ENDOPHYTIC FUNGI ASSOCIATED WITH EDIBLE GREENS AND ITS POTENTIAL APPLICATIONS

Abstract

Endophytic microbes are well-known for producing secondary compounds, that shield the host from invading pathogens. Without causing any adverse effects on the host plant, they survive within the tissues of plants in either symbiotic or mutualistic relationships. Fungal endophytes are epitomes of numerous secondary metabolites. Endophyte-derived secondary metabolites are gaining popularity due to their numerous potential applications in agriculture, industry and medicine. Furthermore, it is necessary to have a solid understanding of the dynamics between edible greens and endophytic fungi, as well as how these fungi are an important source of phytochemicals and may impact their hosts and produce a range of positive effects. Thus, with a focus on understanding the interface between endophytic fungi and edible greens, this chapter will provide an information regarding novel aspects of some natural products associated with fungal endophytes that have substantial medicinal value in combating diseases and eventually enable discovering new products. In the near future, this insight could impact the adoption of enhanced therapeutic products based on edible greens.

Keywords: Edible greens, Endophytic fungi, Bioactive compounds, Industry, Medicine.

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I. INTRODUCTION

Microorganisms are essential sources of bioactive natural chemicals, which hold immense promise for creating new drugs useful in industry and agriculture [1]. Most of the current antibiotics and therapeutic drugs were derived from Bacteria, fungal filaments and actinomycetes. Secondary metabolites derived from the aforementioned categories have proven to be highly effective. Approximately one-third of all medications today are derived from natural sources [2]. In the year 1866, De Bary was the first one to coined the term "endophyte." Endophytes complete their life cycles within a plant without exhibiting noticeable disease signs and are located locally or all over a plant [3]. Endophytes have modified themselves to the specific conditions of the plant's microenvironment through genetic variety, including the acquisition of DNA from some plants [4]. Endophytes started to produce plant metabolites or the precursors of those metabolites depending on how well they adapted and acquired genetic material [5]. There have only been a handful of plant species for which endophytes have been studied; hence,there is a great deal of room for the discovery of new endophytic microbes and the bioactive substances they produce, particularly in traditional medicinal plants. Endophytic microorganisms are transported vertically from one host to another or mutually develop with their hosts and have interactions ranging from opportunistic to highly mutualistic [6]. They thrive in accomplishing this by making their hosts more resistant to environmental and biological threats, which in turn contribute to the overall health of the plant [7] [8]. Many useful metabolites, which include phenols, terpenoids, alkaloids, quinones, isocoumarin derivatives, steroids, flavonoids, peptides, and phenolic acids, are produced by endophytes these chemicals are further used as novel structural metabolites that can be utilized to study various biological phenomena. Some of the natural products for example antibiotics, antimycotics, immunosuppressants and anticancer compounds has been discovered after the separation and cultivation of individual endophytes, followed by the purification and characterization. Few research have been done on the connection of endophytes with edible greens, compared to other types of plants, such as medicinal plants, mangroves, tropical forest trees, and grasses. As a result, we investigated the endophyte relationships in these delicious greens that we eat. The purpose of this review was to give a quick overview of the naturally occurring bioactive substances as well as possible uses for endophytic fungus found in edible green plants.

II. IMPORTANCE OF EDIBLE GREENS

Humans historically relied on plants for nourishment, therapeutic, as well as architectural materials. It is commonly known that people have used plants as healthcare for centuries [9]. However, there is not much knowledge about implementing edible green plants for food. Several residents who live in villages and remote areas rely on edible green plants that grow naturally. Some edible greens were historically documented in ancient Greece and in the middle ages. The leaves that were part of ancient traditional diets are still to be found in gentle generally they are easily cultivated in the inhabited places, indicating that they are the leftovers of ancient cultivated plants. Some of the edible greens are consumed in Indian cuisine example curry leaf (*Murraya koenigii*), fenugreek (*Trigonella foenum graecum*), Brahmi (*Centella asiatica*), keerae (*Amaranthus sp.*) and are also recognised for their therapeutic properties. Since edible greens have many health benefits such as low in fat, high in dietary fibre, nutrients like folic acid, vitamins, potassium, and magnesium. Additionally, they contain phytochemicals like lutein, beta-cryptoxanthin, zeaxanthin, and beta-carotene which have been proven to reduce the risk of developing certain cancers like breast and lung cancer and also assisted in avoiding heart disease and stroke. The following are some examples of edible green plants:

Table 1: List of Few Edible Greens and their Modes of Consumption

Botanical name	Family	Season Consumed	Parts used	Mode of consumption
Basella alba L.	Basellaceae	Year round	Stem & leaves	Cooked as samber and tambuli
Talinum fruticosum	Basellaceae	Year round	Stem & leaves	Cooked as samber and tambuli
Plectranthusa mboinicus	Lamiaceae	Year round	Stem & leaves	Cooked as chatney, tambuli and samber.
Amaranthus mangostanus	Amaranthaceae	Year round	Leaves & young shoots	Cooked as curry and samber along with other leaves.
Solanum nigrum	Solanaceae	Aug-Oct	Tender stem & leaves	Cooked as samber.
Alternanther asessilis (L) R. Br.	Amaranthaceae	Raining & summer	Tender stem & leaves	Cooked as samber and palyam.
Coriandrum sativum	Apiaceae	All year round	Leaves & tender stem	Used for garnishing foods and in salads
Cucurbita pepo	Cucurbitaceae	All year round	Leaves	Cooked as curry and samber.
Alternanther asessilis	Amaranthaceae	Wet season	Tender leaves & stem	Cooked as curry and samber
Trigonella foenumgraecu m (L.)	Apaiaceae	All year round	Fresh leaves	Used for palya preparation
Moringa oleifera L.	Moringaceae	All year round	Leaves	Eaten after frying and roasting also used in samber and curry
Mentha spicacata	Lamiaceae	All year round	Leaves	Used for garnishing foods, in tea and also in some foods.
Spinace aoleracea	Amaranthaceae	All year round	Young leaves	Boiled in water and mixed with flour of ragi to prepare roti.

III. IMPORTANT OF ENDOPHTIC FUNGI

Endophytes have been found in almost all vascular plant species that have been investigated. Endophytes play an essential role in edible greens and are called plant mutualists since they acquire nutrients and protection from the host plants simultaneously the host plant my benefit from enhanced competitive abilities. Studies suggests that plants infected with endophytic fungus have a discernible advantage over non-endophytic plants against stress (biotic and abiotic) [10] [11]. Certain metabolites that promote disease resistance are known to be produced by endophyte-associated plants that exhibit symbiotic activity. These endophytes also create secondary metabolites including lytic enzymes and antibiotics that provide defence against different infections[12]. Because of their symbiotic relationship with endophytes, which causes their reaction to be more dependable and quick than in non-symbiotic plants, the plants connected with endophytes were also shown to be resistant to abiotic stresses including heat, salt, and drought [10]. Endophytic fungus live in symbiotic relationships with their host plants, mimicking their chemistry and, astonishingly, producing identical compounds to those of their hosts. As a result, they are used in the synthesis of significant beneficial compounds including vinblastine, vincristine, taxol, and podophyllotoxin, among others. Endophytes are less expensive sources of bioactive substances than the traditional approach of using plants, which is expensive and scarce [13]. Many plants have been reported to harbour fungal endophytes, including *Fusarium*, *Colletotrichum*, *Acremonium*, *Cryptosporiopsis*, *Eutypella*, *Alternaria*, *Apiospora*, *Aspergillus*, *Bartalinia*, *Cephalosporium*, *Chaetomium*, *Chloridium*, *Choanephora*, *Trichoderma*, *Emericella*, *Eupenicillium*, *Eutypella*, *Fusarium*, *Gliocladium*, *Hypoxylon*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Pseudomassari*, *Quercina*, and *Talaromyces*. These endophytes are recognised for their natural products, which display a wide range of biological activity. Furthermore, there has been an increase in global publications on the secondary metabolites produced by endophytic fungi on a variety of hosts. It is currently recognised that a number of natural substances possess antimicrobial, antiviral, anticancer, antioxidant, insecticidal, antidiabetic, and immunosuppressive qualities, among other qualities [14] [15].

Isolation and Identification of Endophytic Fungi: The process of isolating endophytic fungi begins with gathering plant material and pre-processing it using conventional techniques, such as surface sterilisation and slicing [16][17] thereafter the plant bits are placed on the "agar medium" in Petri plates, calibrated with the proper antibacterial agents, and then placed in liquid/solid media that is rich in nutrients. After a sufficient incubation period, fully developed endophytic fungi were detected using standard morphological characteristics such as spore shape, size, and colour, as well as other reproductive structures. Through the use of publications and authentic species descriptions, the sporulated strains are identified [18]. The selected strains can be verified and validated using molecular biology methods [19]. Internal transcribed spacer (ITS) sequencing using polymerase chain reaction (PCR) and fungal species identification using 18S rRNA are two emerging methods for identifying endophytic fungi utilising molecular biology techniques [20]. Following identification, liquid medium (potato dextrose broth) has to be inoculated with the active endophytic fungus, and the mixture must be incubated for several weeks in order to facilitate large-scale cultivation. For the extraction of bioactive compounds, the ideal culture conditions must be maintained. For example the concentration of components for the endophyte *Pestalotiopsis microspora* in the production of taxol has been thoroughly researched. The production of taxol has risen due to the optimised formulation with less phosphate and a high concentration of sodium benzoate in the "medium." Inhibitors of cholesterol production, like triadimefon and tebuconazole, have also demonstrated enhanced taxol output. [21]. It has been shown that endophytic fungus and their host plants engage in many kinds of interactions throughout their lives. Hence, even a slight variation in the in vitro growing conditions can have a direct impact on the nature and variety of secondary metabolites which the endophytes generate [22].

IV. ISOLATION OF BIOACTIVE COMPOUNDS

The separation and purification of bioactive substances from endophytes is a critical step. Following the endophyte's successful cultivation, the liquid filtrate from the fully developed fungus, or fractions isolated from growth medium, must undergo bioassays to verify the endophyte's suitability for isolating the essential bioactive compounds. The most popular technique uses an organic solvent to extract liquid-liquid from fungal growth medium. Depending on the target metabolite's solubility, several solvents were utilised either alone or in combination throughout the extraction process. The most popular solvents for extracting metabolites from culture broth and drying them by flash evaporation include ethanol, methanol, dichloromethane, hexane, and ethyl acetate. The resulting fractions undergo chromatographic procedures, including TLC and HPLC, for further purification as needed. Furthermore, mass spectrometry (MS), gas chromatography (GC), or nuclear magnetic resonance (NMR) will be used to validate the recovered fraction. For determining structure, NMR and MS are the two primary methods utilised. In addition to these, X-ray diffraction (XRD) is also used as a potential method for crystallized Biomolecule [20]. Very recently combination of separation methods with detection techniques known as hyphenated techniques have shown greater impact on isolation, purification and identification of bioactive from the crude extracts which help for both qualitative and quantitative investigation of unidentified molecules in complex natural extracts or fractions. Taxol was obtained from Taxus cuspidate culture medium by using dichloromethane, purified and quantified employing HPLC, and the structure was confirmed by LC–MS and 1H NMR spectroscopic analyses [21]. Extraction of vinblastine and vincristine from fungal endophyte *Fusarium oxysporum* were carried out using ethyl acetate and purification was performed employing silica gel column chromatography, followed by HPLC. The molecular mass of the purified compounds was identified through Electrospray ionization mass spectrometry (ESI-MS) and Tandem mass spectrometry (MS-MS) analysis followed by 1H NMR analysis [16]. In general, natural extracts that represent extremely complex mixtures of various compounds, could be determined effectively by employing suitable hyphenated techniques. LC-PDA and LC-MS are the two mainly used methods for the analysis of natural compounds. LC-NMR, along with various multiple hyphenated techniques such as LC-PDANMR- MS, has also become well-accepted technique. The analysis of a broad range of sample from small nonpolar compounds to large polar constituents can be carried out by available types of LC-MS systems [24]. “Metabolomics” is a newly emerging area involving the study of detailed analysis of metabolites from natural sources. The metabolite profiling essentially requires highly sophisticated analytical techniques, for example, various types of hyphenated techniques which can analyze metabolites without the isolation and fractionation of individual one. Many studies are reported on metabolomics by different researchers, for example the metabolomic study of the metabolite profiling and gene expression of an anthocyanin chemotype in red and green forms of *Perilla frutescens* using LCPDA- MS. Further, studies were reported on cell-specific anthocyanin accumulation and localization of anthocyanidin synthase followed by gene expression through the mRNA differential display of two chemo-varietal forms of *P. fructescens* [25] [26].

V. POTENTIAL APPLICATION OF ENDOPHYTIC FUNGI

1. **In Pharmaceutics:** The ability of fungal endophytes to produce a variety of secondary metabolites that are utilised in a variety of biological processes has made them an extremely important subject of study in recent years. According to the above mentioned information, it was discovered that the secondary metabolites isolated from endophytic fungus have cytotoxic, antibacterial, anticancer, and antidiabetic properties. These bioactive substances could aid in the invention of novel natural products that have a significant potential for usage in medicine to advance humankind and society. The following list includes the various endophytic fungal bioactivities that have been described by various researchers:

2. **Anticancer:** Cancer refers to a category of diseases that are distinguished by the unregulated multiplication and proliferation of aberrant cells. Any tissue in the body is capable of developing into cancerous tissue. It is possible that death will occur if the spread of the infection is not stopped. As a result, there is an immediate demand for the development of new sources of innovative medicinal chemicals. Nature has always been a potential source of novel pharmaceutical compounds, and plants have a long history of success in treating cancer. It is estimated that between 75 to 80 % of chemically active chemicals are obtained from natural sources. Vinblastine, Vinca alkaloids, and Vincristine were the first plant-derived anticancer drugs that were discovered and developed in the early 1950s. Other plant-derived anticancer medications came shortly thereafter. This cleared the door for the identification of many novel chemotherapeutic drugs displaying a wide spectrum of activities that are cytotoxic [27]. The bioactive compounds found in hundreds of plant species, animal species, marine species, and microbes have already been utilised in treatment as a possible source of anticancer biologically active agents. The active compounds contain a wide spectrum of chemical variety [28]. The majority of these medicines have anticancer action, and this is achieved by inhibition of topoisomerase II [29]. *Pestalotiopsis microspora* that was isolated from the critically endangered *Torreya taxifolia* tree yielded toreyanic acid, which is a particular cytotoxic quinone dimer. Camptothecin, Vincristine [13], chaetominine [30], Ergoflavin [31], Phomoxanthone B [32], and a great number of other compounds isolated from endophytic fungi had demonstrated substantial in vitro anticancer activity. For the treatment of cancer, paclitaxel, camptothecin, emodin, and hypericin are the most common substances utilised [33]. Many of the endophytic fungi have high cytotoxicity against a variety of cancer cell lines, which may be helpful in the search for primary anticancer medicines [34] [35][31].

3. **Antimicrobial Activity:** Endophytes carry out a resistance strategy to withstand the invasion of pathogens by creating secondary metabolites that have antibacterial activity. Several compounds from endophytic fungi have been discovered to have antibacterial action. Periconicins A and B, phomopsichalasin, and javanicin are all examples of antimicrobial compounds that possess antibacterial action. Because certain antimicrobial compounds produced by endophytic fungi are effective not only against human infections but also against plant pathogens, these agents have found widespread use in the agricultural industry. It is considered that testing endophytes for the presence of antimicrobial chemicals is a viable strategy to combat the growing problem of drug-resistant bacteria in human and plant pathogens [36]. There are several publications on

the antibacterial action of fungal endophytes, such as those published by [37] [38]. Microorganisms of many different kinds are continually invading the human body but only a tiny fraction of these, less than 1% of bacteria, are capable of invading our body (the host) and causing infection. The gram-positive bacteria known as *Staphylococcus aureus* is predominantly carry responsibility for infections that occur after surgical procedures as well as food poisoning. *Escherichia coli*, a gram-negative bacterium that is found in human intestines and can lead to infections of the lower urinary tract or septicemia, usually present in humans [39]. In animals, zoonotic diseases such as gastroenteritis can be caused by some strains of pathogenic bacteria such as *Salmonella* and *Camphylobacter* [40]. *Salmonella* are gram negative bacteria responsible for gastroenteritis [41]. Infections caused by *Pseudomonas aeruginosa* can occur in individuals who are normally healthy as well as in people who are hospitalised or have a compromised immune system [42]. Antibiotic medication is a tried-and-true remedy for the treatment of infections caused by microorganisms. The discovery of penicillin leads to the development of a wide variety of antibiotics and have proven to be effective in the treatment of infectious disorders and a potent weapon that can be used to prevent and treat sickness [43].

4. **Antioxidant:** Antioxidants have been seen as a potentially life-saving medicine for the treatment of diseases caused by reactive oxygen species (ROS) [44]. It would suggest that the endophytic fungi that are linked with plants are a rich source of new antioxidants. Many studies have shown that fungal endophytes possess powerful antioxidant properties [45]. Endophytic fungus has been exploited for a large number of chemicals that have antioxidant properties. Pestacin and isopestacin were extracted from *Pestalotiopsis microspora,* an endophyte that was isolated from the plant *Terminalia merobensis* found in Papua New Guinea. Isopestacin was shown to scavenge both superoxide and hydroxyl free radicals, which accounts for its antioxidant action. It has been calculated that pestacin's antioxidant activity is at least 10 times stronger than that of trolox, a derivative of vitamin E. This is achieved through the breaking of an extremely reactive C-H bond and, to a lesser extent, through the abstraction of O-H from the molecule.

5. **Diabetes:** Diabetes is one of the most significant health concerns and emerged as the most prevalent disease. It has been discovered that a significant portion of this rise in diabetes is taking place in developing countries, with the majority of the population at risk falling between the ages of 55 and 60 [46]. Diabetes is a disease that can lead to severe health complications, permanent impairment, a significant amount of suffering, and even death in some people. Diabetes is a condition that affects multiple functions and is caused by insulin not working as well as it should. The International Diabetes Federation estimated that there were 366 million cases of diabetes in 2011, and they anticipate that number will climb to approximately 530 million cases by the year 2030. [47]. Vascular complications in diabetes, particularly type 2 diabetes, are thought to be greatly influenced by oxidative stress [48]. The scavenging effects of catalase, superoxide dismutase, and glutathione peroxidase may all contribute less to scavenging and more to producing reactive oxygen species (ROS) in people with diabetes. Microvascular and macrovascular damage, diabetic retinopathy, cancer, cardiovascular disease, and stroke are some of the consequences that can result from having diabetes [49] [50]. Insulin analogues, sulphonylureas, biguanides, thiazolidiones, and -glucosidase inhibitors are some of the types of antidiabetic medications that are currently on the market for the

treatment of diabetes. However, due to the need for long-term medication and the adverse effects of the medications that are currently on the market, there is a significant demand for effective and cost-effective agents for the treatment of diabetic conditions [51]. Synthetic drugs that are now in use include meglitinides like prandin and starlix, as well as alpha glucosidase inhibitors like acarbose and miglitol [52].

6. **Drug Development:** There is a growing demand for the commercialization of bioactive metabolites that have the potential to be used in medicine, particularly those that have a high therapeutic efficacy, low toxicity, and only a moderate effect on the environment. To find a solution to this issue, it is necessary to investigate the natural products and bioactive metabolites that are produced by plants and bacteria [53]. In order to have an impact on microorganisms, bioactive substances must bind to receptor sites on those organisms. As a result of recent advancements in computer science, we now have in silico technologies that enables high throughput screening for possible drug candidates but also introduces pharmaceutical research that is more scientifically informative and reasoned.

   The processes of finding new drugs and developing them typically involve the use of either naturally occurring or synthetically produced substances [54]. The use of herbal products is not restricted to nutritional applications; rather, it also plays a particular role in the treatment of a variety of disorders. A multidisciplinary method to the process of drug discovery, which begins with an analysis of the biological activity of a crude extract derived from its natural source and is then followed by fractionation. The activity fractions are then subjected to additional fractionation based on the biological activity, and this process continues until active molecules are obtained. Computational drug design is a relatively recent field that aims to make use of many information sources to speed up the production of novel medications that can modify the behaviour of therapeutically important protein targets. The majority of these computational strategies can be grouped into two distinct families: structure-based and ligand-based methods. Ligand-based methods make use of what is already known about active chemicals in relation to the target to make predictions about novel chemical entities that exhibit behaviour that is comparable [55]. Structure - based model may be developed from a library of molecules. There are several advantages to employing the structure-based drug design approach, one of which is that it does not need any previous knowledge of active ligands. By studying the drug's three-dimensional structure, it is possible to design new ligands with therapeutic potential. As a result, structure-based techniques provide a contribution to the development of new medications by facilitating the discovery and improvements made to the original lead chemical. It has been theorised that the integration of ligand- and structure-based methods can enhance the strengths of each method while also reducing the downsides of each method. As a result, the combination of these two types of methodologies has become a frequent approach in the practise of virtual screening.

VI. CONCLUSION

Endophytic fungi from edible greens are rich source of bioactive chemicals may be and may be exploited as a possible source of novel pharmaceuticals, since there has recently been a lot of interest for very effective antibacterial and anticancer treatments. There has been little extensive investigation along these lines. As a result, there is opportunity for future investigation. A single endophyte may create many bioactive metabolites. As a result, further *in vivo* investigations are required to learn more about their individual biological features to approach innovative natural products.

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