Bioactive potential of endophytic fungi isolated from *Eucalyptus globulus*

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IN

BIOTECHNOLOGY

By

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CERTIFICATE

I hereby certify that the work that has been presented in this thesis **"Bioactive potential of endophytic fungi isolated from *Eucalyptus globulus*"** submitted by **Mr. Sunny Yadav** in the partial fulfillment of the requirement for the award of the degree of Master of Technology in Biotechnology, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, is a record of a student's own work carried out under my supervision and guidance. This report has not been submitted for the award of any other degree in this institute or any other institute.

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DECLARATION

I, hereby declare that the work presented in this thesis entitled "**Bioactive potential of endophytic fungi isolated from *Eucalyptus globulus***" for the award of degree of Master of Technology in Biotechnology is an authentic record of my work. The work has been performed under the supervision of Dr. M. Vasundhara, assistant professor, Department of Biotechnology at Thapar Institute of Engineering and Technology (Patiala). This is my own original research work done during the period August 2022 to July 2023. This report has not been submitted for the award of any degree or certificate in this institute or any other institute.

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Dated:

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ABBREVIATIONS

Symbols	Abbreviations
%	Percentage
C	Degree Celsius
L	Litre
ml	Millilitre
mg	Milligram
µg	Microgram
O. D	Optical Density
MHB	Muller Hinton Broth
MHA	Muller Hinton Agar
PDB	Potato Dextrose Broth
PDA	Potato Dextrose Agar
w/v	Weight by Volume
pH	Potential of hydrogen ion
Temp	Temperature
Rpm	Rotation per Minute
H_2SO_4	Sulfuric Acid
MIC	Minimum Inhibitory Concentration
EtBr	Ethidium Bromide
DNA	Deoxyribose Nucleic Acid
spp.	Species
EDTA	Ethylene diamine tetra acetic acid
TLC	Thin Layer Chromatography
Rf	Retention factor
DPPH	2,2-diphenyl-1-picryl-hydrazyl- hydrate

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ABSTRACT

This study aimed to isolate and characterize antimicrobial compounds from *Eucalyptus globulus*, a widely known medicinal plant with potential therapeutic properties. The plant material was collected, dried, and subjected to solvent extraction using a non-polar organic solvent ethyl acetate. The crude extract obtained was then fractionated using column chromatography, and the resulting fractions were tested for their antimicrobial activities. The bioactive fraction was further purified using various chromatographic techniques, such as thin-layer chromatography (TLC) and Column chromatography.

A total of 8 fraction from extract ES1 and 13 fraction from extract ES2 were obtained. Pooling of fraction on the basis of TLC was performed. The antimicrobial of the isolated compounds was evaluated against a panel of clinically relevant bacterial strains using standard antimicrobial assays. The results revealed potent inhibitory effects of the isolated compounds against both Gram-positive and Gram-negative bacteria.

The characterization of the isolated compounds using phytochemical analysis confirmed their chemical structures and provided valuable insights into their properties. The identified compounds belong to different chemical classes, such as terpenoids, phenolics, or alkaloids, known for their antimicrobial properties. These findings highlight the potential of *Eucalyptus globulus* as a valuable source of bioactive compounds with significant antimicrobial activities.

In conclusion, in this study we successfully isolated and characterized antimicrobial compounds from *Eucalyptus globulus*. The identified compounds exhibited promising activities against a range ofbacterial pathogens. Further studies are warranted to elucidate the mechanisms of action and potential therapeutic applications of these compounds, with the ultimate goal of developing novel antimicrobial agents for clinical use.

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Chapter-1

INTRODUCTION

Plants that are identified as "medicinal plants" are those whose parts contain substances that can be employed as either therapeutic agents or as a starting point for the synthesis of efficient drugs. Since the very beginning of time, plants with medicinal properties have been used for a variety of healing and curative purposes (Tlau, L., & Lalawmpuii, L. 2020).

One of the earliest scriptures, the Rig Veda, describes the various ways that plants were used in the Indian subcontinent for many different purposes, including healing, treatment, and recovery. Earlier studies concentrated on the bioactive compounds in medicinal plants (Petrovska *B.B., 2012*). but today's studies are focusing on the microorganisms that live inside the plants and how they interact with them to provide support to the plants (Delaux, P. M., & Schornack, S. 2021). There has been a lot of study on various medicinal plants and how to use them, but the microbiome as a whole is still being researched.

The endophytes are a particular and significant source of research and relevance to scientists among the microorganisms. Inside the plants, a group of microorganisms known as endophytes live without causing any harm to the plants. These microbes have been the subject of in-depth research in recent years, and it has been determined that they are crucial to plant growth and disease defense. It has been shown that endophytes have significant potential for use in the fields of biotechnology, medicine, and agriculture. They can be found in practically all types of plants, including those used in different fields. One of the many fascinating things in the world are microorganisms. Among the most important and frequently discussed topics among scientists are endophytes. In areas with substantial plant variety, such as tropical rainforests, the greatest diversity of endophytic organisms with novel and diverse chemical metabolites may be identified. There may be a million endophytic fungi in the world, according to estimates. Microbial endophytes are present in all recognized plant species (Nisa *et al*.,2015)

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The ability of endophytes to enter and thrive in plant tissues illustrates their uniqueness and reveals their relationships with the host plant (Khare et al., 2018). There is proof that endophytes can have an impact on several important host plants. Endophytes are microscopic organisms that live inside plants, according to themost fundamental and widely recognized understanding of them. In 1866, De Bary identified microbial cells in the tissues of plant specimens that were being studied under a microscope. He also gave the first official definition of the word "endophyte" in 1866, stating that "any organism that grows within plant tissues can be referred to as an endophyte. Endophytes are fungi that exist inside and are asymptomatic for at least a portion of their existence. He also talked about the endophyte's symptomless habitation of plant tissue and its mutualistic interdependence with its host. Endophytes are plant-associated organisms that colonize and interact with the internal tissues of their host plants, making them beneficial for agriculture as a method to improve crop performance (Azevedo *et al*.,2000).

Endophytes can also colonize dead and hollow hyaline cells of plants, according to Hata and Sone (2008), Specian et al., (2012), and Stpniewska and Kuzniar (2013). Additionally, they can develop on a plant's stem, roots, petioles, leaf segments, weeds' inflorescences, fruit, buds, and seeds. Dudeja and Giri (2018) assert that a plant species' population of endophytes is very variable and influenced by a variety of variables, including the species of the host, host stage of development, inoculum population density, and the surrounding conditions.

Researchers have shown that endophytes, or microorganisms that dwell in the intercellular gaps of plant tissues. These endophytes might be creating bioactive compounds with potential medical uses. Endophytes are currently the focus of an international scientific effort to isolate and investigate their natural compounds. According to a recent thorough study, only 38% of novel chemicals from soil microflora were previously known, compared to 51% of physiologically active molecules recovered from endophytic fungi (Schulz *et al*.,2002).

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It's possible that endophytic interactions started to develop hundreds of millions of years ago, when higher plants first started to grow on earth. The fossilized tissues of stems and leaves contain traces of microorganisms that are connected to plants. It's possible that these endophytic microorganisms have created genetic systems that enable information to be transferred from them to higher plants and vice versa (Rizzo *et al.*, 2013).

Furthermore, it's possible that microbes that are connected with plants have evolved metabolic pathways that produce plant growth hormones. Last but not least, the endophytic microorganisms may have evolved independently, enabling them to better adapt to a plant host and possibly progressing to the point where they could contribute to the link by carrying out functions like disease, insect, and grazing animal defense.

Numerous natural products produced by microorganisms have a wide range of uses, including medications, agriculture, biofuel, antimicrobials, ecological adaptation, and environmental remediation. Endophytes are a broad group of microbial symbionts that live in plants and are known to create bioactive secondary metabolites (Bano et al., 2016). These secondary metabolites mainly cause certain phenotypic functions in the host plant and/or endophyte, such as crosstalk with allied organisms, chemical warfare or defense, and stress adaptation. One of the possible mechanisms for this is hypothesized to be horizontal gene transfer between the host plant and the endophytic microbial partner. Some endophytes are known to produce secondary metabolites that mimic the metabolites of the host plant. One well-known example of endophytes producing secondary metabolites that mimic the host plant is the case of Epichloë endophytes in grasses. Epichloë is a group of fungal endophytes that live in symbiosis with grasses, such as perennial ryegrass and fescue. The endophytes produce alkaloids, such as ergovaline and lolitrem B, which are toxic to herbivores. These alkaloids can help the host plant by deterring herbivores from feeding on it. Interestingly, some of these alkaloids are structurally similar to alkaloids produced by the host plant itself. This mimicry helps the endophyte hide among the plant's natural chemistry and potentially evade the plant's defense mechanisms that might otherwise recognize and cause harm (Simpson *et al.*,2014).

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Recent years have seen a rise in the demand for bioactive chemicals in pharmaceuticals and naturopathy due to their numerous health advantages for people and plants. Microorganisms produce bioactive substances on their own or in collaboration with plants. Endophytes are microorganisms that live inside the living tissues of plants and are symbiotically or mutualistically associated with the host plant. Despite having no immediate or obvious negative consequences, endophytes create a variety of bioactive chemicals.

Nearly 75% of the plants analyzed to date contain endophytes (Griffin, E. A., & Carson, W. P. 2018). Endophytes are a treasure trove of organically produced, environmentally friendly products for industrial, medical, and agricultural uses with the least detrimental effects on the environment. Endophytic microorganisms with enormous biotechnological potential are being actively sought after through bioprospecting due to their rich biodiversity and capacity to biosynthesize bioactive compounds (Puspita *et al.*,2020) to give people a better healthcare system, there is a need for new, better, and more beneficial substances.

The problems of feeding and managing the natural resources grow in light of this projection. Effective and sustainable agricultural methods must be used to increase productivity and meet the growing population's basic nutritional needs. Chemical fertilizers and pesticides are crucial to agricultural output on a large scale because they help plants better absorb nutrients and control pests and pathogens. These intensive farming methods, however, are expensive, unsustainable, and dangerous for the environment. Therefore, the pursuit of global food security requires the search for eco-friendly and sustainable alternatives. Plant microbiomes can serve as such substitutes because they significantly contribute to the stimulation of plant growth without having a negative impact on the environment. Endophytic microbiomes are thought to be more significant because of their direct and effective influence on plant growth (Strobel, G. 2018). Endophytes stimulate plant growth and development by producing phytohormones, fixing nitrogen, producing siderophores, and solubilizing phosphate, zinc, and potassium. They also increase the photosynthetic efficiency of their host plants. They also give resistance to a variety of abiotic variables such as drought, salinity, temperature extremes, reactive oxygen species, and soil

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pollutants while increasing the tolerance of plants to biotic factors such as diseases, pests, and nematodes

Significance of the project

It has been demonstrated through studies that endophytic fungi found in Eucalyptus species produce secondary metabolites that have biological activity in the treatment and overall benefits. More research is needed to examine the utilization of these secondary metabolites against different disease-causing microorganisms because only a small number of endophytes have been examined for their therapeutic potential. This gives a large field of scope for this project.

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OBJECTIVES

1. Isolation of endophytic fungi from *Eucalyptus globulus*.
2. Extraction, and purification of secondary metabolites from endophytic fungi.
3. Evaluation of fungal extracts for Bioactive potential.

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Chapter- 2

Review of literature

2.1 Medicinal plant: History and development in recent years

Since ancient times, people have used medicinal herbs, and today's research has acknowledged their active effects and included them in modern pharmacology. There is historical evidence from written records, preserved monuments, and original plant remedies that links man to his hunt for pharmaceuticals in nature (Phillipson, J. D. 2001). The knowledge of using medicinal plants comes from man's long history of battles with illness, which taught him to look for medications in the barks, seeds, fruit bodies, and other parts of plants. Modern pharmacotherapy currently includes a variety of medications with plant origins due to the active effects that modern science has recognized. Physicians and chemists are better equipped to address issues that have arisen as a result of the expansion of professional services thanks to the information obtained from the development of ideas connected to the use of medicinal plants.

Eucalyptus has a long history of medicinal use by indigenous Australian Aboriginal communities, which have utilized various parts of the plant for their therapeutic properties for thousands of years (Packer et al.,2012). The traditional medicinal uses of eucalyptus were later adopted and popularized by European settlers in Australia. Eucalyptus leaves were commonly used in traditional medicine to treat respiratory ailments such as coughs, colds, and sinus congestion (Sultana *et al.*,2016). The leaves were often brewed as a tea or infused in hot water for inhalation, providing relief from respiratory discomfort (Armutcu, F., & Kucukbayrak, A. 2021). The essential oil extracted from eucalyptus leaves is known for its antiseptic and antimicrobial properties. It was applied topically to wounds, cuts, and burns to help prevent infections and promote healing. Eucalyptus leaves were used to help reduce fever and alleviate inflammation. The leaves were sometimes crushed and applied externally as a poultice

or added to baths to provide relief. Eucalyptus oil was used as a topical analgesic to alleviate muscle and joint pain. It was often massaged into sore areas to provide comfort and reduce pain. The strong aroma of eucalyptus was used to ward off insects and pests. Eucalyptus leaves were sometimes placed in bedding or around living spaces to deter insects (Batish et al., 2008).

Table 1: List of plant part used for different purpose of Eucalyptus species

Plant part	**Traditional Uses**	**References**
Leaves	Respiratory remedies Antiseptic and antimicrobial properties Fever and inflammation Pain relief Oral health benefits Insect repellent	(Sebei *et al.*, 2015)
Essential oil	Respiratory remedies Pain relief Antiseptic and antimicrobial properties Aromatherapy	(Gilles *et al.*, 2010)
Bark	Antiseptic and antimicrobial properties Wound healing and dressing	(Wesolowski *et al.*,2014)
Gum	Chewing gum for oral health As an adhesive in traditional practices	(Martins *et al.*,2020)
Flowers	Nectar sources for bees and other insects Ornamental use in floral arrangements.	(Morrant *et al.*,2010)
Wood	Timber for construction and furniture Essential oil extraction	(Pangh et al.,2019)

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The traditional uses of eucalyptus described in table 1 are based on historical and cultural practices of indigenous Australian groups, and they might not fully cover all of their modern applications. These are just afew of the many Indian medicinal plants that are utilized in traditional medicine to treat a variety of human illnesses and health issues. While traditional methods have been appreciated for ages, it's crucial to remember that modern study is still exploring and validating the medicinal properties of these plants. The table 2 shown below represent shows list of plants used for medicinal purposes.

Table 2: The below lists include Indian medicinal plants used to treat various kind of human diseases

	Botanical name	Common Name	Part used
1.	*Abutilon indicum*	Monkey bush	The plant is used to treat rheumatoid arthritis and erectile dysfunction.
2.	*Bacopa monnieri*	Brahmi	The leaves are used to treat nerve illnesses like epilepsy and anxiety.
3.	*Bombax ceiba*	Red-silk cotton	The plant's bark has ulcer-healing, expectorant, tonic, and demulcent properties.
4.	*Chlorophytum laxum*	Siam lily	Root is used for diarrhoea and dysentery are both treated using the bark.
5.	*Holoptelea integrifolia*	Indian elm	Stem and bark Used in inflammation and treatment of skin diseases.

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6.	*Justicia zeylanica*	Malabar nut	Leaf used for bronchitis, asthma, and microbial infections.
7.	*Saraca asoca*	Ashok tree	Bark is used to treat intestinal issues, acne, weakness, menorrhagia, uterine sedation, and bleeding.
8.	*Tamarindus Indica*	Tamarind	Whole plant used to treat diarrhea, pustules, ulcers, asthma, and amenorrhea with this plant.

(Anand *et al.*,2019)

2.2 Endophytes as a source of bioactive molecules

Endophytes, which are microorganisms that live unharmed in a plant's internal tissues, have drawn a lot of interest as a potential source of bioactive compounds with a variety of pharmacological actions. Numerous studies have documented the incredible variety of bioactive chemicals that endophytes produce. Alkaloids, terpenoids, phenolics, flavonoids, and peptides are some of these substances. Numerous of these substances have effects that are antibacterial, anticancer, antioxidant, anti-inflammatory, and immunomodulatory (Anand *et al.*,2023).

Endophytes have been shown to produce novel compounds that are not found in the host plant or other conventional sources. These novel compounds are formed as a result of the plant's particular biochemical pathways and environmental interactions. Numerous endophytic extracts and substances have demonstrated strong antibacterial action against different pathogens, such as bacteria, fungi, and viruses (Manganyi et al., 2020). These results point to the possibility of endophytes as a source for fresh antibacterial substances. In preclinical trials, endophytic substances showed a strong anticancer effect. A viable contender for further research is a chemical that exhibits selective cytotoxicity against cancer cells while protecting normal cells.

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Many endophytic substances contain antioxidant and anti-inflammatory properties, which may be useful in treating inflammatory disorders and oxidative stress-related diseases. Some endophytic substances have demonstrated neuroprotective properties, indicating the potential for their use in the treatment of neurological conditions. The potential of endophytes to assist plants in coping with and recovering from environmental challenges, such as heavy metal contamination and soil pollutants, has been investigated.

It has been discovered that some endophytes can increase plant growth and yield by releasing phytohormones or encouraging nutrient uptake. Endophytes' genetic potential has been utilized for biotechnological purposes, including the development of biocontrol agents, improvements to agriculture, and the biotransformation of natural materials (Hamedi *et al*., 2015).

Table 3: List of examples of endophytes species and their bioactive molecule isolated from host plants

Endophyte Species	Host Plant	Isolated Bioactive Molecules	Pharmacological Activities	References
Phomopsis sp.	Azadirachta indica	Azadirachtin derivatives (Nimbin, Nimbidin, Salanin)	Antimicrobial, Antifeedant, Antioxidant	Rajeshkumar et al., 2018
Alternaria sp.	Catharanthus roseus	Vindoline, Vinblastine, Vincristine	Anticancer, Antimicrobial	Kumaran et al., 2012
Nigrospora oryzae	Aegle marmelos	Marmelosin A, Marmelosin B, Marmesinin	Antioxidant, Anticancer	Raghu et al., 2013
Aspergillus sp.	Garcinia dulcis	2,4-Di-tert-butylphenol	Antioxidant, Antimicrobial, Anti-	Song et al., 2011

			inflammatory	
Fusarium sp.	Dioscorea bulbifera	Diosbulbin B	Anticancer, Cytotoxicity	Li et al., 2010
Pestalotiopsis sp.	Camptotheca acuminata	Camptothecin derivatives (10-hydroxycamptothecin)	Anticancer, Topoisomerase I inhibition	Zhang et al., 2009
Colletotrichum sp.	Camptotheca acuminata	Camptothecin	Anticancer, Topoisomerase I inhibition	Strobel et al., 1996

Despite the potential of endophytes as a source of bioactive compounds, challenges remain, including the need for standardized isolation and identification methods, optimization of compound production, and safety assessment for potential pharmaceutical and agricultural applications. Continued research and exploration of endophytic communities are likely to unveil new compounds with diverse applications in medicine, agriculture, and environmental management. However, further studies are required to fully understand the ecological and genetic aspects of endophyte-host interactions and harness their full biotechnological potential.

2.3 Endophytes: Metabolite Mimicry and Its Implications in Host Plant Interactions

Metabolite mimicry in endophytes is a remarkable phenomenon seen in some interactions between plants and microbes. Microorganisms known as endophytes reside inside the tissues of plants without clearly harming their host. Numerous endophytes establish mutually advantageous connections with plants, offering a variety of benefits like greater nutrient uptake, increased resiliency to environmental challenges, and defense against viruses or herbivores. Endophytes produce substances that structurally resemble or imitate specific host plant metabolites

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in metabolite mimicry. These mimicking substances can serve several purposes and have a variety of consequences for the host plant (Abu Taher *et al*.,2023).

Some endophytes produce substances that closely resemble the host plant's secondary metabolites, which the plant uses to defend itself from herbivores or pathogens. The endophyte can remain unnoticed and live within the tissues of the plant by mimicking these chemicals in order to evade detection or recognition by the plant's defensive mechanisms. Endophytes' ability to mimic metabolites can help them colonize host plants. Endophytes may take advantage of communication routes employed by the host to entice advantageous bacteria by generating substances that imitate plant signaling molecules. During the early stages of invasion, this can give the endophytea competitive edge. Some endophytes create mimicry compounds that resemble vital nutrients or molecules thataid in growth, enabling them to more successfully scavenge or acquire these nutrients from the plant or the environment (Mattoo *et al.*,2021).

The mutualistic relationship between the endophyte and the host plant may also benefit from metabolite mimicry. Endophytes may cause particular plant responses or physiological changes that are advantageous to both parties by mimicking particular chemicals. Leguminous plants have the unusual capacity to develop symbiotic partnerships with rhizobia, a type of nitrogen-fixing bacterium. The rhizobia cause nodules, which are specialist structures, to develop on the roots of leguminous plants. Rhizobia in these nodules use nitrogen fixation to turn atmospheric nitrogen into ammonia, which the plant can use as a source of nutrients. Some endophytic bacteria have the ability to imitate the signals or substances that the leguminous plant uses to identify and attract rhizobia for symbiosis. These endophytes have the ability to make molecules that resemble or imitate the signaling substances that the plant ordinarily recognizes to start the development of nodules and the symbiotic interaction with rhizobia (Badri et aL.,2009).

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Table 4: Metabolite Mimicry in Eucalyptus-Endophyte Interactions

Eucalyptus metabolite	Endophyte	Mimicry compound	Functions
Terpenoids	*Phyllosticta spp.*	Eucalyptol and other terpenoids	Endophyte-produced terpenoids mimic the host plant's natural terpenoids, contributing to the characteristic aroma of eucalyptus leaves and enhancing plant defensce against herbivores and pathogens.
Gibberellins	*Fusarium spp.*	Gibberellins	Endophyte-produced gibberellins mimic plant hormones involved in growth regulation. The presence of these mimics can influence the host plant's growth and development, possibly leading to improved plant performance.
Phenolics	*Pseudomonas spp.*	Phenolic Compounds	Endophytic *Pseudomonas* species may produce phenolic compounds that resemble host plant phenolics, possibly affecting plant-microbe interactions, pathogen defense, and other metabolic processes.
Flavonoids	*Burkholderia spp.*	Flavonoids	Some *Burkholderia* endophytes have been found to produce flavonoids similar to those found in eucalyptus plants. Flavonoids play various roles in plant-microbe interactions and may contribute to the mutualistic relationship between the endophyte and the host.

(Rodriguez *et al* 2016)

The table above shows metabolite mimicry in Eucalyptus-endophyte Interactions and explains how the metabolites made by endophytes are similar to other compounds in the host by imitating them. An area of plant-microbe interactions that is still being researched is metabolite mimicry in endophytes. Understanding how to harness these linkages could result in novel approaches to pest control and improve the health and productivity of plants, which has significant consequences for agriculture. While metabolite mimicry has been seen in some endophytic partnerships, it's crucial to remember that the precise mechanisms and outcomes rely largely on the type of plant, the endophyte in question, and the surrounding environment.

2.4 Demand for new antimicrobial compounds

Antimicrobial resistance is a serious threat to the health of the entire world. The urgent creation of novel chemicals is required to battle diseases that are resistant to currently used antibacterial drugs. The need for new antimicrobial

compounds, stressing the difficulties caused by antimicrobial resistance as well as various methodologies and strategies for finding and creating new antimicrobial agents. Worldwide, the prevalence of antimicrobial resistance has reached worrying proportions, rendering many frequently used drugs useless. Pathogens have developed a number of defense strategies to counter-attack the effects of antibiotics, including the development of resistance genes and other mechanisms to evade or neutralize the antibiotics. The treatment of infectious diseases is compromised by this phenomenon, which significantly raises mortality rates and healthcare expenditures. In order to address the current global healthcare problem, there is an urgent need for novel antibacterial chemicals. Antibiotics, antifungals, and antivirals are only a few examples of the antimicrobial substances that are currently available. The overuse and abuse of these medications has increased pathogens' capacity to develop resistance mechanisms. The pipeline for developing new antimicrobial drugs has also been considerably reduced as a result of legislative, economic, and scientific obstacles. As a result, there is an urgent need for unique chemicals that have fresh mechanisms of action, a wider range of activity, and improved efficacy against pathogens that are resistant to common medications.

Numerous obstacles, such as the need for improved screening and evaluation techniques, the high cost of drug development, and regulatory difficulties, must be overcome in order to find and develop novel antimicrobial compounds. It is essential for regulatory agencies, businesses, and academia to work together to translate research findings into clinically effective antimicrobial medicines. Additionally, to maintain the efficacy of novel antimicrobial drugs, cautious use of currently available antibiotics, infection control procedures, and public awareness campaigns are crucial.

It is obvious that novel antimicrobial substances are urgently needed to fight drug-resistant bacteria. The difficulties caused by antimicrobial resistance as well as the methods and techniques being used to find and create new antibacterial substances. In the fight against antimicrobial resistance, there is ongoing efforts to investigate a variety of sources, cutting-edge technology, and alternative treatment targets. The successful translation of

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scientific results into efficient antimicrobial medicines for the advancement of global health requires a multifaceted and collaborative approach.

Table 5: Antimicrobial Compounds Extracted from *Eucalyptus globules*

Compound	Type	antimicrobial	Reference
Eucalyptol	Terpenoid	Antibacterial, Antifungal	(Seol *et al.*,2016)
Globulol	Sesquiterpenoid	Antibacterial, Antifungal	(Darji et al.,2021)
Cineole	Monoterpene	Antibacterial, Antiviral	Mishra et al., 2017
Pinene	Monoterpene	Antibacterial, Antifungal	Chaudhary et al., 2014
Flavonoids	Phenolic compound	Antibacterial, Antifungal	Sartorelli et al., 2007

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Chapter -3

MATERIAL AND METHODS

3.1 Collection of plant material:

The plant samples were collected from the campus of TIFAC-CORE TIET, Patiala. The bark, stem and leaves of *Eucalyptus globulus* were collected in autoclaved bottles were brought to the TIFAC-CORE laboratory.

3.2 Surface sterilization of the sample

To clear the surface of dust and debris, the samples were first washed in running tap-water and then rinsed with double-distilled water. Collected samples were first submerged in 70% ethanol for 1 minute, followed by 3 minutes in an aqueous solution of 1% sodium hypochlorite, and then another 10 seconds in 70% ethanol. Double-distilled autoclaved water was used for the final wash.

3.3 Isolation of endophytic fungi from the sample

The samples were sliced from the edges after drying, and a sterile blade was used to scrape off the outer layer. The samples were then split in half horizontally. These were then put on PDA plates, which were then incubated for 7–14 days at 27±2°C and checked every two–three days for the development of endophytic fungi.

3.4 Purification of fungal endophytes

In order to obtain the pure strains, fungal endophytes had to be separated from a mixed population. Utilizing a sterile blade, the inoculum was transferred from the master plate to the potato dextrose agar medium. At 27±2°C, the plates were observed for 7–14 days. The isolated fungi were preserved for further use at 4°C

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3.5 Naming of fungal endophytes

The isolated endophytes were named after the parts from which they were obtained. E in the name represents the name of the species, and the second letter represents a part of the plant. For example, in EL1, where E represents Eucalyptus, L represents leaves, and number 1 represents the fungal isolate from a specific plate.

3.6 Sub-culturing of the endophytic fungi

Periodically, the endophytic fungi were sub cultured by removing 1-2 cm discs of mycelia off the plate with sterile blades. These discs were placed in the center of a brand-new PDA plate and incubated there for 7 days at a temperature of 27±2.

3.7 Production of secondary metabolites by fermentation

Fresh mycelia that had been sub cultured on PDA were then put into a 500 ml Erlenmeyer flask with 250 ml of PDB. The culture flasks were kept stationary and incubated for 21 days at 27±2. The culture was filtered using sterile muslin cloth after the 21-dayincubation period in order to remove the mycelia from the broth. Each separated fungus mycelia were dried to a reduced weight, and the dry weight was calculated.

3.8 Solvent extraction to obtain metabolites

The solvent extraction method was employed to extract the fungal metabolites using ethyl acetate as the solvent. In a separating funnel, equal parts of filtrate and ethyl acetate were added and mixed for 10 to 15 minutes. Further, it was permitted to remain standing until the organic and inorganic phases had fully separated. A different flask was used to collect the organic phase. The same was repeated

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once more. The obtained organic phases were mixed in a flask and dried using a Rota-evaporator with a water bath temperature of 32°C and a chiller temperature of 4°C. The remaining crude extract was then thoroughly dried in a vial while being kept at room temperature, then it was weighed. These extracts were preserved in refrigerator for further use.

3.9 Preliminary bioactive screening of fungal extracts

Preliminary bioactive screening of fungal extracts is a crucial step in identifying potential bioactive compounds with various biological activities. Here is a general outline of the process:

Preliminary screening assays

The crude fungal extract is then subjected to a series of bioassays to evaluate its bioactivity. The procedure of screening assays depends on the intended targets or activities of interest. Some common preliminary screening assays include:

a. antimicrobial activity

To assess the extract's ability to inhibit the growth of microbial pathogens, including bacteria, fungi, or other microorganisms, Agar diffusion methods (such as disc diffusion or well diffusion assays) or broth dilution methods can be employed which are further explained in detail.

b. antioxidant activity

Evaluating the extract's ability to scavenge free radicals or inhibit oxidative damage Common assays include the DPPH.

3.10 Antimicrobial activity using broth dilution method *(Azzam et al.,2020)*

The broth dilution method is commonly used to determine the antimicrobial activity of a substance, such as afungal extract from eucalyptus, against a specific microorganism like Escherichia coli (E. coli). This methodinvolves preparing a series of dilutions of the fungal extract in a liquid growth medium (broth) and then inoculating each dilution with the test organism

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to evaluate its antimicrobial effect.

Materials Required:

Eucalyptus fungal extracts ES1, ES2, and ES3 (dissolved in a suitable solvent)

Sterile broth (e.g., Mueller-Hinton broth for bacteria)

Sterile test tubes or microplates

Inoculating loop or pipette

Incubator

Positive control

Negative control

Procedure:

Prepare a stock solution of the eucalyptus fungal extract by dissolving the extract in dimethyl sulfoxide (DMSO) to achieve a specific concentration.

Prepare a series of dilutions of the stock solution using sterile broth.

Inoculate each dilution with a standardized suspension of E. coli.

Include positive and negative controls in the experiment. The positive control should contain a known antimicrobial agent, while the negative control should contain only sterile broth without the extract or antimicrobial agent.

Incubate all the tubes or microplates at the appropriate temperature for the growth of E. coli (usually 37°C for

After incubation, observe the tubes or microplates for visible growth. The lowest concentration of the fungal extract that inhibits bacterial growth (no visible growth) is the minimum inhibitory concentration (MIC) of the extract against E. coli.

Additionally, perform further subcultures from the tubes or wells showing no visible growth (MIC or higher

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concentration) to determine if the extract exhibits bactericidal or bacteriostatic effects.

Calculate the percentage inhibition or growth inhibition zone (if applicable) to quantitatively measure the antimicrobial activity of the fungal extract

3.11 Free radical scavenging activity by DPPH assay (Akinsanya. M et al.,2015)

DPPH assay: (DPPH 2, 2-diphenyl-1-picrylhydrazyl-hydrate) is an antioxidant assay that is based on electron transfer and gives a violet-colored solution in methanol. These free radicals are stable at room temperature and get reduced in the presence of an antioxidant molecule. It transforms the fluid from violet to colorless by scavenging free radicals.

Materials required: DPPH, ascorbic acid, a 96 well microtiter plate, methanol, a pipette, tips, crude sample, aluminum foil, and an ELISA plate reader.

Procedure: In a 96-well microtiter plate, 50 µl of 100, 250, 500 and 1000 µl dilution used. The endophytic fungal extracts were added to 150 µl of DPPH (100 µM). Methanol was used as the negative control. The plate was kept in the dark for 45 minutes after being wrapped in aluminum foil. Using an ELISA reader, a change in color intensity (from deep violet to alight yellow) was recorded at 517 nm.

Free radical scavenging activity was calculated using formula,

Free radical scavenging activity = [(A Control - A Sample)/A Control] x100

Phytochemical analysis of crude extracts *(Kaur et al.,2019)*

Test for phenols and tannins (Ferric chloride test)

When 1 ml of the extract (x) is treated with the 1 ml of ferric chloride solution (5% w/v), If hydroxylate tannins are present, blue color occurs, and if condensed tannins are present, it shows green color.

Test for amino acids (Ninhydrin test)

1ml of test solution was added to 1 ml of ninhydrin solution, which was then heated until it boiled. The violet color indicated the presence of amino acids in the solution

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Test for carbohydrates (Molisch's test)

To 1 ml of extracts, a few drops of Molisch's reagent were added, and then a few drops of concentrated sulfuric acid were added through the tubes' walls. If carbohydrates were present, a purple to violet ring would emerge at the junction.

Test for alkaloids (Wagner's reagent test)

A few drops of Wagner's reagent were added to 1 ml of extract. The presence of alkaloids was identified by reddish brown ppt.

Test for fats and fixed oils (Saponification test)

To 1 ml of extract a drop of phenolphthalein and a few drops of 0.5 N alcoholic KOH were added. The test tubes were then heated in a water bath for one hour at 55°C. The formation of soap bubbles) indicated the presence of oils and fists.

Test for steroids and triterpenoids (Salkowski's test)

When 1 ml of the extract were treated with a few drops of concentrated sulfuric acid, the formation of a yellow-colored lower layer indicated the presence of triterpenoids, while the presence of steroids is indicated by formation of red color at lower layer.

Test for glycosides (Keller-Killiani test for Deoxy sugars)

1ml of extracts was added with 1 ml of glacial acetic acid, 1 drop of 5% FeCl, and a few drops of concentration. The existence of deoxy sugars was determined by the appearance of a reddish-brown color at the junction of two liquidlayers and a blue green color in the top layer.

3.12 Purification of crude extracts

Thin layer chromatography

Instruments: Glass plates, sample applicator, capillary tube, glass chamber, UV torch Reagents: All solvents of A.R. grade was employed throughout the determination.

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TLC is an efficient and simple method to determine how many components are present in a crude extract. The fungus' ethyl acetate extracts have been identified on TLC plates (Silica Gel 60 F254- Merck) above 2 cm from the base. The spotted TLC plates were run in optimized mobile phase. The components were divided up into different Rf value ranges by the mobile phase. The developed chromatograms were examined with UV light of wavelength 365nm during this process. By dampening the background's greenish yellow fluorescence, sample components that absorb light in this area show as a black patch (Ishnava, K. B., Chauhan, J. B., & Barad, M. B. 2013). Various steps were involved:

·Selection of stationary phase

·Selection of mobile phase

·Sample application

·Type and size of developing chamber

·Mode of development

·Visualization and detection.

Separation of active compounds by silica gel column chromatography

Requirements: Glass column, stationary phase-Silica gel G (60-120 mesh), Mobile phase- Hexane, ethyl acetate and methanol, cotton, test tubes, test tube stand, Silica gel G, TLC plates

Sample Preparation: The fermented culture media ethyl acetate extract was dried by evaporation, weighed. To separate the different fractions, 150 mg of material was employed.

Column preparation: Adsorption of the extract, charging, and saturation of the column were all steps in the column preparation process. On stationary phase, the extract selected for fractionation was adsorbed. For the purpose of getting removal of impurities, a solvent was washed through the selected column. To prevent the stationary phase from flowing, a cotton pad was firmly set at the bottom. Wet packing was used to charge the column with stationary phase. In order to avoid messing the

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stationary phase while adding eluting solvents from the top, a second layer of cotton pad was put above the mixture.

Procedure: In order to avoid the formation of air bubbles, the slurry was softly placed on top of the column and packed with gentle pushed air. A sufficient amount of the initial solvent combination was then passed through the column after it had been cleaned with hexane. The crude extract was dissolved before being placed onto the column. The column was then eluted using a gradient of hexanes and ethyl acetate (5% ethyl acetate to 100% ethyl acetate), 100% hexane, and lastly methanol in ethyl acetate (35% ethyl acetate). Pure methanol was used as the final wash for the column.

In test tubes, the eluted solvents from the column were collected. On a TLC plate, each fraction emerged concentrated and patchy. The TLC was created using an appropriate mobile phase and observed in an iodine or long UV (365 nm) chamber. Similar fractions were combined based on the TLC profile, and the fractions that contained several compounds were again subjected to small-sized column chromatography.Final fractions were evaporated using a rotary evaporator.

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Chapter- 4

RESULTS AND DISCUSSION

The flowering plant species *Eucalyptus globulus*, also referred to as southern blue gum or blue gum, belongs to the family Myrtaceae. It is a large, indigenous tree to south-east Australia. This species of Eucalyptus has generally smooth bark, juvenile leaves that are waxy on the underside and are whitish, adult leaves that are glossy green and lance-shaped, glaucous, ribbed flower buds that are placed singly or in groups of three or seven in the leaf axils, white flowers, and woody fruit.

Fig 1: Shows *(A) and (B) tree of E. globulus.*

Table 6: Classification of *Eucalyptus globulus*

Botanical name	*Eucalyptus globulus*
Kingdom	Plantae
Order	Myrtales
Family	Myrtaceae
Genus	*Eucalyptus*

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Species	*globulus*
Common name	Tasmanian Blue Gum
Phylum	Tracheophyta

Collection of samples

The samples were collected from the campus of TIET Patiala. The bark, stem and leaves of *Eucalyptus globulus* were collected from the TIFAC-CORE. The samples collected in autoclaved bottles were brought to the TIFAC-CORE.

Fig 2: Collection of samples from different parts of *Eucalyptus globulus (A) bark of tree, (B) leaves and stem of tree.*

Isolation of endophytic fungi from the stem leaves and bark of *Eucalyptus globulus*

The plant's tissues were surface sterilized before being placed on water agar plates, which were then incubated at 27±2°C for 4–14 days. Regular inspections of the plates were made to monitor endophyte growth.

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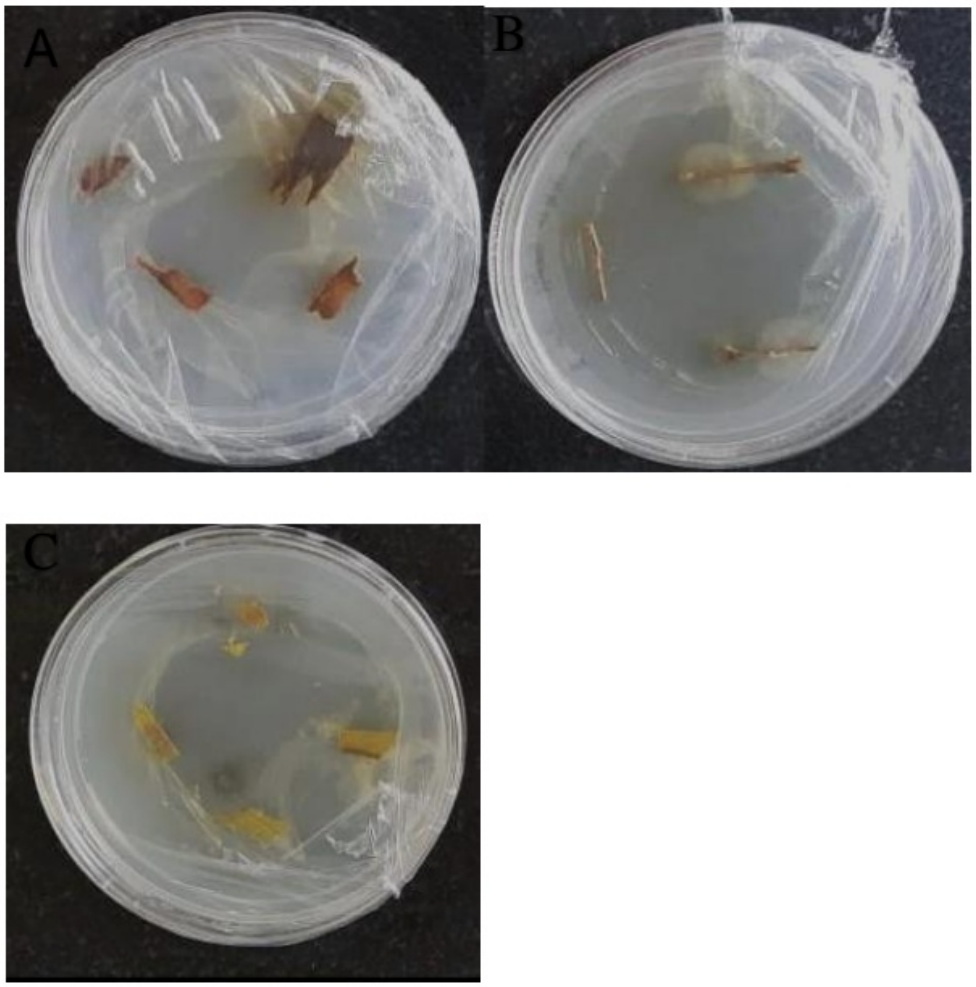

Fig 3: Water agar plate with bark(A), stem(B) and leaves(C).

After the growth of fungi in water agar plates, the fungi were transferred to the PDA plates. The plates were incubated for 3-10 days at 27±2°C. Subculturing was performed for suitable media for the growth of fungi. The three media used were SDA, GYP and PDA. The maximum growth was observed in PDA plates. Total of 7 endophytic fungi were obtained out of which the fastest growing fungi were sub-cultured further. Figure 3 shows 7 endophytic fungi obtained from *Eucalyptus globulus*. Most fungi were found from stems and leaves which were further used for study.

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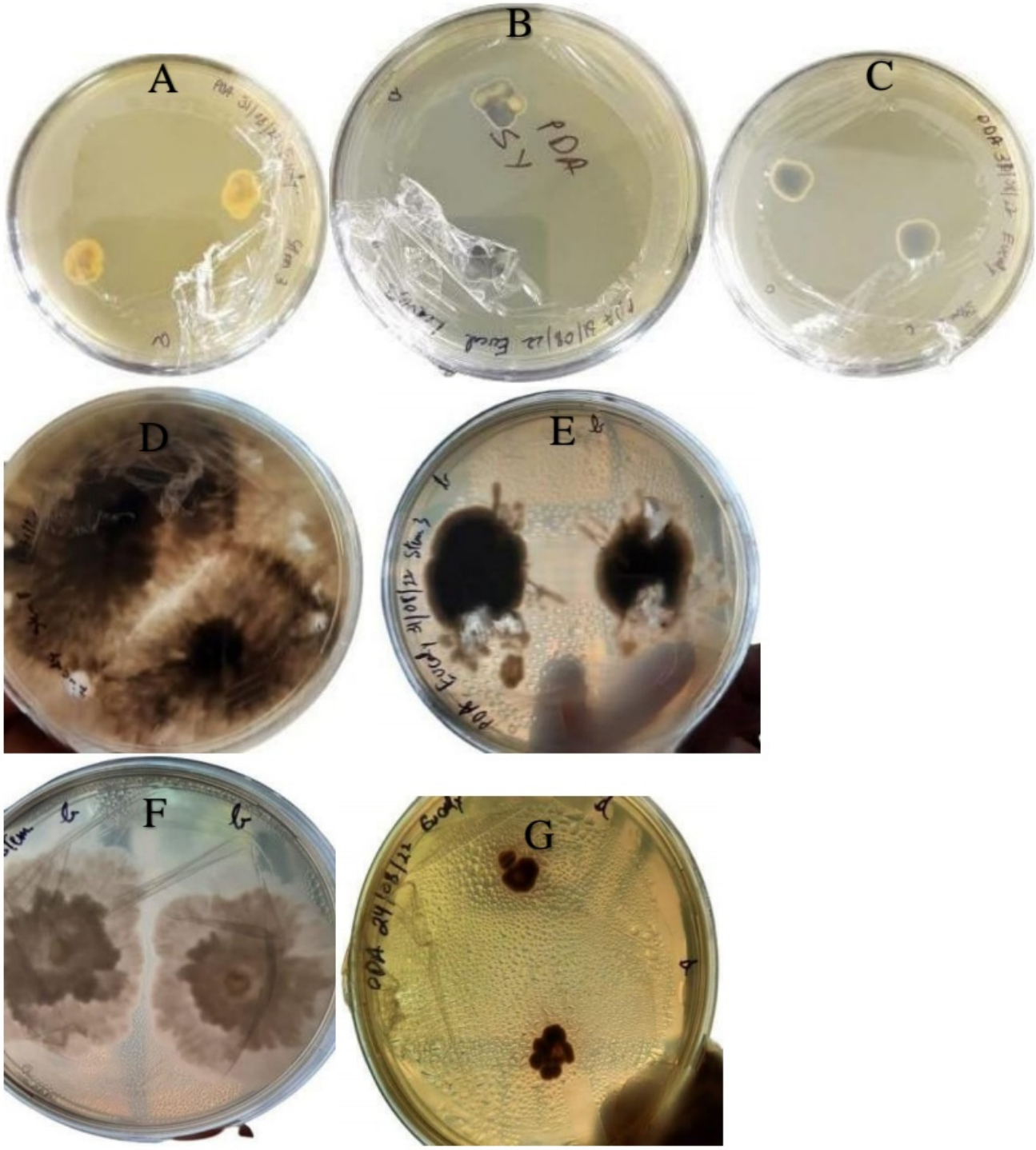

Fig. 4 Different fungal isolates obtained from *Eucalyptus globulus*.

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Production of Secondary metabolites by fermentation

The isolated endophytic fungi obtained from *eucalyptus globulus* were incubated for 21 days for 25-27°C under stationary conditions. The growth was monitored under controlled condition.

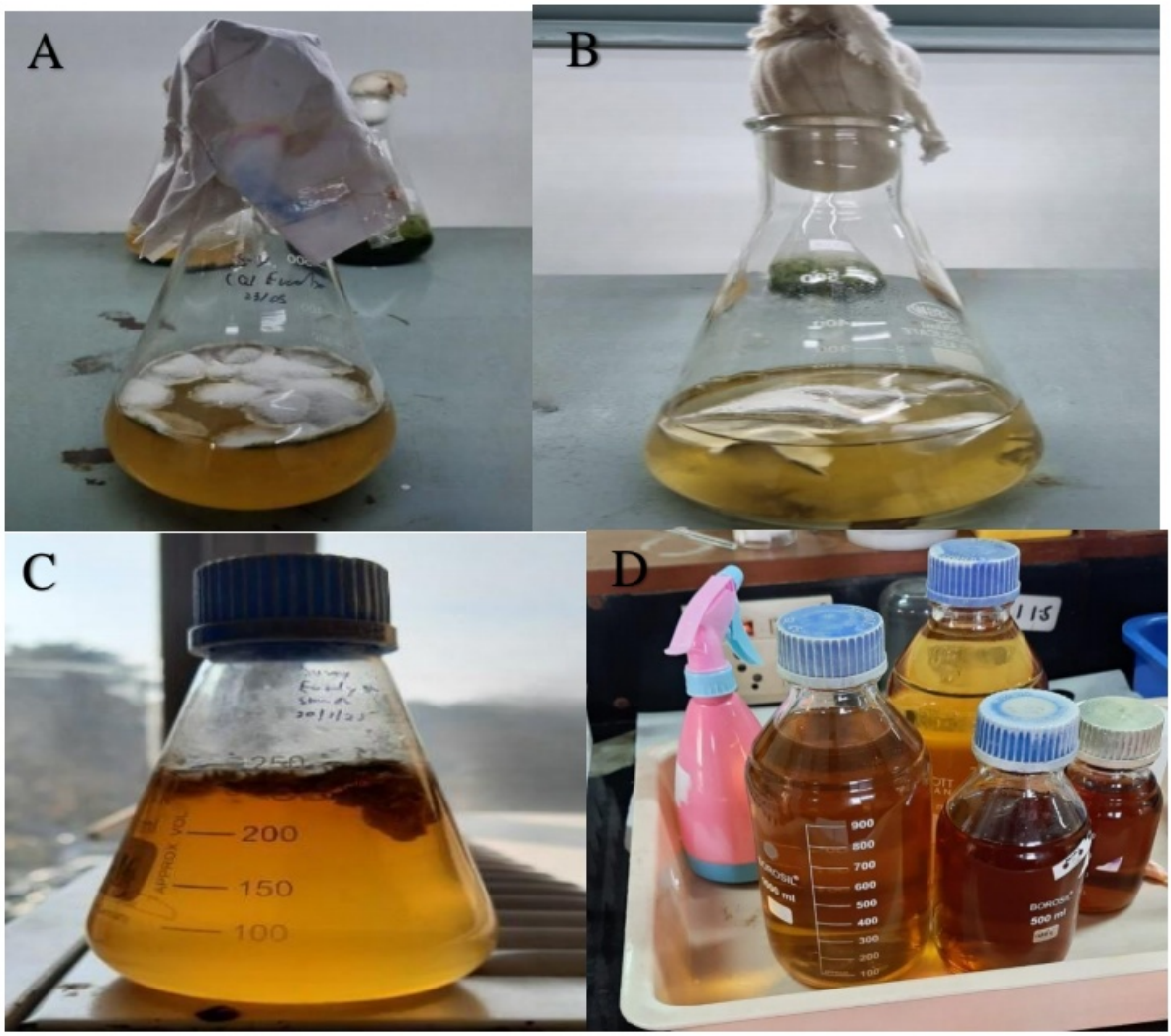

Fig 5: Production of secondary metabolites by fermentation (A) Broth having inoculum of fungus during fermentation (B) Production of metabolites after 21 days (C)Broth having inoculum of fungi for fermentation (D) Collection of broth after filtration

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Filtration and extraction of the crude extract from broth

After the mycelia and broth had been incubated for 21 days, ethyl acetate was used to extract the organic phase, which comprised the metabolites. To get the fungal crude extract, this was further dried. Preliminary bioactive investigations were conducted on the obtained crude fungal extracts.

Fig 6 Purification and extraction of crude extract (A) collection of broth after filtration (B) Extraction of broth using ethyl acetate as organic solvent.

Dry weight of mycelia

The dry weight of mycelium refers to the weight of fungal mycelium (the vegetative part of the fungus) after removing the water content. Collect a representative sample of the fungal mycelium from the culture or substrate. Transfer the collected mycelial sample to a pre-weighed filter paper, a pre-weighed container, or a pre-weighed crucible. Place the filter paper, container, or crucible containing the mycelial sample in an oven or a desiccator. Calculate the dry weight of mycelium by subtracting the weight of the filter paper, container, or crucible (Measured before adding the mycelial sample) from the combined weight of the filter paper, container, or crucible with the dried mycelium.

Table 7: The dried mycelia weight and metabolite production in isolated fungi.

	Sample	Mycelia weight (gms)	Crude extract (mg/l)
1	ES1	2.1	15
2	ES2	1.8	12
3	ES3	2.5	18
4	ES4	1.6	10
5	EB1	2.3	16

Preliminary bioactivities for the screening of crude extracts

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a commonly used antioxidant assay to evaluate the radical scavenging activity of compounds. Ascorbic acid (vitamin C) is often used as a positive control in DPPH assays due to its well-known antioxidant properties. Three fungal extracts were tested for their scavenging activity at different concentrations.

A

	1	2	3	4	5	6	7	8	9	10	11	12
1	M	DPPH+M	AA+M+DP	DPPH+M	E1a	E1b	E1c	E1d	E2a	E2b	E2c	E2d
2	M	DPPH+M	AA+M+DP	DPPH+M	E1a	E1b	E1c	E1d	E2a	E2b	E2c	E2d
3	M	DPPH+M	AA+M+DP	DPPH+M	E1a	E1b	E1c	E1d	E2a	E2b	E2c	E2d
4	E3a	E3b	E3c	E3d	E1a*	E1b*	E1c*	E1d*	E2a*	E2b*	E2c*	E2d*
5	E3a	E3b	E3c	E3d	E1a*	E1b*	E1c*	E1d*	E2a*	E2b*	E2c*	E2d*
6	E3a	E3b	E3c	E3d	E1a*	E1b*	E1c*	E1d*	E2a*	E2b*	E2c*	E2d*
7	E3a*	E3a*	E3c*	E3c*	E3c*	blank	blank	blank	blank	blank	blank	blank
8	E3b*	E3b*	E3b*	E3d*	E3d*	E3d*	blank	blank	blank	blank	blank	blank

B

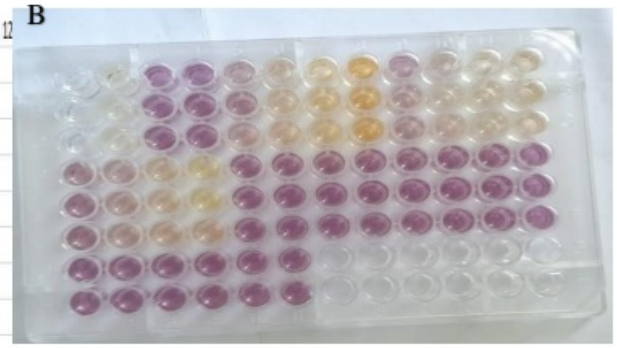

Fig 7: Scavenging activity of crude extract (A) template (B) 96-well plate

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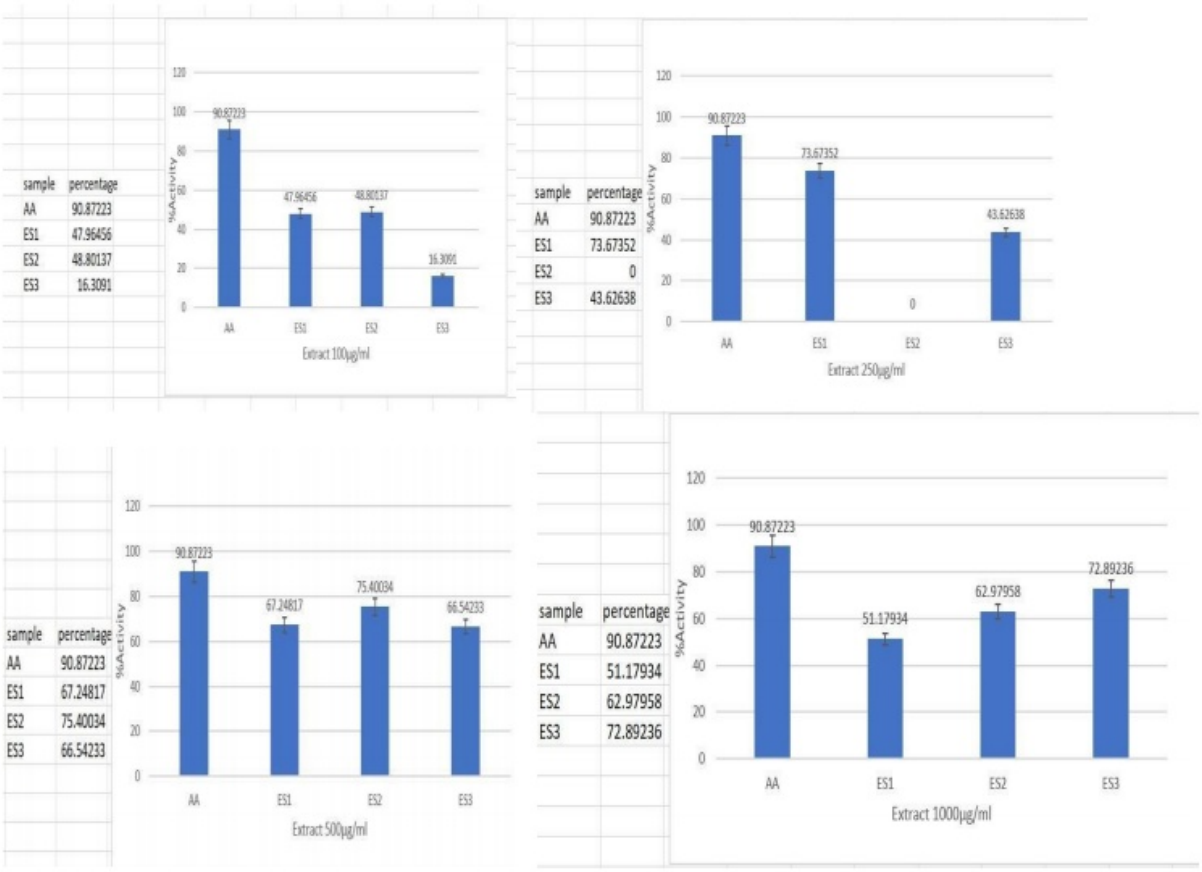

Fig 8: Scavenging activity at different concentrations

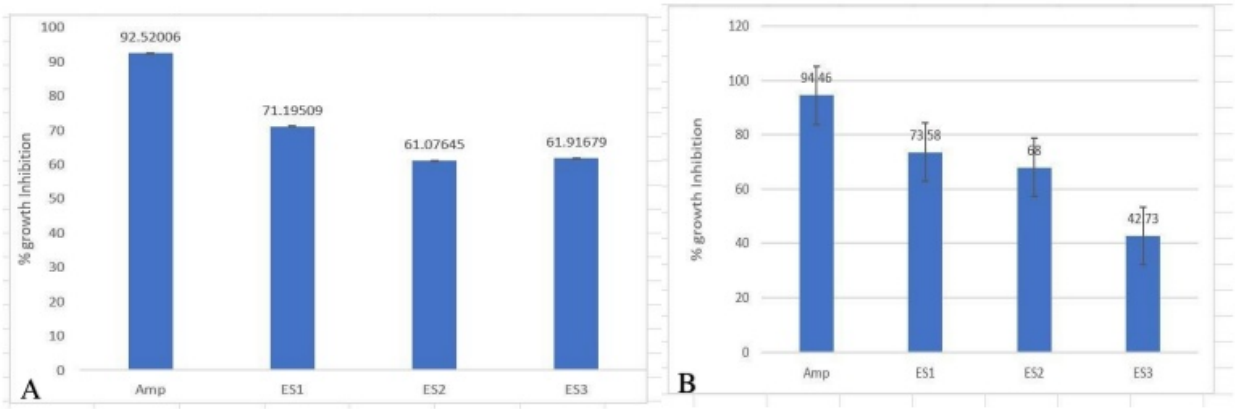

Fig 9: Antibacterial activity against *Staphylococcus aureus* and *E. coli*

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Phytochemical analysis of crude extract

Phytochemical analysis is a process used to identify and quantify the bioactive compounds present in a given extract. These substances, sometimes referred to as phytochemicals, are secondary metabolites that contain a variety of components such as alkaloids, flavonoids, phenolic compounds, terpenoids, glycosides, and many others. Phytochemical analysis is an essential step in understanding the chemical composition and their potential bioactive properties.

Table 8: Phytochemical analysis for fungal crude extracts

	ES1	ES2	ES3
1 Tannins	+++	-	-
2 phenolic	-	+	++
3 Carbohydrates	-	-	-
4 Alkaloids	+	+	+
5 Saponins	-	+	-
6 Steroids and triterpenoids	+	-	-
7 Glycosides	++	-	++

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The results showed the presence of tannins, alkaloids, steroids, and glycosides in crude extract ES1. The result showed the presence of phenolics, alkaloids, and saponins in crude extract ES2. The resultshowed presence of phenolics, alkaloids and glycosides in crude extract ES3.

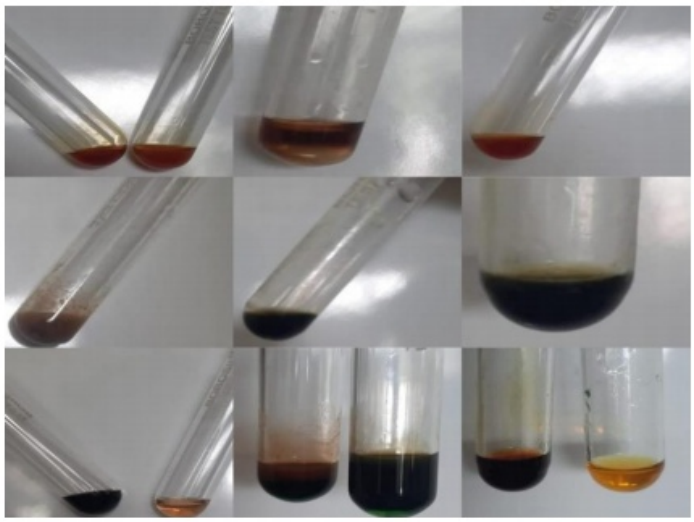

Fig 9: shows phytochemical analysis of samples ES1, ES2 and ES3

Purification of crude extract

Thin layer chromatography

TLC was performed for the extracts ES1 and ES2. Solvent-system toluene and ethyl acetate (93:7) were used. The solvent system separated the extract into different fractions. Multiple spots indicate different compounds in the extract. The Rf value was calculated for each spot. The Rf values were compared to the known standard. The Rf value matching the known compound suggests the presence of that compound in the extract.

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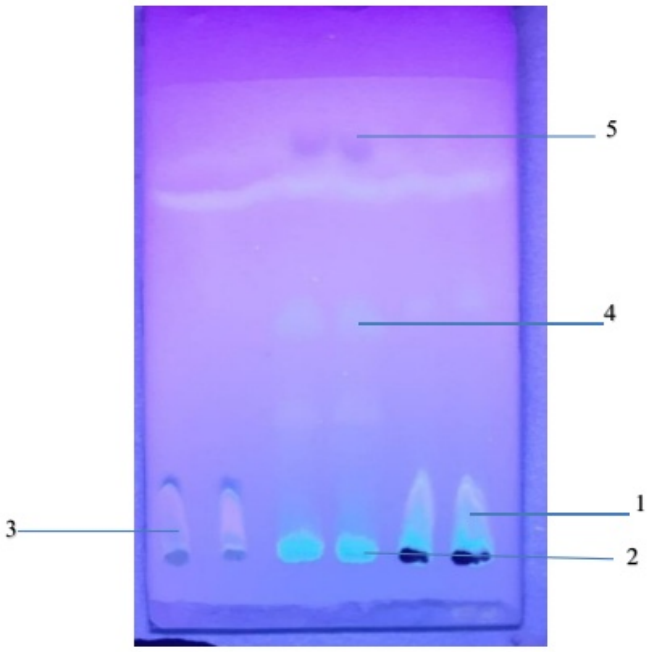

Fig 10: Chromatogram obtained by toluene and ethyl acetate for ES1 ES2 and ES3

Column chromatography

A common method for isolating and purifying chemicals from complicated mixtures, such fungal extracts, is column chromatography. Based on how differently each component interacts with a stationary phase (often silica gel or alumina) and a mobile phase (a solvent or combination of solvents), it separates the components of a mixture. Silica G60 was used as a stationary phase. The selected extract was absorbed into the stationary phase for fractionalization. The column was rinsed with hexane. Cotton-dipped hexane was used for flow rate (A. Luis et al., 2016). A different gradient of hexane, ethyl acetate, and methanol was used. Eight fractions of crude extract ES1 and 13 fractions of crude extract ES2 were obtained.

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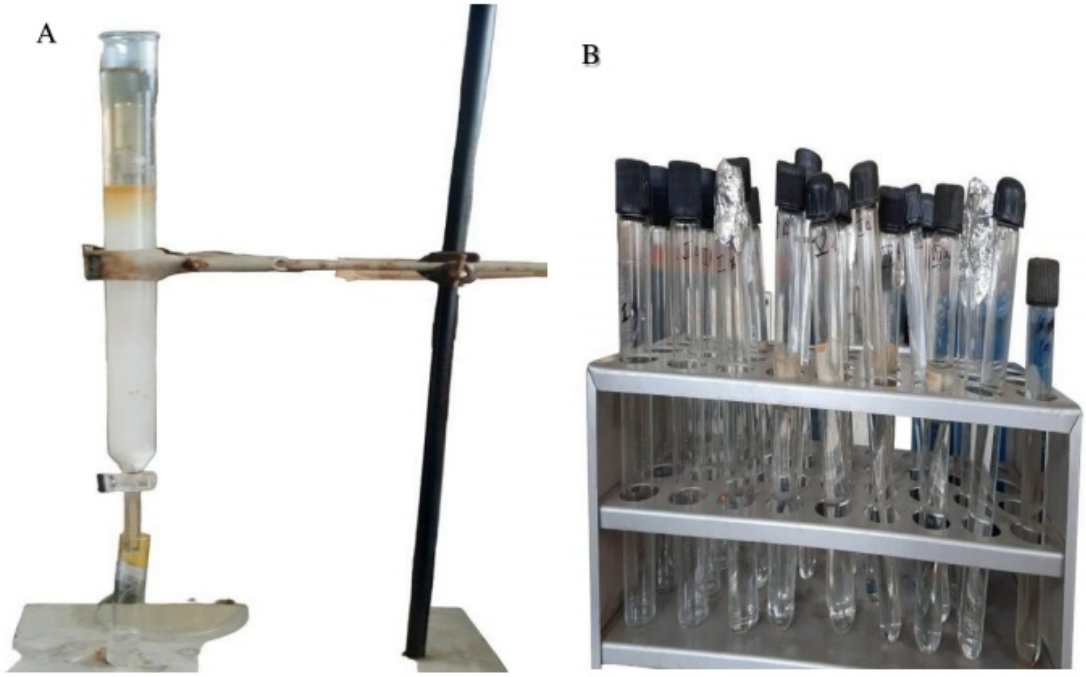

Fig :11 Column chromatography of fungal extract (B) Fraction collected in test tube.

The test tube containing the fractions was reduced by a rotary evaporator. Reduced solvents were collected in the vials and dried.

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BB

C

Fig :12 (A) Rota-evaporator (B) Eight fraction of extract ES1 (C) thirteen fraction of extract ES2

Pooling of fractions based on TLC is a common practice during the purification process in chromatography. After eluting the column and collecting fractions, performed TLC for each fraction. Based on the TLC analysis, the fractions that contain the desired compounds or groups of compounds were selected. By pooling fractions based on TLC analysis, we obtained a more purified sample of the desired compounds, making it easier to isolate specific components from complex mixtures.

A

B

Fig :13 (A) Pooling of fraction ES1 (B) Pooling of fraction ES2

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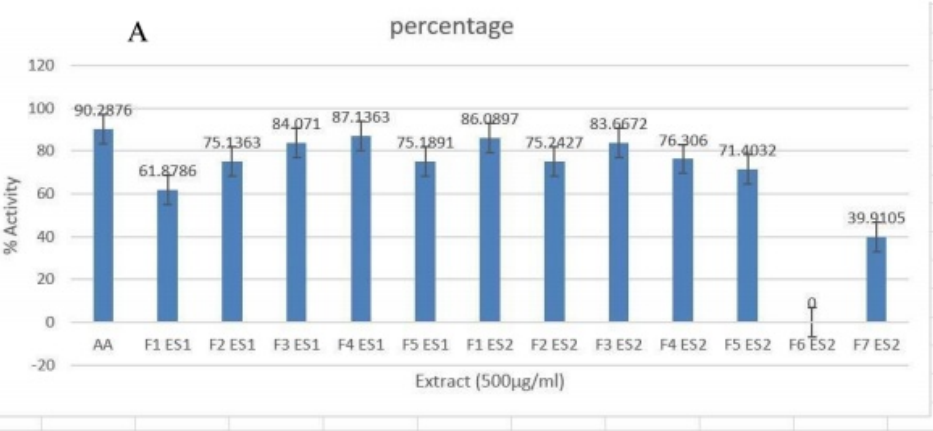
All the collected fractions were tested at various concentrations for their antioxidant potential to identify themost active fractions for further investigation.

fraction	percentage
AA	90.2876
F1 ES1	61.8786
F2 ES1	75.1363
F3 ES1	84.071
F4 ES1	87.1363
F5 ES1	75.1891
F1 ES2	86.0897
F2 ES2	75.2427
F3 ES2	83.6672
F4 ES2	76.306
F5 ES2	71.4032
F6 ES2	0
F7 ES2	39.9105



fraction	percentage
AA	90.2876
F1 ES1	60.8786
F2 ES1	72.1363
F3 ES1	86.071
F4 ES1	80.1363
F5 ES1	52.1891
F1 ES2	72.0897
F2 ES2	75.2427
F3 ES2	67.6672
F4 ES2	75.306
F5 ES2	71.4032
F6 ES2	0
F7 ES2	39.9105

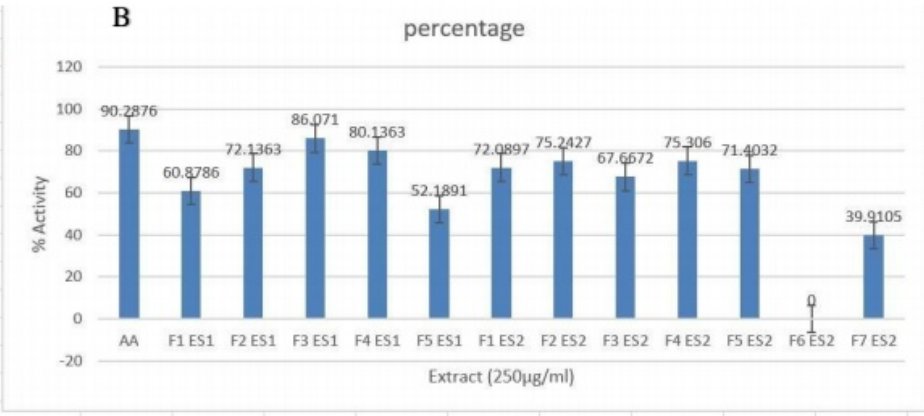


Fig :14 Percentage scavenging activity of fractions at different concentrations (A) at 500 µg/ml (B) at 250 µg/m

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From the above (Fig 14 A) results conclude that F4 ES1 and F1 ES2 shows maximum radical scavenging activity of 87.13% and 86.08% at 500 µg/ml. From the above (fig 14 B) it shows that maximum scavenging activity was shown by F3 ES1 and F2 ES2 at 250 µg/ml.

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CHAPTER 6:- CONCLUSION

In this study, we aimed to investigate the diversity and ecological significance of endophytic fungi associated with Eucalyptus globulus, a widely distributed and economically important plant species. The isolation of endophytic fungi from *Eucalyptus globulus* has been successful, and the results demonstrate the presence of diverse fungal communities within the plant tissues. The study showed that *Eucalyptus globulus* has a large and unique population of endophytic fungi. This shows how important it is to study how plants and microbes interact in this ecosystem. A total of seven endophytic fungi were obtained from *Eucalyptus globulus*. To collect samples, we carefully sampled stems, leaves, and bark from healthy *Eucalyptus globulus* trees. We employed rigorous surface sterilization techniques to eliminate any external contaminants and isolated endophytic fungi from each tissue type separately. Out of which three were selected for growth on PDA plates. The diversity of endophytic fungi isolated from *Eucalyptus globulus* highlights the potential for these microorganisms to play crucial roles in the health and growth of the host plant. Some of these endophytes may help the plant make secondary metabolites, which can help the plant protect itself from pathogens and herbivores, take in more nutrients, and handle abiotic stress better.

Using cultures of *E. coli* and *Staphylococcus aureus*, the crude extract produced from the isolates was examined for preliminary antibacterial activity using the broth dilution method. ES1 demonstrated the highest levels of inhibition against *E. coli* and *Staphylococcus aureus*, at 73.58% and 71.19%, respectively. This indicates that ES1 crude extract possesses significant antibacterial properties against both gram-negative (*E. coli*) and gram-positive (*Staphylococcus aureus*) bacteria. The ability of the crude extracts to scavenge free radicals was also examined. Maximum radical scavenging activity was shown by ES2 at 500 µg/ml, 75.4%, and by ES1 at 250 µg/ml, 73.67%. We investigated the potential of endophytic microorganisms isolated from *Eucalyptus globulus* to produce secondary metabolites through fermentation. After subjecting the isolated and selected endophytes ES1, ES2 and ES3 to a 21-day fermentation process in a controlled environment, we observed substantial

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production of secondary metabolites and bioactive compounds.

The phytochemical analysis of the crude extracts from ES1, ES2, and ES3 showed the presence of bioactive compounds like tannins, alkaloids, steroids, glycosides, phenolics, and saponins. These findings suggest that these crude extracts hold significant potential as sources of natural compounds with diverse pharmacological properties. In particular, ES1 had tannins, alkaloids, steroids, and glycosides. This shows that it has a rich chemical composition, which may be a factor in its high antibacterial activity against E. coli and Staphylococcus aureus as well as its ability to get rid of free radicals. On the other hand, ES2 showed the presence of phenolics, alkaloids, and saponins, which could be responsible for its remarkable radical scavenging activity at a concentration of 500 µg/ml. ES3, with its phenolics, alkaloids, and glycosides content, also exhibits interesting bioactive components. These compounds have been associated with various medicinal properties, and their presence in ES3 may contribute to its potential biological activities.

The purification method of column chromatography was successfully employed to fractionate the crude extracts of ES1 and ES2, resulting in the collection of several fractions. Subsequently, based on thin-layer chromatography (TLC) analysis, a total of 5 fractions from ES1 and 7 fractions from ES2 were selected for further evaluation of their free radical scavenging activities. Fraction F4 displayed the maximum free radical scavenging activity at a concentration of 500 µg/ml. This indicates that F4 from ES1 contains potent antioxidant compounds that effectively neutralize free radicals. Fraction F3 showed the highest free radical scavenging activity at a concentration of 250 µg/ml. This suggests that F3 from ES1 also contains active compounds with antioxidant properties. Fraction F1 demonstrated the highest free radical scavenging activity at 500 µg/ml, indicating the presence of potent antioxidants in F1 from ES2. Fraction F2 exhibited the maximum free radical scavenging activity at a concentration of 250 µg/ml. This indicates that F2 from ES2 also contains active compounds with antioxidant capabilities.

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ABSTRACT

This study aimed to isolate and characterize antimicrobial compounds from Eucalyptus globulus, a widely known medicinal plant with potential therapeutic properties. The plant material was collected, dried, and subjected to solvent extraction using a non-polar organic solvent ethyl acetate. The crude extract obtained was then fractionated using column chromatography, and the resulting fractions were tested for their antimicrobial activities. The bioactive fraction was further purified using various chromatographic techniques, such as thin-layer chromatography (TLC) and Column chromatography.

A total of 8 fraction from extract ES1 and 13 fraction from extract ES2 were obtained. Pooling of fraction on the basis of TLC was performed. The antimicrobial of the isolated compounds was evaluated against a panel of clinically relevant bacterial strains using standard antimicrobial assays. The results revealed potent inhibitory effects of the isolated compounds against both Gram-positive and Gram-negative bacteria. The characterization of the isolated compounds using phytochemical analysis confirmed their chemical structures and provided valuable insights into their properties. The identified compounds belong to different chemical classes, such as terpenoids, phenolics, or alkaloids, known for their antimicrobial properties. These findings highlight the potential of Eucalyptus globulus as a valuable source of bioactive compounds with significant antimicrobial activities. In conclusion, in this study we successfully isolated and characterized antimicrobial compounds from Eucalyptus globulus. The identified compounds exhibited promising activities against a range of bacterial pathogens. Further studies are warranted to elucidate the mechanisms of action and potential therapeutic applications of these compounds, with the ultimate goal of developing novel antimicrobial agents for clinical use.

Chapter-1

INTRODUCTION

Plants that are identified as "medicinal plants" are those whose parts contain substances that can be employed as either therapeutic agents or as a starting point for the synthesis of efficient drugs. Since the very beginning of time, plants with medicinal properties have been used for a variety of healing and curative purposes (Tlau, L., & Lalawmpuii, L. 2020).