ENDOPHYTIC FUNGI ASSOCIATED WITH EDIBLE GREENS AND ITS POTENTIAL APPLICATIONS

Tejaswini HK. and Jayashankar M.

Department of Studies and Research in Microbiology, Jnana kaveri campus, PG Centre, Mangalore University, Kodagu, Karnataka, India.

tejaswinigowda1393@gmail.com

Abstract

Endophytic microbes are known for the production of secondary metabolites, which shield the host from invading pathogens. They reside in plant tissues, in either a symbiotic or mutualistic relationship, without harming the host plant. Fungal endophytes are epitomes of numerous secondary metabolites. Endophyte-derived secondary metabolites are gaining prominence due of their many potential applications in medicine, agriculture, and industry. In addition, it is necessary to have a solid understanding of the dynamics between edible greens and endophytic fungi, as well as how these fungi are a significant source of phytochemicals and can influence their hosts and produce a range of positive effects. Thus, with a focus on comprehending the interface between endophytic fungi and edible greens, this chapter will offer an insight into the novel aspects of some natural products of fungal endophytic origin that may have significant therapeutic potential in disease control and ultimately facilitate product discovery. This understanding may lead to a greater use of improved plant-based medications in the near future.

Key words: Edible greens, Endophytic fungi, Bioactive compounds, Industry, Medicine.

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 today's antibiotics and therapeutic drugs were derived from filamentous fungi, actinomycetes and bacteria. Secondary metabolites derived from these groups have proven to be quite effective. Approximately one-third of all medications today are derived from natural sources [2]. Endophytes are microorganisms that may complete their life cycles within a plant without creating visible disease signs. Endophytes can be located locally or all over a plant [3]. In the

year 1866, De Bary was the first person to use the term "endophyte." Endophytes have adapted 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 co-evolve with their hosts and have interactions ranging from parasitic to strongly 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]. Endophytes produce various functional metabolites, including terpenoids, alkaloids, quinones, isocoumarin derivatives, steroids, flavonoids, peptides, phenols and phenolic acids. These chemicals have further use as novel structural metabolites that can be utilized to study various biological phenomena. Novel antibiotics, antimycotics, immunosuppressants and anticancer compounds are only a few examples of what has been found after the isolation and culturing of individual endophytes followed by purification and characterization of some of their natural products. The association of endophytes from various groups of plants including medicinal plants, mangroves, tropical forest trees and grasses have been done but there are only few studies on the endophytes of edible greens. Therefore, we studied the endophyte associations of these edible greens that we consume. The aim of the present review was to provide a brief overview of the natural bioactive compounds and also potential applications of endophytic fungi associated with edible green plants.

Importance of Edible Greens

Since early times man had used plants for medicine, fuel, timber and food. The traditional knowledge on the use of plants as medicine is well documented [9]. However, the knowledge on the use of wild plants as food is very much limited. Large sections of population of Karnataka living in villages and remote forests depend on edible wild plants. Even the people in cities purchase wild edible plants marketed by village folk. There is no accurate documentation of folk knowledge on wild edible plants. The folk knowledge is fast disappearing due to factors such as migration of villagers to cities, input of high-quality food in markets, etc. Some of the edible plants are listed below:

Table 1: **List of few Edible Greens with mode of consumption**

Botanical name	**Family**	**Season Consumed**	**parts used**	**Mode of consumption**
Basella alba L.	Basellaceae	Year round	Stem and leaves	Cooked as samber and tambuli
Talinum fruticosum	Basellaceae	Year round	Stem and leaves	Cooked as samber and tambuli
Plectranthusam boinicus	Lamiaceae	Year round	Stem and leaves	Cooked as chatney, tambuli and samber.
Amaranthus mangostanus	Amaranthaceae	Year round	Leaves and young shoots	Cooked as curry and samber along with other leaves.
Solanum nigrum	Solanaceae	Aug-Oct	Tender stem and leaves	Cooked as samber.
Alternanther asessilis (L) R. Br.	Amaranthaceae	Raining and summer	Tender stem and leaves	Cooked as samber and palyam.
Coriandrum sativum	Apiaceae	All year round	Leaves and 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 and stem	Cooked as curry and samber
Trigonella foenumgraecum (L.)	Apaiaceae	All year round	Fresh leaves	used for palav 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.

The endophytes play an important role in edible greens and they are considered plant mutualists as they receive nutrition and protection from the host plants while the host plant may benefit from enhanced competitive ability. Evidence suggests that plants infected with endophytic fungi have distinguishable advantage against stress (biotic and abiotic) over non-endophytic [10] [11]. Some edible leaves 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. Brahmi (*Centella asiatica*), curry leaf (*Murraya*

koenigii), fenugreek (*Trigonella foenum graecum*) and keerae (*Amaranthus* sp.) are used in Indian culinary and are also known for their medicinal value. Since edible greens are low in fat, high in dietary fiber, and rich in folic acid, vitamin C, potassium, magnesium, phytochemicals such as lutein, beta-cryptoxanthin, zeaxanthin, and beta-carotene have the cancer protective properties and also reduce the risk of certain types of cancer, such as breast and lung cancer, and may contribute to the prevention of heart disease and stroke.

Important of Endophtic fungi

The endophyte-associated plants (mainly associated with the symbiotic activity with the endophytic fungi) known to produce some metabolites that induce resistance to pathogens. Further, the secondary metabolites produced by these endophytes such as antibiotics, lytic enzymes, etc. also impart protection from various pathogens [12]. The plants associated with endophytes were also found tolerant to the abiotic stresses such as drought, salt and heat, mainly because of symbiosis with endophytes triggers their response that is reliable and rapid when compared to non-symbiotic plants [10]. Endophytic fungi occur in a symbiotic mode within the host plants, imitate the chemistry of the host and amazingly they are able to produce the similar products like hosts. Thus, they are being employed for the production of important high-value compounds such as taxol, podophyllotoxin, camptothecin, vinblastine and vincristine, etc. Endophytes as sources of bioactive compounds that are less expensive when compared to the conventional method employing plants which is non-abundant and uneconomical [13]. Fungal endophytes such as *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* have been reported from different plants and are known for the natural products that exhibit a broad range of biological activity. Moreover, reports on the secondary metabolites from endophytic fungi of different hosts have been on the rise from all over the world. Several natural compounds are now known to have antibiotic, antiviral, anticancer, antioxidants, insecticidal, antidiabetic, immunosuppressive properties, etc. [14] [15].

Isolation and identification of Endophytes

Generally isolation procedure of endophytes starts from collection of plant material and pre-processing including surface sterilization, slicing etc., using standard methods

[16][17] followed by placing the plant bits on the ‘agar medium’ in Petri plates, amended with appropriate antimicrobial agents and later in nutrient-rich liquid/solid media. After a proper incubation time, fully grown endophytic fungi were identified based on conventional morphological characters such as shape, size and colour of spores and other reproductive structures. The strains that are sporulated are identified through the monographs and original descriptions of species [18]. The identified groups can verify and confirmed employing molecular biology techniques [19]. The recent approaches to identify endophytic fungi using molecular biology techniques are polymerase chain reaction (PCR) based sequencing of internal transcribed spacer (ITS) and identification of fungal species through 18S rRNA [20]. After identification, the active endophytic fungi are to be inoculated into liquid media (potato dextrose broth) and incubated for a number of weeks for cultivation (large scale). It is necessary to maintain the optimum culture conditions for the production of bioactive compound have systematically studied the concentration of ingredients for the endophyte *Pestalotiopsis microspora* in the production of taxol. The optimized formulation of lower phosphate and the high sodium benzoate concentration in the ‘medium’ has shown increased taxol production. Sterol biosynthesis inhibitors, such as tebuconazole and triadimefon, also shown increased yield of taxol [21]. It is confirmed that endophytic fungi always remain in versatile interactions with the host plant and other endophytes. Hence, the kind and range of secondary metabolites produced by these endophytes can be directly affected by even a minor change in the conditions of *in vitro* culture. It is known by the fact that endophytic fungi always remain in versatile interactions with the host plant and other endophytes and even slight variation in the in-vitro cultivation conditions can directly affect the kind and range of secondary metabolites they produce [22].

Isolation of Bioactive Compounds

The isolation and purification of bioactive compounds from endophytes is a crucial process. After successful culture of the endophytes, the fractions extracted from growth media (liquid filtrate from the completely grown fungi) are to be subjected to the bioassays to confirm the suitability of the endophyte in order to isolate the desired active compounds. The most common methodology involves liquid-liquid extraction employing organic solvent from liquid media of fungal culture. Different solvents alone or in combination were used for the extraction based on the solubility of the desired metabolite. Ethyl acetate, methanol, dichloromethane, hexane, ethanol are the most commonly used solvents used for the extraction of metabolites from the culture broth and ultimately dried by flash evaporation.

The selection of the solvent for the extraction is mainly on the solubility of the desired component to be extracted. The obtained fractions were subjected to chromatographic techniques such as TLC and HPLC for further purification if required. Finally, the recovered fraction will be confirmed by Gas chromatography (GC), nuclear magnetic resonance (NMR) or mass spectrometry (MS). NMR and MS are the two main techniques used for structure determination. 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].

Potential application of Endophytic fungi

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:

i) 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].

ii) 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].

iii) 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.

iv) 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].

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.

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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