland, and Manipur. The transfer of genes involved in taxol biosynthesis to endophytes harboring in various parts of yew trees may be explained by the endosymbiotic nature of several endophytic fungi, which may also inherit the ability to produce taxol. (Garyali,*et.al*.,2019). Also Subban and Kempken (2023) reported that Taxol is transferred from *Taxus* plants to endophytic fungi via horizontal gene transfer (HGT).

This can be explained by the fact that the genes that produce taxol are either naturally present in the *Fusarium* fungus or that the tomato plant produces it as a metabolic byproduct. Future studies are necessary to examine the facts behind this finding. And This study provides crucial information and a novel method for fermenting endophytic fungi to produce the significant medication taxol.

This results uncording with (El-Bialy and El-Bastawisy, 2020) which reported that The Northern Himalayan yew was chosen as the source for endophytic fungal isolation because, as of yet, no yew tree endophytes with the capacity to produce taxol have been identified.

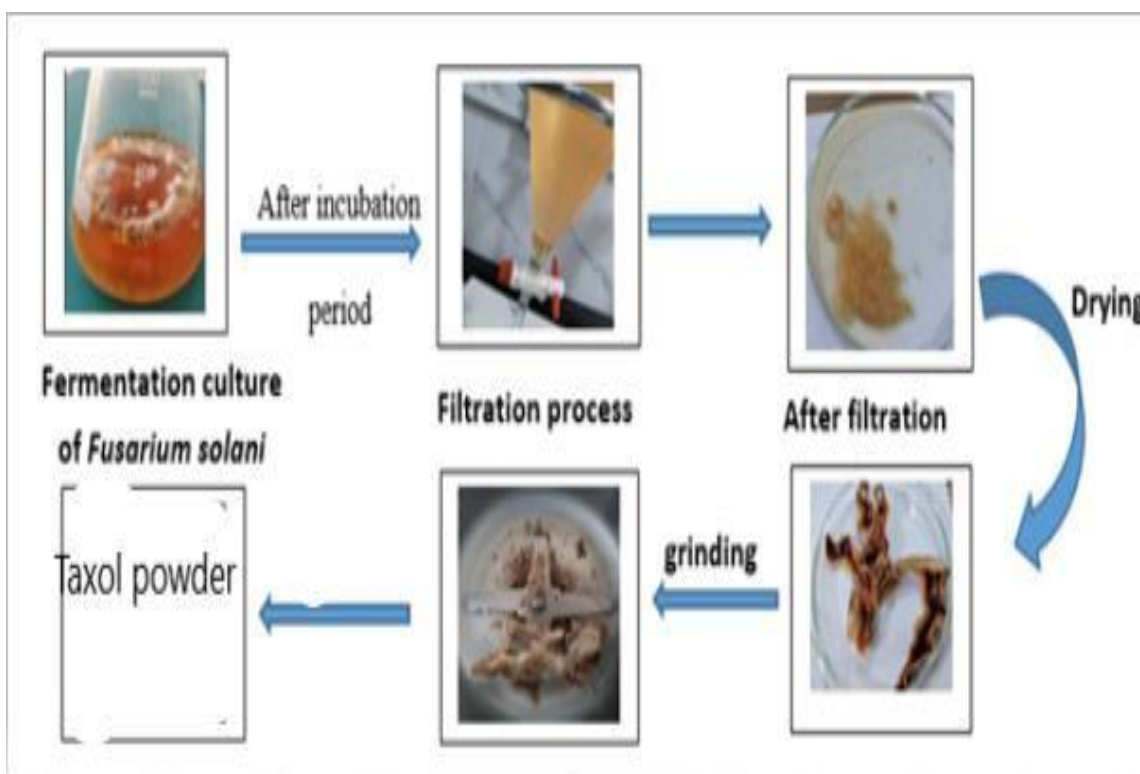


Figure. (4-5) the scheme of Taxol production by endophytic

4.3.1. Qualitative HPLC analysis of the generated taxol

The production of taxol by *F. solani* was demonstrated by qualitative HPLC analysis of the extracts, which displayed (Fig.4-6) relative retention times (10.724 min) and peak shapes that were exactly the same as those of the taxol reference substance. Remarkably, *F. Solani's* production of taxol was approved by the results of HPLC analyses, and HPLC also verified the purity of taxol (. Gou,*et.al.*,2015; Zhao,*et.al.*,2019;Xia,*et.al.*,2013; El-Sayed, *et.al.*,2015; El-Sayed, *et.al.*,2021). And by contrasting the fungal samples' peak areas with those of standard taxol, the taxol was validated. It concluded from our findings that the purified sample contained taxol.

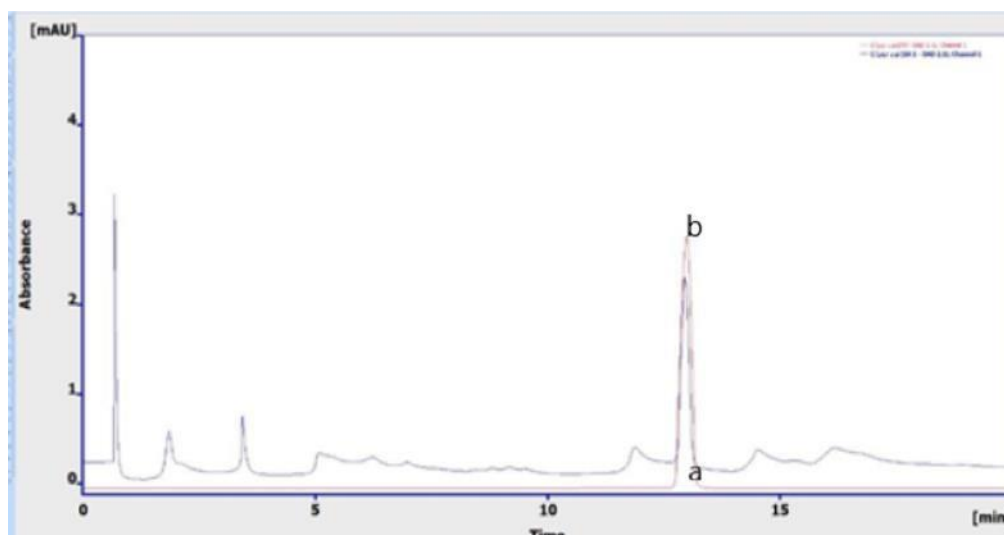


Figure.4-6 concentration of taxol determined using HPLC. (a) The reference Taxol's standard curve. (b) extracts of taxol from *F. solani*, Taxol's relative retention times (10.724 min)

The fungal taxol in this study was simple quantified with HPLC method due to the production was found to be higher (in μg). While in the previous reports, it was quantified with used of immunoassay since the yield level was recorded to be low (in ng) (Jiang *et al.*, 2024; Roshankhah *et al.*, 2020).

4.3.2. The effects of various media on *F. solani* taxol yields

Different media chosen for liquid fermentation affected the taxol yields of *F. solani*, according to quantitative HPLC assays (Table4-6, Figure (4-7)). The strain grown in the medium (type1) had the highest level of taxol ($66.483892 \mu\text{g/mL}$). The taxol yields of these reported fungi, however, 24 ng/L under ideal culture conditions ,(Garyali,*et.al.*,2018),, suggesting that the strain has a higher potential for taxol production. The presence of certain elicitors in medium (type1) may be the cause of the increased taxol yield. Xu *et.al* (2016) reported that the primary way that yeast extract contributes to the production of taxol by fungi is by supplying vital nutrients and growth factors that promote fungal growth and the

biosynthesis of taxol. In particular, yeast extract is a great source of vitamins, amino acids, and other nutrients that help support fungal metabolism and make it easier for the enzymes involved in the biosynthesis of taxol to be produced. Also, Obiedallah, *et.al.* (2024) find the effect of medium composition on the production of paclitaxel. To increase the yield of paclitaxel, researchers have employed a variety of unique and modified media. The production of taxol is significantly influenced by the induction of elicitors and inhibitors in the fermentation media. Pathways for elicitor signal transduction can increase taxol yield.

Table (4- 6) Taxol yields from *Fusarium solani* grown in two media

Type of medium	Taxol yield (μ g/ml)
Type(1)	66.483892
Type(2)	14.121202

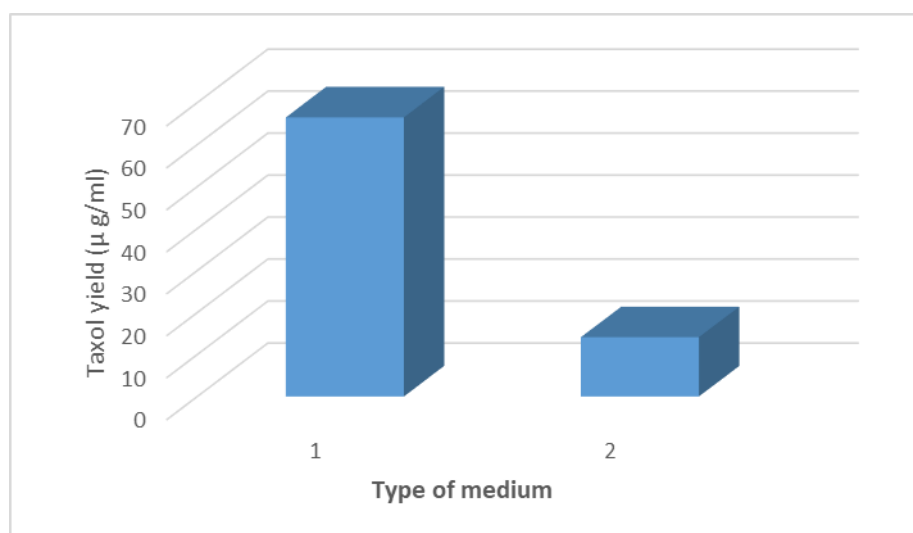


Figure 4-7- Assessment of taxol yields from *Fusarium solani* grown in two media

4.3.3. Characterize of extracted taxol 1- FTIR analysis

In Figure (4-8), the obtained taxol showed FTIR peaks. The broad hydroxyl (OH) and amide NH peak was observed at around 3403.70 cm^{-1} . In contrast, the ester group stretch and the aliphatic CH stretch were related

to the peaks at close to 2926.21 cm⁻¹ and 2855.21 cm⁻¹, respectively. The peak was attributed to keto groups (C=O, amide) at approximately 1657.28 cm⁻¹. The peaks near 1456.22 cm⁻¹ were attributed to the bending of aromatic CH₃. The peak frequency of COO⁻ stretching was nearly 1242.31 cm⁻¹. The -O- ester peaked at approximately 1155.83 cm⁻¹, and the aromatic CH bends were assigned a peak at approximately 1074.80 cm⁻¹. (Véléz,*et.al.*,2022). This results accordant with DAS, *et.al.*(2017).

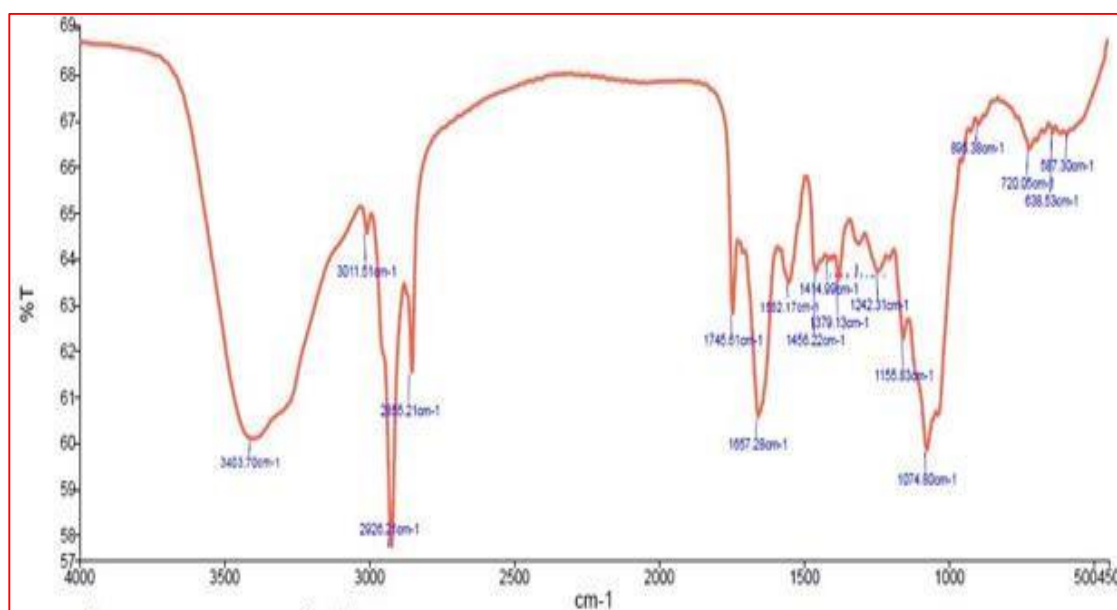


Figure (4-8) FTIR of extracted taxol

2- UV spectroscopy analysis

With a peak at 227 nm, the UV absorption spectrum of the fungal compound obtained from the strain resembled that of authentic taxol (Figure 4-9). It is evident that the maximum absorbance wavelength for taxanes' UV spectrum is 227 nm. This results accordant with (Pandy,*et.al.*,2023).

Trace amounts of different chemicals other than taxol can disrupt absorbance values in the UV spectrum, making weight estimates for taxol higher than the actual value. UV absorbance values for the test fungus (220–270 nm) were linked to the real taxol when compared to the standard

fungal taxol. The reported fungus was confirmed to produce taxol in the medium based on the results of the UV analyses, which was also evident in earlier publications (Suresh *et al.*, 2020; Abdel-Fatah *et al.*, 2022; Jagan *et al.*, 2021).

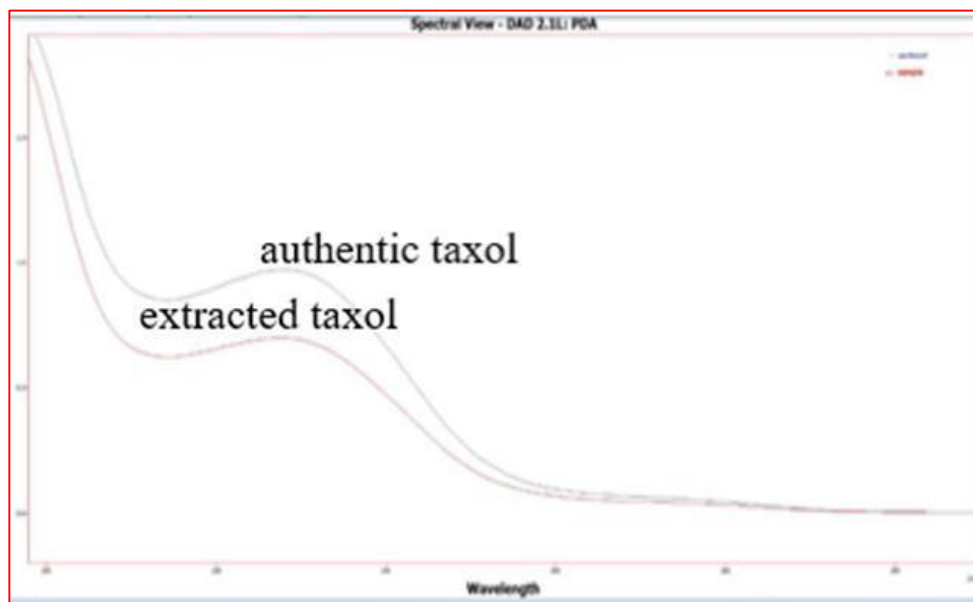


Figure (4-9) UV spectroscopy of extracted taxol

3- XRD analysis

XRD examination. Using Ni-filtered Cu K α radiation and an X-ray powder diffractometer, the taxol powder XRD patterns were produced.

X-ray diffraction patterns were acquired using the XRD-6000 series, which also included crystallinity computation and the overlaying of X-ray diffraction patterns to analyze crystallite size/lattice strain materials. Figure (4-10)

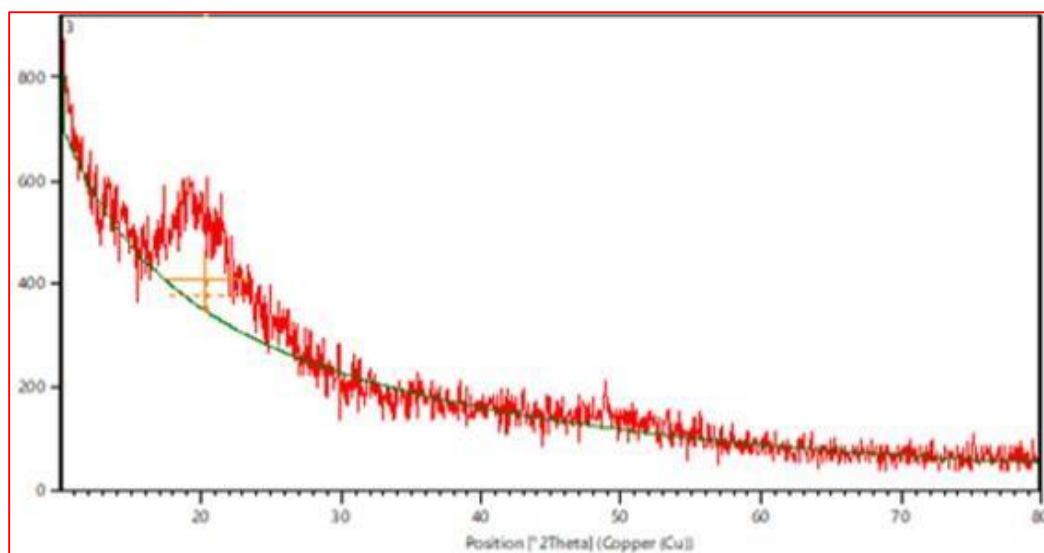


Figure (4-10) X-Ray Diffraction Analysis of Taxol

4- SEM analysis

Taxol's morphology and size have been examined using scanning electron microscopy (SEM). figure (4-11). which are 39.39 nm irregular lamelliform crystals. Due to the possibility that smaller particles can more easily enter cells, particle size may have an impact on how well cells absorb taxol. Smaller particles may interact with cells more readily than larger ones, which could have an impact on how taxol interacts with cells. The effectiveness of taxol in treatment may be enhanced by smaller particles. Reduce adverse effects: Taxol's adverse effects may be lessened by smaller particles. Taxol's morphology under a scanning electron microscope can take the form of crystals or nanoparticles, and its physical and chemical characteristics can change based on the preparation process. (Gallego-Jara,*et.al.*,2020).

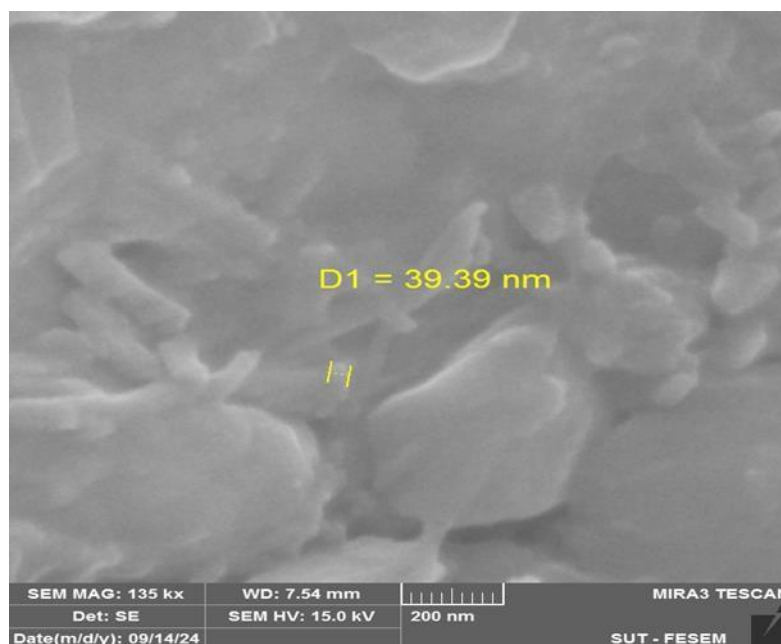


figure (4-11). SEM (scanning electron microscope), displaying the Taxol's surface morphology

The chemical structure of the taxol extracted was determined by instrumental analyses using UV, XRD, FTIR, and SEM. It was confirmed that this fungal taxol substance demonstrated a promising source for the natural bioactive. According to studies on the isolation of taxanes produced by *Fusarium* spp., the fungus produces taxanes, such as Taxol, in vitro in the culture broth. HPLC and additional analyses of the culture broth extracts support this finding. To the best of our knowledge, this is the first report of Taxol being isolated from the tomato (*Solanum lycopersicum*) plant by *Fusarium* sp.

A major category of microorganisms, taxus-associated fungi were recognized for producing secondary metabolites that were effective against tumors and pathogens. (Han,*et.al.*,2020). At present, taxol producing endophytic fungi that have been isolated are mainly related to *T. andreanae*, *P. microspora*, *S. nepalense*, and *N. sylviforme* (Zaiyou,*et.al.*,2021). Currently, *T. andreanae*, *P. microspora*, *S. nepalense*, and *N. sylviforme* are the primary taxol producing endophytic fungi that

have been isolated (Xiong,*et.al.*,2018). But as of right now, no endophytic *Fusarium* sp. that produces taxol has been identified. We identified *F. solani*, a novel taxol-producing endophytic fungus, from *Solanum lycopersicum*. The increased yield of taxol indicates that more thought should be given to various hosts with particular conditions in order to isolate taxol-producing fungal species.

4.3.4. Biological activities of extracted Taxol.

4.3. 4..1. Anticancer activity of Taxol (Cytotoxic Effect of Taxol)

The cytotoxic bioassay, which is frequently used to identify cytotoxic effects, is thought to be a helpful method for estimating an average lethal dose, or LC50, and for the initial evaluation of overall toxicity (Li *et al.*, 2015). The antitumoral effect of taxol, which was isolated from *Fusarium solani*, was assessed against MCF-7 cells for 24 hours using an MTT assay, given the significance of using compounds extracted from natural sources as alternative therapies for treating a variety of diseases, including cancer. Figure (4-12) show, HdFn has an IC₅₀ value of 118.0, meaning that this concentration reduces cell viability by 50%. While MCF7 has a lower IC₅₀ value of 91.75, indicating that it is more sensitive to the tested taxol compared to HdFn.

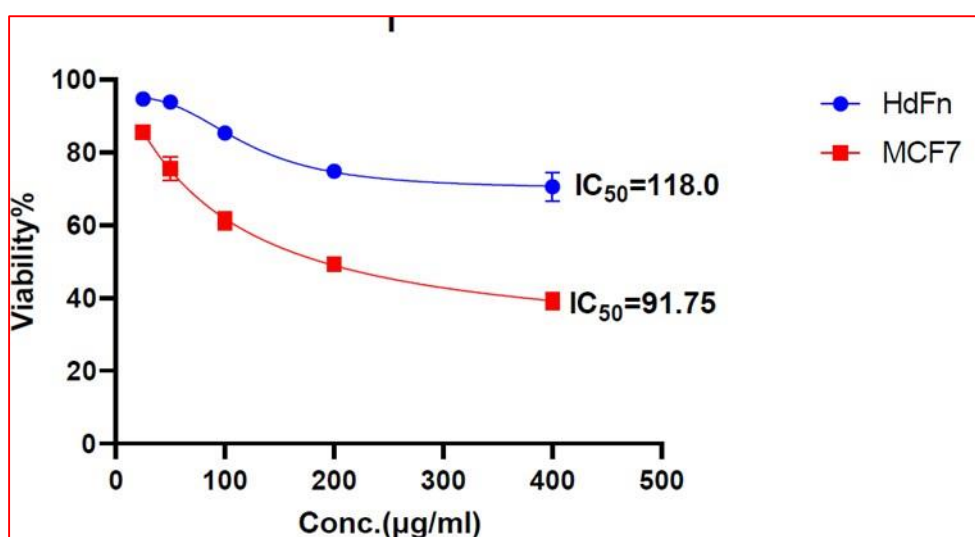


Figure (4-12) the effect of different concentrations of a taxol (measured in $\mu\text{g/ml}$) on cell viability, indicated in percentage.

Table (4-7), Figure (4-13) show Purified Taxol's effectiveness against cancerous cell lines in different concentrations.

Table (4-7) Efficacy of purified Taxol on cancerous cell lines

Conc.($\mu\text{g/ml}$)	HdFn		MCF-7	
	mean	SD	mean	SD
400	70.56333	3.884527	39.12033	2.23531
200	74.807	0.868624	49.344	1.075329
100	85.26233	1.853211	61.227	2.438719
50	93.78867	1.124029	75.50167	3.245626
25	94.63733	0.481986	85.571	1.446894

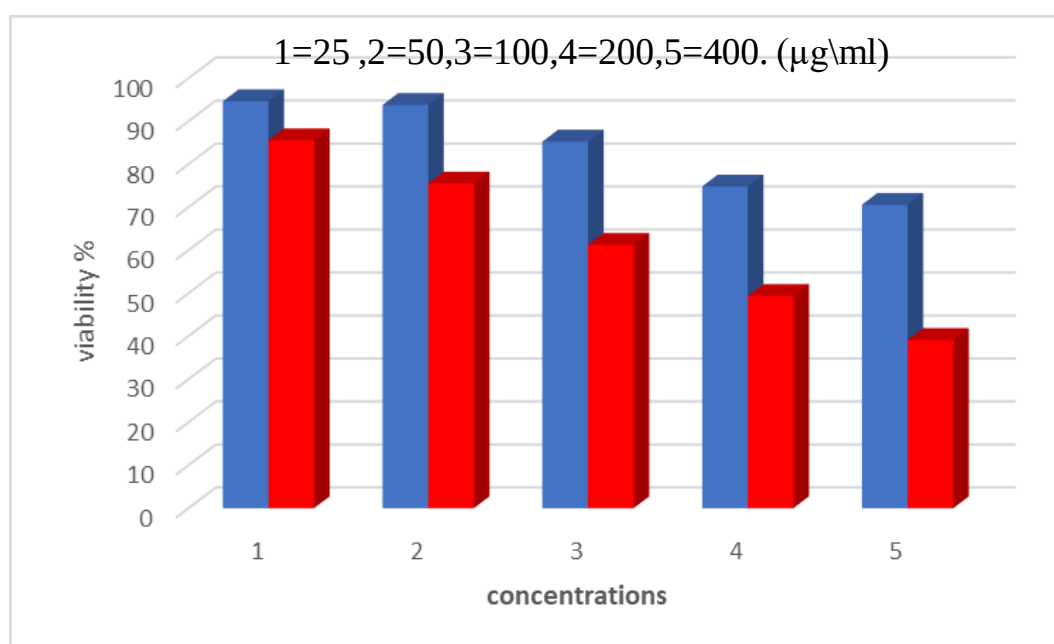
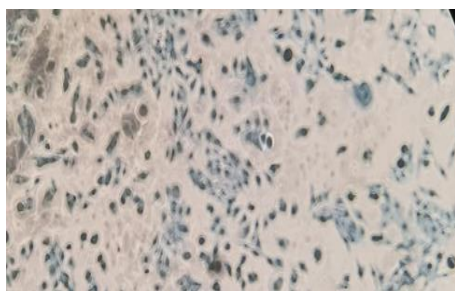


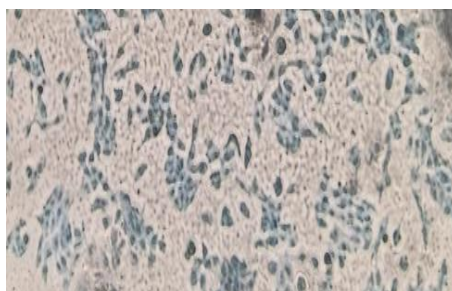
Figure (4-13) Purified Taxol's effectiveness against cancerous cell lines in different concentrations

After being cultivated on a slide for 72 hours, an untreated breast cancer cell line appears as a monolayer. The cytoplasm is stained violet, while the nucleus is dark, giving the appearance of elongated cells with ovoid nuclei. However, microscopic analysis of taxol-treated breast cancer cells revealed cell culture dissociation, cell separation, and the emergence of sizable cell-free regions. Figure 4-14.

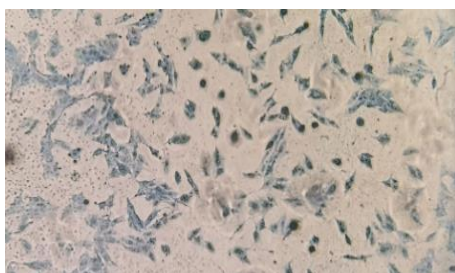
Both cell types show a decrease in viability as the concentration of the substance increases. MCF7 cells show a more pronounced decline in viability at lower concentrations than HdFn cells, suggesting that MCF7 cells are affected more readily by the substance. Pandey, *et.al.* (2023) investigated the cytotoxic effects of fungal taxol against the human liver cancer cell line HepG2 using the MTT assay, and the results showed After 24 hours of treatment, there was a noticeable drop in cell viability (0.05–0.5 μM), and the IC50 dosage was determined to be 0.25 μM .



Cell culture without treatment



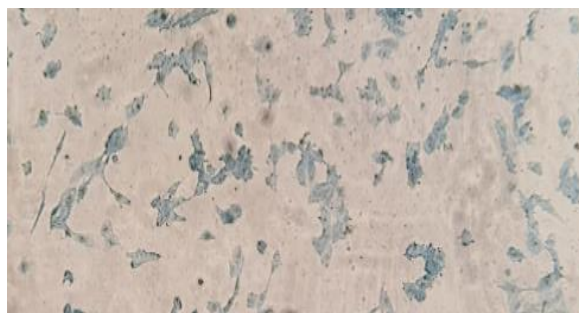
Cell culture with treatment (50mg/ml)



Cell culture with treatment (100mg/ml)



Cell culture with treatment (200mg/ml)



Cell culture with treatment (400 mg/ml)

Figure (4-14). Effect of taxol-treated breast cancer cells

The taxanes family, which includes Paclitaxel (PTX) and Docetaxel (DTX), generally interfere with microtubule growth, which inhibits cell division and causes cancer cells to undergo apoptosis or programmed cell death. Taxol causes cell death during the G2/M phase of the cell cycle by inhibiting microtubules. (Lim,*et.al.*,2022). Taxol is frequently used to treat a variety of cancers, including lung, ovarian, and breast cancers, because it has an anti-mitotic effect on cancerous cells. In a nutshell, Taxol's main mechanism is its capacity to stabilize and stop microtubules from depolymerizing, which results in cell death and cell cycle arrest at the G2/M phase. Nevertheless, this effect might depend on dose and cell lines.

In addition to controlling mitosis, taxol also affects cell survival by influencing the Bcl-2 family, apoptotic-regulating proteins, and other signaling pathways like the NF κ B and JNK/SAPK pathways. To induce cell death in cancer cells, taxol either activate or inhibit the pro-apoptotic (BAX/BAK) or anti-apoptotic (Bcl-2/MCL-1) Bcl-2 proteins. Taxol may also attack and release calcium that is stored in the endoplasmic reticulum, disrupting calcium homeostasis and making cell survival more difficult. It was discovered that taxol had an impact on immune cells, including macrophages and regulatory T cells (Tregs). Because of its LPS-mimetic properties, taxol can trigger apoptosis by activating signaling pathways that are dependent on the Toll-like receptor 4 (TLR4). Moreover, taxol may change the polarity of macrophages from the pro-tumor (M2) to the anti-tumor (M1) phenotype, enhancing the immune system's ability to eliminate cancer cells. (Lim,*et.al.*,2022; - Gallego-Jara,*et.al.*,2020).

Numerous fungal species have been utilized as natural sources of anti-cancer compounds. Of these, *Aspergillus* and *Penicillium* species have drawn a lot of interest recently due to their capacity to produce a number of secondary metabolic products with anticancer properties against a range of cancer cells, including HeLa cells, breast cancer cells, hepatocellular carcinoma, and other types (Deshmukh,*et.al.*,2018; El-Gendy,*et.al.*,2021; Kareem and Jasim,2024).

Also The cytotoxic and anticancer efficacy of *A. terreus* taxol was additionally determined by Nabila (Zein,*et.al.*,2019) in comparison to commercial *T. brevifolia* taxol, highlighting the fungal source as an interesting platform for commercial manufacturing of taxol.

4.3. 4..2. Antioxidant activity of extracted Taxol

An imbalance between ROS and the cell's antioxidant capacity is known as oxidative stress. (Campos,*et.al.*,2014). In contrast to the commercial taxol that used in cancer treatment and used by Uzma,

(Faridi,*et.al.*,2017) fungal taxol, especially the taxol extracted from *Fusarium* in this study, showed antioxidant activity by reducing free radicals. As seen in Figure (4-15), table (4-8), the DPPH assay exhibits a notable activity to eliminate free radicals and for two hours of reaction, the mean of three repetitions. This capability is crucial and a significant benefit to this material, which makes it useful in medicine. This finding referred that natural taxol that isolated from microorganisms especially fungi have antioxidant activity and it accordant with (Das,*et.al.*,2023).

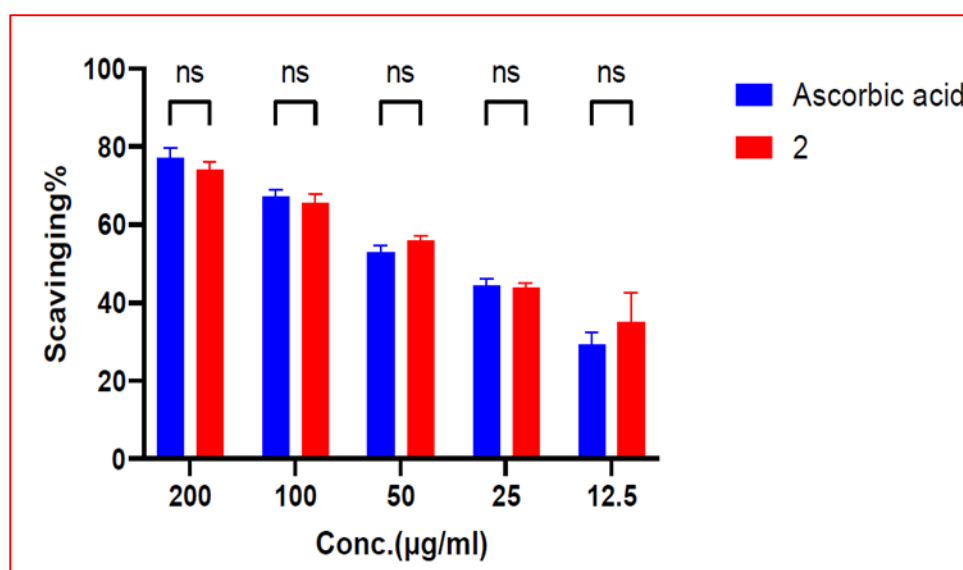


Figure (4-15), Effect of Taxol on Scavenging Activity 2- Extracted Taxol

Table (4-8) Scavenging Activity of taxol

conc(µg/ml)	Ascorbic acid		Extracted Taxol	
	mean	SD	mean	SD
200	77.23767	2.400792	74.11267	2.022381
100	67.20667	1.793807	65.625	2.363498
50	52.93233	1.771683	55.82533	1.380876
25	44.29033	1.801796	43.98133	0.988588
12.5	29.205	3.119173	34.954	7.592459

4.3. 4.3. Antibacterial of extracted Taxol

The antibacterial activity of pure fungal taxol against bacterial pathogens was evaluated using the agar well diffusion method. Figure (4-16), (4-17), appear effect of extracted crud taxol on two types of bacteria (G^+ , G^-), results show the significant effect of taxol on *S. aureus* (G^+). more than *E. coli* (G^-)

Perhaps this is to many natural antimicrobial substances, such as certain metabolites of fungi, have a tendency to be more effective against Gram-positive bacteria than Gram-negative ones. In antimicrobial research, this is a typical pattern that Taxol occasionally follows. The two bacterial types' different cell envelope structures are the main cause of this effect.

Taxol, which has been isolated from a variety of fungal species, particularly endophytic fungi, has been shown in numerous studies to have antibacterial properties such as Pandey, *et.al.*(2023) which discover that the fungal taxol have antibiotic-like effects on *S. aureus*, *E. coli*, and *S. typhi*. According to Gauchan *et al.* (2020), the antimicrobial activity of fungal taxol was assessed against two gram-positive bacterial strains, *Bacillus subtilis* (ATCC6633) and *Staphylococcus aureus* (ATCC12600), as well as a gram-negative bacterial strain, *Escherichia coli* (ATCC 25922).

Compared to its well-established mechanism in eukaryotic cancer cells, the mechanism of Taxol's effect on bacteria is still being studied and is not as well understood. The main cause of this is that bacteria do not have the intricate microtubule cytoskeleton that eukaryotic cells' Taxol targets. the mechanisms that have been suggested and examined (Busiek,*et.al.*,2015; Ortiz,*et.al.*,2016):

1 -FtsZ Interaction (The Leading Hypothesis): Bacterial "Tubulin": FtsZ (Filamenting temperature-sensitive mutant Z) is a protein found in bacteria that is thought to be a structural and functional homolog of

eukaryotic tubulin. An essential part of the bacterial cell division apparatus is FtsZ.

Given that FtsZ and tubulin share structural similarities, it is hypothesized that Taxol may interact with FtsZ in a manner akin to that of tubulin. In the end, this interaction prevents the growth and proliferation of bacteria.

-Role in Cell Division: FtsZ polymerizes to create the Z-ring, a ring-like structure, at the mid-cell during bacterial cell division. The mother cell eventually divides into two daughter cells as a result of this Z-ring constricting and a septum forming.

Although more conclusive proof and thorough mechanistic understanding are still required, some research has investigated the direct interaction between Taxol and FtsZ. If verified, focusing on FtsZ is a very alluring approach to creating novel antibiotics because it is crucial for bacterial survival and is mostly lacking in human cells, which may lessen off-target toxicity.

2- Membrane Disruption: The molecule taxol is comparatively lipophilic, or fond of fat. It can interact with and possibly insert itself into biological membranes, like bacterial cell membranes, due to this property. Increased permeability could result from such interaction, which would compromise the integrity of the bacterial cell membrane.

leakage of vital intracellular elements, such as proteins, ions, ATP, and nucleic acids. membrane potential loss .and Cell death may eventually result from this disturbance.

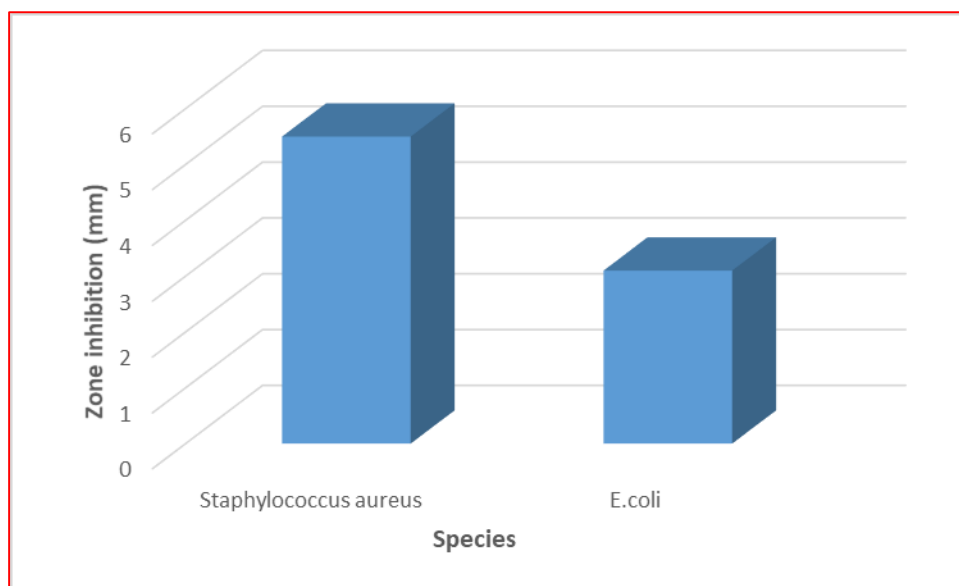


Figure (4-16), Effect of crude fungal taxol on isolated bacteria



Staphylococcus aureus



E. coli

Figure (4-17) Inhibition effect of crude fungal taxol

Because we must present scientific facts in order to maintain the integrity of science. Since the current study looked at the effects of different dilutions of the taxol substance that was extracted from the fungus, it should be noted that no apparent inhibition was observed (Figure 4-18). This might be because, in addition to the bacteria's natural defense mechanisms, which are more effective when dealing with low concentrations of the compound, the concentration did not reach a

concentration high enough to cause damage that the bacteria could not repair or overcome.



Figure (4-18).effect of different concentration of extracted Taxol on bacteria spp.

Conclusions & Recommendation

Conclusions

- 1- The most common cancer diagnosed in women is breast cancer.
- 2- Compared to other patients, cancer patients have higher risk factors for bacterial infections.
- 3- The most prevalent pathogens were *Staphylococcus* species especially *S. aureus*.in cancer patients.
- 4- The existence of specific bacteria in cancer patients' blood samples is a risky sign that could endanger their lives.
- 5- The Endophytic fungus *F. solani* that isolated from tomato plant can conceder as good source of nature source of Taxol with high yield.
- 6- The medium with the highest concentration of taxol is the one that contains yeast extract.
- 7- fungal Taxol that extract from *Fusarium solani*., has anti-cancer properties, especially against breast cancer.
- 8- Crude Taxol more effect on bacterial strains than any concentration of it.
- 9- Taxol effect on positive gram more than negative gram.
- 10- Natural taxol that isolated from microorganisms especially fungi have antioxidant activity

Recommendation

- 1- Patients with cancer more susceptible to bacterial infections.
- 2- Because cancer is immunosuppressive, secondary bacterial infections significantly affect the overall prognosis
- 3- *F. solani* conceder as natural friendly source of bioactive Taxol.
- 4- Searching for other sources other than the Taxus plant to produce Taxol
- 5- Identifying the genes involved in the Taxol biosynthesis pathway by utilizing precursors, elicitors
- 6-. To get the most taxol out of this fungus, more research is needed to optimize the culture conditions.
- 7- The high growth rate and short generation time of the fungi will make further research on the taxol isolated from *F. solani* worthwhile.
- 8- Fungal taxol may be used as an anticancer medication following additional research on lab animals. (in vivo)
- 9- Conducting extensive research on the stability of fungal taxol.
- 10- Based on the references that are currently available, this study is the first to isolate Taxol in Iraq. It also demonstrates that endophytic fungi that grow on plants other than yew trees can be used to produce taxol.

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جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة القادسية كلية العلوم
قسم علوم الحياة

نشاط التاكسول للفيوزاريوم كمضاد للبكتيريا مضاد للأكسدة ومضاد للسرطان

أطروحة مقدمة الى

مجلس كلية العلوم / جامعة القادسية كجزء من متطلبات نيل درجة دكتوراه فلسفة في علوم
الحياة / الاحياء المجهرية

من قبل

عبدالله حسن جاسم

ماجستير في علوم الحياة / الاحياء المجهرية / كلية العلوم للنبات / جامعة بابل 2021

بإشراف

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