Study of Isolation, Identification, Fermentation, Extraction and Antimicrobial Effect of Endophytic Fungi by Using Panax Ginseng Roots.

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Abstract— Ginseng (Panax ginseng) is a renowned medicinal plant with a long history of use in traditional medicine due to its therapeutic properties, such as boosting the immune system and enhancing vitality. Recent research has highlighted the potential of endophytic fungi, microorganisms that live within plant tissues without causing harm, as producers of bioactive compounds with antimicrobial properties. Exploring these fungi may lead to the discovery of novel antimicrobial agents, which are urgently needed in the face of rising antibiotic resistance. This study aimed to isolate and identify endophytic fungi from different parts of ginseng, optimize their fermentation conditions to maximize the production of bioactive compounds, extract these compounds, and evaluate their antimicrobial efficacy against a range of pathogenic microorganisms.

Index Terms- Ginseng, Endophytic Fungi, Antimicrobial activity, Secondary metabolites, Fermentation, Bioactive compounds.

I. INTRODUCTION

Endophytic Fungi:

It has been revealed that plant endophytes are a crucial source for the discovery of novel microbes [1]. Numerous investigations conducted in the past several years have revealed the presence of a vast number of endophytic fungus in plants, which form symbiotic relationships with their hosts that are mutually advantageous [2]. The majority of them also demonstrated the ability to support plant growth [4], enable the host plant survive external conditions [3], and biosynthesize certain bioactive chemicals to safeguard the host plants [5].

Numerous bioactive substances, including quinones, flavonoids, terpenoids, alkaloids, steroids, and isocoumarin derivatives, are produced by some endophytic fungi and have the potential to be valuable industrial, agricultural, and medicinal products [6,7, 8].

These molecules have varied functions in plants, such as in defense against phytopathogens and the promotion of development, which happens owing to the creation of such phytohormones as auxin and gibberellins and the absorption of such minerals as nitrogen and phosphorus from the soil [9,10]. The fungal endophytes found in ginseng, however, have only been the subject of a few reports [7, 11, 12]. Thus, the purpose of this study was to investigate the variety of endophytic fungus found in P. ginseng plants grown in Korea, spanning a range of ages and tissues. A remarkably adaptable and biodiverse microbial population that appears to be found everywhere in nature are endophytic fungus. Research has demonstrated that endophytic fungi are present in nearly all plants, including those that have colonized deserts, oceans, tropical rainforests, and the Arctic and Antarctic [13, 14].

A variety of plants, including algae, mosses, ferns, gymnosperms, and angiosperms, have had their roots and aboveground sections extracted and cultured. Endophytic fungi co-evolved distinct biosynthetic pathways and metabolic mechanisms to synthesize complex secondary metabolites during the approximately 400 million-year-long history of plant-endophytic fungal interactions, according to evidence from microorganism records in fossil plant tissue [15].

Just 5% of the 1.5 million fungal species that exist on Earth have detailed descriptions to date, and only 16% (or 11,500 species) of these 69,000 fungal species have been cultivated and investigated. Results from next-generation sequencing methods indicate that there are between 0.035 and 5.1 million fungus species on Earth [16].

Ginseng:

The perennial plant Panax ginseng Meyer, a member of the Araliaceae family, has been utilized for thousands of years as a major medicinal herb. Ginsenosides, phenols, oleanic acids, and volatiles are among the several pharmacologically active components found in ginseng [17]. The molecular structure of ginseng saponins, often referred to as ginsenosides, differs from that of saponins from other plants. As a result, they have distinct, wide-ranging effects as antioxidant, antidiabetic, and anticancer chemicals as well as cholesterol-lowering agents [18–21]. Ginseng plants need to be grown in shadow for four to six years, and because they must be grown for such a long time in the same soil, the soil environment has an impact on ginseng culture [22]. The illness complications brought on by various pathogenic fungi over the protracted cultivation period are one of the main issues. Gray Mold (Botrytis cinerea), alternaria blight (A. panax), anthracnose (Colletotrichum gloeosporioides), Sclerotinia white rot (Sclerotinia sp.), phytophthora blight (Phytophthora cactorum), and root rot (Cylindrocarpon destructans) are the most common pathogenic fungi found in ginseng [23, 24]. Consequently, ginseng growers place a high value on the prevention and treatment of these diseases. We go over the pharmacological and structural characteristics of ginseng as well as the active ingredients, such as polysaccharides, polyacetylenic alcohols, and ginsenosides. This paper discusses the pharmacological and clinical applications of ginseng, with a focus on ginsenosides, and its potential benefits for reducing cancer, diabetes, immune system dysfunction, and neurological illnesses.

Aqueous ginseng extracts include minerals, amino acids, saponins, and a variety of water-soluble low- and high-molecular-weight substances. It was observed that in a mouse model with a lung infection caused by pseudomonas aeruginosa, the production of cytokines was controlled by ginseng extract. The lung cells in the ginseng extract-treated group produced greater ratios of IFN-γ/IL-4, more interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α), and less interleukin 4 (IL-4). The mice with a lung infection caused by Panax aeruginosa showed signs of a Th1-like immune response (cellular immune response) when treated with ginseng extract [25].

II. METHOD USED

Isolation of Fungal Endophytes from Roots of Ginseng:

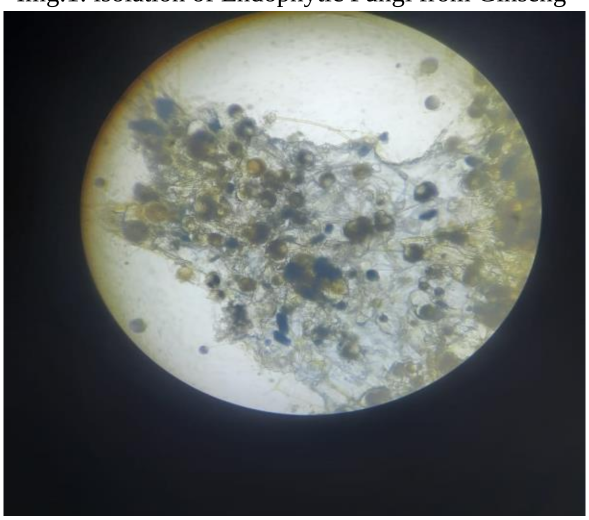
Examples of ginseng plants collected as dried roots from the forest. The samples of ginseng were promptly placed in sterile bags and kept at room temperature. The roots was cleaned scrupulously under running tap water to eliminate adherent soil and dust particles which was followed by washing with sterile distilled water and chopped into little pieces (3-4 cm) under sterile circumstances. The root fragment was first dipped in 70% (v/v) ethanol for 5 minutes, followed by treatment with sodium hypochlorite (6%) for 5 minutes, and finally treatment with 70% (v/v) ethanol for 30 seconds. This surface critical stage was carried out by following the established protocols. Lastly, sterile distilled water was used to rinse the sterilized root pieces. Cutting into tiny pieces (0.5 x 0.5 cm), the sterilized root fragments were arranged on potato dextrose agar (PDA) medium in petri plates and cultured for 30 days at 25 ± 1°C.

Identification of Endophytic Fungi:

Morphological and microscopic identification of endophytic fungi:

The morphology of the cultures, the spore generation methods, and the spore properties were used to identify the fungal species. In order to conduct morphological research, the fungus were plated on PDA and incubated for seven days. On both the upper and lower surfaces of the culture plates, the growth appearances were noted. Microscopic slides of every fungal endophyte were generated for provisional identification. Under a microscope, slides were made using the teasing mount procedure and lactophenol cotton blue staining.

Img.1: isolation of Endophytic Fungi from Ginseng



Img.2: Microscopic Identification of Ginseng Fungi

Morphological and Microscopic View of the Endophytic Fungi Isolated from Ginseng.

Table 1: Morphological and Microscopic Identification:

Sr. No.	Macroscopic characteristics	Microscopic characteristics
1	White cotton colonies. Black colour on the surface and corner of petri plate	Conidia is multi-celled with three darkly pigmented centre cells and clear pointed end cells, two or more clear, whisker-like appendages arising from the end cells Macroconidia were ovoid, 1-2 celled and slightly curved. Sterile mycelia

Fermentation and Extraction of Endophytic Fungi:
The inoculum was prepared by inoculating 1 cm2 mycelia agar plugs age 7 days into two 1000 mL Erlenmeyer flasks, each containing 500 mL of the SD broth medium. &e cultures were cultivated at 28°C with speed of 150 rpm. After three weeks of incubation, the fermented broth and fungal biomass were separated out by centrifugation for 15 min. Supernatant was then extracted thrice with equal volume of ethyl acetate (1:1, v/v). &e upper organic phase was concentrated to dryness under reduced pressure to obtain the crude organic extract. &e crude organic extract of the isolate AS2 was kept at 4°C [26].

Img.3: Fermented Broth

Img.4: Phase Separation

Antimicrobial Effect of Endophytic Fungi:

Endophytic fungi, residing within the internal tissues of plants without causing apparent harm, represent a rich source of bioactive compounds with diverse biological activities. Among these activities, the antimicrobial potential of endophytic fungal metabolites has garnered significant attention. These metabolites exhibit inhibitory effects against a wide range of pathogenic microorganisms, including bacteria, fungi, and even certain parasites. Harnessing the antimicrobial properties of endophytic fungi holds promise for the development of novel therapeutics, agricultural pesticides, and industrial antimicrobial agents.

Mechanisms of Antimicrobial Action:

Endophytic fungi produce a plethora of secondary metabolites, including alkaloids, terpenoids, polyketides, and peptides, many of which possess antimicrobial properties. These metabolites exert their antimicrobial effects through various mechanisms, including:

- Cell Membrane Disruption: Certain secondary metabolites disrupt microbial cell membranes, leading to leakage of intracellular contents and eventual cell lysis.
- Enzyme Inhibition: Some fungal metabolites inhibit key microbial enzymes involved in essential metabolic processes, disrupting microbial growth and survival.
- DNA/RNA Interference: Certain metabolites interfere with microbial nucleic acid synthesis or function, leading to inhibition of replication or transcription processes.
- Biofilm Inhibition: Endophytic fungal metabolites have been shown to prevent or disrupt microbial biofilm formation, thereby reducing microbial resistance and enhancing susceptibility to antimicrobial agent.

Img. 5: Antimicrobial Effect of Endophytic Fungi

Table: Antimicrobial Activity

Sr. No	Sample	Zone of Inhibition (mm) 1 µl	Zone of Inhibition (mm) 2 µl	Zone of Inhibition (mm) 3 µl
1	Ampicillin (Standard)	12.5	16.8	17.2
2	Ginseng (Test)	7.2	9.8	12.5

III. RESULT AND DISCUSSION

The study on endophytic fungi from panax ginseng revealed its antimicrobial properties. The fungi were extracted from plant roots and analyzed using ethyl acetate. The resulting alcoholic extracts were tested for antimicrobial activity, with the highest activity observed in panax ginseng extract. The ginsenosides, the active compounds found in panax ginseng species, exhibit a wide range of pharmacological activities like antioxidant activity, anti-inflammatory effects, anticancer properties, immunomodulatory effects, antidiabetic effects and cardioprotective activities.

CONCLUSION

Endophytic fungi isolated from ginseng exhibit significant antimicrobial properties. These fungi, which live within the roots of ginseng plants without causing apparent harm, produce a variety of bioactive compounds. Research indicates that these compounds have the potential to inhibit the growth of a wide range of pathogens, including bacteria, fungi, and viruses. The antimicrobial activity of endophytic fungi from ginseng is attributed to secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolics. These metabolites interfere with microbial cell walls, membranes, and essential enzyme functions, leading to the inhibition of pathogen growth. The study of these fungi highlights their potential as sources of new antimicrobial agents, which could be crucial in the fight against antibiotic-resistant bacteria and other pathogens. Further research and development could lead to the isolation and synthesis of these compounds for use in medical, agricultural, and pharmaceutical applications. In conclusion, endophytic fungi from ginseng represent a promising area of study for discovering novel antimicrobial agents. Their diverse and potent bioactive compounds offer a natural and effective means to combat various microbial infections, presenting an alternative approach to conventional antibiotics.

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