International Journal of Secondary Metabolite
2018, Vol. 5, No. 4, 288-303

DOI: 10.21448/ijsm.482798

Published at http://www.ijate.net http://dergipark.gov.tr/ijsm **Review Article**

Brief Review on Fungal Endophytes

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Abstract: Fungal endophytes are the critical organisms in the environment residing in the plant tissues without showing any harmful effects on its host life cycle. The finding of fungal endophytes in natural habitat has been insufficient due to some non-sporulating and non-culturable fungal endophytes by traditional method. Several investigations on fungal endophytes in plants have resulted in an excessive knowledge of the group. This review emphasis on the biology of fungal endophytes, their discovery, isolation, identification by morphological and molecular methods, production, purification and structure elucidation of the bioactive compounds.

ARTICLE HISTORY

Received: 28 July 2018
Revised: 25 October 2018
Accepted: 01 November 2018

KEYWORDS

Fungal endophytes,
endophytism,
isolation,
bioactive compounds,
structural elucidation

1. INTRODUCTION

In present scenario, we are dealing with different emerging diseases in our society due to rehabilitated environment and life style. Several researchers are working on the different emerging diseases to understand and cure them by using different natural and chemical formulations however still many areas are untouched due to poor knowledge, techniques and myths [1,9,12,31,47,57]. One such area is fungal endophytes. As far as fungal endophytes are concerned, researchers failed to explain their potential medicinal applications in emerging disease [19,37,47,57,61]. Some attempts are made to purify fungal endophytes from natural habitats though not effective as compared to other microbes [41,42]. Researchers are trying to isolate new bioactive compounds from newer species of fungal endophytes for medicinal, agricultural and industrial applications though they are unable to explain their origin, pathways

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ISSN-e: 2148-6905 /© IJSM 2018

and mechanism of actions [4,17,27,46,48]. The need of the hour is to encourage researchers to isolate novel bioactive compounds [1,2,4,5,6,7,28,34,45,61].

The finding of novel bioactive compounds involves isolation, structural elucidation and knowing the biosynthetic pathway of the secondary metabolites. This is an area of significant curiosity to scientists due to the structural diversity, complexity and various bioactivities of isolated compounds [7,16,42,59]. Crude natural products have been used unswervingly in drugs manufacturing which forms the basis for deriving semi-synthetic natural products [13,16,50,51].

The objective of this review is to acquaint the researcher with information about the biology of fungal endophytes, and in particular, on the aspects of endophyte research.

2. HISTORY

Ecosystem has fungi as significant module. Certain critical practices in ecosystems such as transportation of nutrients from one environment to other, decomposition, recycling etc. are carried out by fungi. Diverse evaluations suggest that the earth may be hosting more than a million fungal species and approximately 3% have been truly identified [10,46,50] which constitute the second largest cluster of tropical ecosystems of the world. Since they are heterotrophic, they have saprophytic or parasitic association with the hosts. However, in the path of evolution, the fungi developed various types of relationships with them. One of these associations is ‘endophytes’ [10,12,37]. The fossil archives shows that plants are linked with endophytic and mycorrhiza fungi for approximately 400 million years and most doubtless they were associated when plants started colonizing the land, thus they played a very lengthy and vital role in the evolution [10,42,50,51].

In 1866, De Barry introduced the term "endophyte" for those microbes that reside inside the healthy tissues of plants without causing any apparent diseases [12]. Carroll (1986) coined the term 'endophyte' for those organisms that causes asymptomatic infections within the plants [12]. Petrini (1979) elucidated Carroll's definition as a commensalism in plants [37]. Wilson (1995), further expanded endophytes and included both fungi and bacteria as a commensals [39,53]. Stone et al. (2004) have defined endophytes as “infections are inconspicuous, the infected host tissues do not show symptoms at least for transient period and the microbial colonisation can be demonstrated to be internal.” This definition attributes the tenure endophyte to a short-lived grade. It may therefore include an accumulation of microorganisms with dissimilar life history approaches as well as latent pathogens and virulent pathogens in the premature stages of infection [16,47].

Inappropriately, if we take the correct definition, it can include all pathogens at some stage of their development. Therefore, additional characteristic of “not causing apparent harm” as described by Petrini is important as it refers to reflect the lack of macroscopically visible symptoms. Nowadays this term is prolonged as endophytic microorganisms, which includes fungi and bacteria with Actinomycetes, resides intra or intracellularly for their whole or at least part of life cycle [3,4,5,6,7,9].

In 20th century, extensive work has been carried out for the isolation of fungal endophytes from temperate and rainforest region plants because of their largest tropical biodiversity [10,11,13,17,58]. Although most studies are on flowers, leaves and stem as compared to bark, fruits, roots and seeds [5,30,31,41]. In Indian natural therapy and Ayurveda, seeds, bark, fruits and roots have been proved safe and natural remedies for different aliments therefore one can go for bark, fruits, roots and seeds in the view of fungal endophytes purification [2,6,7].

3. ENDOPHYTISM

Darwin proposed the idea that diversity can have tough effects on ecosystem practices. Many hypothetical models and investigational test revealed important functions for diversity including the improvement of primary efficiency, nutrient holding, nutrient flow, water availability and resistance to pathogen invasion [8,9,21]. The diversity of fungal endophyte is confirmed not only in the specificity of the hosts and their morphology, but also in the types of assistances that they offer to the host. Today fungal endophytes have been isolated from plants ranging from large trees, palms, sea grasses and even from lichens. The numbers of strains and species of endophytes vary drastically and generally depend on the concentration of the research study [11,12,13,28,46]**.**

In general, very little information is available about distributions and factors affecting the shape of fungal endophytes at local and regional level [14,15,43,44]. However research has been focussed on relative importance of host and habitat features in shaping local level. As far as tropical endophytes are considered different abiotic factors such as humidity, ultra violet radiation, desiccation and density of leaf litter play a major role [16,18].

It is expected, that the collection of endophytes from a selected plant species is an association of diverse ecological groups of fungal endophytes. For occurrence, fantasy is that at least some of the fungal endophytes endure to survive in the dead leaf tissue as leaf litter decomposers. Long ago, it is reported that some of the foliar endophytes of the mangrove *Rhizophora apiculata* continue to rise in dropped leaves and produce extracellular enzymes, which act on wall polymers of plant cells. Several research endorse that some fungal endophytic species are also litter decomposers [19,21,22,60] and they have capacity to remain as saprotrophs in dropped leaves. Endophytism represents a stage of fungal endophytes which surrogates between a saprotrophic and an endophytic lifestyle. Spores of such biphasic fungal endophytes in forests undergoing periodic ground fires unveil constitutive heat tolerance proving this hypothesis [20,51].

Expression of unambiguous genes during endophytic and saprotrophic phases have been observed for a root endophyte [23,24,58,61]. Comparable genomic studies are required to question whether multi-host endophytes, which were not subjected to host-guided specialization, have also evolved as an endophyte saprotroph biphasic directly by expressions of specific genes [8,25,59,61].

4. ISOLATION OF FUNGAL ENDOPHYTES

Isolation of fungal endophytes is the critical procedure as far as plant selection and impingement methods are concerned. One should understand the criteria used for the plant selection and methods.

4.1. Criteria for selection and identification of plants

The criteria used for the selection and identification of plants by the local peoples have been investigated by researcher [25,26,]. Thus, currently there are mainly two hypotheses for the selection of plants namely apparent and non-apparent. Apparent hypothesis involves shrubs and trees while non- apparent deals with only herbs. Apparent plant species produces high molecular weight organic compounds with low toxicity while non-apparent plants harvests low molecular weight organic compounds with high toxicity and bioactivity [27,29,32].

Criteria used for selection and identification of plants are as follows [33,37,41,42,4831].

1. Plants from a distinctive ecological environmental niche, and growing in unusual habitats and holding novel strategies for survival should be considered.

2. Plants with an ethno botanical antiquity, and used for traditional medicines should be designated for study, as squatting endophytes may be the source of the medicinal stuffs of this plant.
3. Plants that are widespread, having an unusual longevity, or have occupied a certain prehistoric land mass, are suitable for study.
4. Plants growing in areas of great biodiversity have the prospective for housing endophytes with prodigious multiplicity.
5. Plants enclosed by pathogen-infected and showing no symptoms are gatehouse for endophytes.
6. Fresh plant tissue is fit for isolation than older tissues, which often contain many other fungi that make isolation of slow growing fungi easy.

Which part of plants is effective for fungal endophytic research? Somehow, the answer for this question is under debate. According to literature, not only leaves, flowers and fruits are effective but also stem and roots are effective in the view of ethnobotany and pharmacognosy.

The collected plant samples are stored at 4°C. Isolation should be carried out as soon as possible after collection to avoid contamination by air microspore (Bacon & White 1994).

After the selection of plants, one can go for authentication with consultation with experts and voucher specimens to be deposited in a herbarium [41,42,48,58].

4.2 Surface sterilization of selected plant parts.

In laboratory**, p**lant materials are surface sterilized with surface sterilants as shown in Table 1, 2, 3 and 4. After sterilization, samples are dried and used for isolation process. The species of host plant, and host tissue type sampled and surface sterilization procedures vary according to the investigator. Some investigators have compared carefully the effects of different surface-sterilization procedures [36,40,43], isolation medium and sample-unit size [10] on isolation frequencies. We recommend that investigators experiment with these factors prior to initiating detailed investigations so that protocols optimal for recovery of endophytes from individual host species or specific organs and tissues can be established. For root tissues, serial washing may be preferable to surface sterilization to obtain demonstrative frequencies of fungal colonists [34,35,46].

Table 1. Surface sterilization method used for selected plant samples (1,2,3,4,30,37,41,53].

Plant samples	Sterilants immersion duration (seconds)			
	Running tap water	70% Ethanol	4% Sodium Hypochlorite	Distilled water
Leaves	300	30	60	60
Flowers	300	30	60	60
Roots	300	30	60	60
Stem	300	30	60	60
Fruits	300	30	60	60

Table 2. Surface sterilization method used for selected plant samples [7,24,27,28,61].

Plant samples	Sterilants immersion duration (seconds)		
	70% Ethanol	0.5 % Sodium Hypochlorite	Distilled water
Leaves	180	60	60
Stem	180	60	60
Seeds	180	60	60

Table 3. Surface sterilization method used for selected plant samples [25,31,34,45].

Plant samples	Sterilants immersion duration (seconds)		
	Running tap water	75% Ethanol	Distilled water
Leaves	300	30	60
Flowers	300	30	60
Roots	300	30	60
Stem	300	30	60
Fruits	300	30	60

Table 4. Surface sterilization method used for selected plant samples [50,51,55,59].

Plant samples	Sterilants immersion duration (seconds)			
	Running tap water	70% Ethanol	10% Commercial bleach	95% Ethanol
Leaves	300	120	120	30
Flowers	300	120	120	30
Roots	300	120	120	30
Stem	300	120	120	30
Fruits	300	120	120	30

4.3 Methods for isolation of fungal endophytes

4.3.1 Isolation by cutting of selected plant parts

Surface sterilized plant samples are dissected under sterilized conditions into small pieces (0.2cm X 0.21cm) and placed on selected artificial media at room temperature [27,31,37,34,38,40].

4.3.2 Isolation by Blender shaft

Surface sterilized plant samples were added to 250 ml of distilled water in beaker and shredded with blender shaft with solvent. Such shafted extract further spread on artificial media for the growth of endophytic fungi [21,38,45,55].

4.3.3 Isolation by Mortar and Pestle

To the surface sterilized plant sample, add 1ml of distilled water and 1g of sterile sand into mortar and crushed with a pestle. Resulting suspension were spread on artificial media for further growth of endophytic fungi [54,55,56].

Table 5. Media used for isolation of fungal endophytes

Media Name	Composition (g/L)	References
Wickerham medium	Malt extract 3, Peptone 5, Yeast extract 3, Glucose 10 pH- 7.2	[41,45,60]
SAB	Peptone 10, Dextrose 20, agar 15	[3,11]
YM agar	Malt extract 10, Yeast extract 2, Agar 20.	[48,55]
CYA	Czapek 10, Yeast extract 5, Sucrose 30, K_2HPO_4, Agar 15	[21,24,46]
YES	Sucrose 150, Yeast extract 20, $MgSO_4.7H_2O$ 0.5, $CuSO_4.5H_2O$ 0.005, $ZnSO_4$. $7H_2O$ 0.01.	[31,54]
MEA	Malt extract 30, Peptone 5, agar 15, Chloramphenicol 0.1	[33,43,53]
PDA	Potato 200, Dextrose 20, agar 15	[2,3,4,5,112,13 41,42,60]

5. IDENTIFICATION OF FUNGAL ENDOPHYTES

Morphological identification of endophytic fungi by mycologists is a very critical step [26,48,54]. It includes development of fungus on standard media, examination of sexual reproduction and determination of growth characteristics. In recent years, according to taxonomical groups, few tests were added such as sub culturing, quick test for purity, examination of sexual reproduction, conidiogenesis and estimation of radial growth and media temperature [2,3,26,43].

Further reference sequences can be created from this identified culture [47,48,49,53]. The axenic culture is disinfected and Sequencing the ITS barcode and a subsequent NCBI Gen Bank BLAST search provide a match with an accessioned sequence. Further, fungal DNA extracted from the needles and ITS barcodes are amplified using different platforms such as NGS (e.g: Illumina MiSeq platform). A bioinformatics pipeline processes raw sequence reads and designates OTUs. Sequence does not result in an identification; e.g: 100% match with unidentified endophyte sequence or no similar sequences present in database [27,28,54,58].

Briefly, **m**olecular characterization of isolated fungal endophytes can be carried out by isolation of genome DNA, PCR amplification of ITS regions, DNA sequencing and sequences analysis [43,46,53,57,59].

Table 6. Fungal endophytes isolated from various plants

Host plant	Identified Endophytic fungus	References
Oryza sativa	*Alternaria alternata, Cladosporium tenuissimum, Epicoccum purpurescens, Fusarium equiseti, F. oxysporum, Hymenula cerealis, Phoma sorghina, Pleospora herbarum, Pythium* sp., *Trematosphaeria sp., Fusarium* sp. *Penicillium* sp. *Aspergillus* sp. *Paecilomyces* sp. *Pyricularia Sacc, Helminthosporium* sp. *Yeast, Sterile mycelium.*	[15,31,37]
Manilkara bidentata	*Xylaria* sp., *Colletotrichum crassipes, Pestalotiopsis versicolor*	[,4,6,27]
Lycopersicon esculentum	*Alternaria alternata, Colletotrichum gloeosporioides, Cladosporium* sp., *Penicillium* sp., *Arthrinium* sp., *Chaetomium globosum, Colletotrichum coccodes, Nigrospora sphaerica, Phomopsis* sp., *Ulocladium alternariae, Stemphylium botryosum*	[25]
Taxus cuspidate	*Alternaria* sp.	[31]
Nothapodytes foetida	*Neurospora* sp.	[2]
Camellia sinensis (Tea)	*Fusarium* sp., *Penicillium* sp., *Diporthe* sp., *Schizophillum* sp.	[2,6,31,56]
Coffee	*Aspergillus, Bipolaris, Cladosporium, Clonostachys, Colletotrichum, Epicoccum, Fusarium,Guignardia, Mycospharella, Phomopsis, Rosellinia, Talaromyces, Trichoderma, Xylaria*	[42,46]
Quercus variabilis	*Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., *Fusarium* sp., *Rhizoctonia* sp.	[56,59]
Azadirachta indica	*Phomopsis oblonga, Cladosporium cladorsporioides, Pestalotiopsis* sp, *Trochoderma* sp., *Aspergillus* sp., *Periconia, Stenella, Drechslera*	[30,48,51]
Huperzia serrata	*Acremonium* sp.	[26,27]
Ananas ananassoides	*Muscodor crispans*	[32,58]
Jatropha curcas	*Leptosphaeria* sp.	[44,52]

Table 6. Continues.

Paris polyphylla var. Yunnanensis	*Fusarium, Gliocladiopsis irregularis, Gliomastix murorum var. murorum, Aspergillus fumigatus, Cylindrocarpon, Podospora* sp., *Plectosphaerella cucumerina, Pichia guilliermondii, Neonectria radicicola*	[6,36,44]
Foeniculum vulgare , Lactuca sativa, Cichorium intybus, Apium graveolens	*Acremonium, Alternaria, Fusarium, Plectosporium*	[14,17]
Antiaris toxicaria	*Trichothecium, acremonium, Rhizoctonia*	[11]
Iris germanica	*Rhizopus oryzae*	[61]
Saussurea involucrate	*Cylindrocarpan* sp. *Phoma* sp., *Fusarium* sp.	[29]
Dendrobium devonianum	*Fusarium sp., Phoma sp., Epicoccum nigrum*	[58]
Podocarpus species	*Aspergillus fumigates*	[59]
Hemionitis ariflora	*Several endophytic fungi*	[21,22]
Oryza granulate	*Dothideomycetes, Arthrinium* sp., *Magnaporthe* sp., *Muscador* sp.	[31,41,46]
Actinidia macrosperma	*Acremonium furcatum, Cylindrocarpon pauciseptatum, Trichoderma citrinoviride, Paecilomyces marquandii, Chaetomium globosum*	[28]
Solanum cernuum Vell.	*Arthrobotrys foliicola, Colletotrichum gloeosporioides, Coprinellus radians, Glomerella acutata, Diatrypella frostii, Phoma glomerata, Mucor* sp., *Phlebia subserialis, Phoma moricola, Phanerochaete sordida, Colletotrichum sp.*	[55]

6. PRODUCTION AND OPTIMIZATION OF ENDOPHYTE DERIVED BIOACTIVE COMPOUNDS

6.1 Production of bioactive compounds from fungal endophytes

The symbiotic relationship among endophytic fungi and plants gives powerful ability to produce new bioactive compounds. However, there are main two substrate-based methods for the production of bioactive compounds such as Solid state fermentation and submerged state fermentation [14,21,56,32,44].

6.1.1 Solid state fermentation (SSF)

Solid State fermentation is widely used for the bioactive compounds production from the fungal endophytes [21,31,41,51,55]. This biomolecules are mostly metabolites generated by endophytic fungi grown on solid support selected for this purpose. In this fermentation process, different solid substrates such as Wheat bran, Rice bran, coconut oil cake, vegetable waste, gram husk, orange peel, sugarcane bagasse etc were used with pure cultures of endophytic fungi [14,21,56,32,44,50,61].

In environment, fungal endophytes breed on the ground; decomposing vegetables combinations under naturally ventilated conditions, Therefore SSF enables the optimal growth of endophytic fungi, permitting the mycelium to spread on the surface of solid compounds through which air can flow [14,32,59,61]. SSF uses culture substratum with low water levels. The solid medium contains both the substrates and solid support [21,56]. After fermentation, fermented media are mixed with effective solvent and further used for purification and analysis [14,32,56,59,61].

6.1.2 Submerged fermentation

In submerged fermentation, enzymes and other reactive compounds are submerged in a liquid such as alcohol, oil, or nutrient broth [21,42,24]. Endophytic fungi are sited in a small closed flask containing the rich nutrient broth with high volume of oxygen. The in situ production of enzymes results in production of bioactive molecule [27,28,35,49,58,60]. Batch Fed fermentation method is used commonly which utilizes the sterilised nutrients under optimized conditions along with fungal endophytes which increase in density [23,36]. The growth rate of fungal endophytes are maintained by the addition of nutrients, also reduces risk of overflow of metabolism [9,23,36].

6.2 Optimization of production of bioactive compounds from fungal endophytes

Optimization of both fermentation processes depends on considerations of carbon homes and nitrogen homes, inoculums, phosphorus, organic acids, surfactants, incubation period, temperature, moisture level and pH level under optimized conditions to achieve greatest production of bioactive compounds from fungal endophytes [13,21,31,33,36,59].

6.2.1. Effect of different medium

Effect of the different medium on the production of bioactive compounds were observed [13,21,31,33,36,59].

6.2.2 Effect of carbon sources

In order to determine the effect of various carbon sources on the production of bioactive compounds, main sugar was replaced by different carbon sources like cellulose, fructose, lactose, galactose, malt dextrin, mannitol and sucrose in the production media [13,33,36,59].

6.2.3 Effect of nitrogen sources

Effect of different organic (beef extract, casein, peptone, malt extract, tryptone, soybean meal) and inorganic (KNO_3, $NaNO_3$ and NH_2CONH_2) at 1% (w/v) as additional nitrogen source were studied [21,31,36,59].

6.2.4 Effect of inoculum amount

To study the effect of inoculum on bioactive compounds production, different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 % (v/v)) of activated culture were used [13,21,31,33,36,59].

Table 7. Media used for the production of bioactive compounds by fungal endophytes

Sr. No	Medium	Conditions	References
1	Liquid Wickerham medium	26^0C, 21 days	[21]
2	S_7 medium	26^0C, 21 days	[36]
3	Minimal medium	28^0C, 10-14 days	[15,16]
4	Lactose & Starch Casein broth	37^0C, 120rpm, 18 days	[24,31]
5	M_2 medium	28^0C, 124rpm, 7 days	[40,43]
6	C2 broth, Sabourauds broth, PDB, MEB	28^0C, 10 days	[21]
7	Nutrient Broth	30^0C, 120rpm, 5 days	[2,4,6,8,11,13,21,22,30]
8	Liquid fermentation	37^0C, 120rpm, 18 days	[30.31]
9	Nutrient Broth	30^0C, 124rpm, 24 hrs	[58]
10	Corn meal medium	26^0C, 21 days	[30,31]

6.2.5 Effect of inoculum time

10-24 h old culture at the interval of 2 h used as inoculum in order to determine the best suitable inoculum age for maximum production of bioactive compounds [13,21,31,33,36,59].

6.2.6 Effect of pH and temperature

The effect of different pH ranging from 4.0 to 10.0 observed on of bioactive compounds production. However to study the effect of different temperature, flasks were incubated at different temperatures (20-45°C) ([13,21,31,33,36,59].

6.3. Phytochemical analysis

The endophytic fungal extract can further be screened by phytochemical analysis, which serves as a key reserve for evidence on analytical and tangential methodology for the presence of bioactive compounds in the extract.

6.3.1. Test for Flavonoids

Test-tube containing 1-2 ml of fungal crude extract, 5-10 drops of dilute HCl, a piece of Magnesium strips were added and the solution was boiled for few minutes. A reddish pink or dirty brown coloration of solution indicates the presences of flavonoids in the extract [13,16,23,25,55].

6.3.2. Test for Alkaloids

The fungal crude extract is evaporated to dryness in boiling water bath. The residue obtained is dissolved in 2N HCl. The mixture treated with equal amount of Wagner's reagent. The reaction shows the appearance of brown precipitate, indicates presences of alkaloids [13,16,23,25,55].

6.3.3. Test for Terpenoids

1 ml of fungal crude extract is mixed in 1ml of chloroform and 3-4 drops of concentrated H_2SO_4 were added. A cherry red colour or reddish-brown precipitate at the interface indicates the presence of terpenoids [13,16,23,25,55].

6.3.4. Test for Cardiac glycosides

To 1 ml of fungal crude extract, add 1ml of $FeCl_3$ reagent. To this solution, add few drops of concentrated H_2SO_4 were added. Appearance of greenish blue colour within a few minutes indicates the presence of cardiac glycosides [13,16].

6.3.5. Test for Steroids

To 1 ml of fungal crude extract, add 1ml of chloroform and 1 ml of acetic anhydride and little concentrated H_2SO_4. A blue green ring indicates the presence of steroids [13,16,23,25,55].

6.3.6. Test for Saponins

The crude dry powder of fungal extract was energetically shaken with distilled water and was allowed to stand for 10 min. No froth indicates lack of saponins and stable forth more than 1.5cm indicated the presence of saponins [13,25,55].

6.3.7. Test for Phenols

The fungal crude extract is dissolved in 5ml of distilled water. Few drops of neutral 5% $FeCl_3$ solution was added. A dark green indicates the presence of phenolic compounds [13,16,23,25,55].

6.3.8 Test for Tannins

The fungal crude extract treated with alcoholic FeCl3 reagent. A bluish black colour, which disappears on adding little dilute H_2SO_4 followed by the configuration of yellowish brown precipitate, indicates the presence of tannins [13,16,23,25,55].

7. PURIFICATION AND STRUCTURE ELUCIDATION ENDOPHYTE DERIVED BIOACTIVE COMPOUNDS

Endophyte derived bioactive compounds include a broad diversity of structures and functionalities that provide a greatest pool of molecules for the production of different in-house products [6,9,13,16,23,35,41,55]. Many of these compounds can be found at very high concentration in nature while some found to be very low in concentration so that extraction is required to obtain sufficient amounts and their structural diversity and complexity create chemical synthesis unbeneficial [3,5,7,11,13,14,16,13,23,35,55]. From long time, Liquid-liquid extraction or solid- liquid extraction are used for the extraction purpose but nowadays pressurized liquid extraction, Subcritical & Supercritical extraction, and ultrasound assisted extraction are highlighted [3,5,7,11,13,14]. Fermented media is exposed to different solvent which takes up compounds of interest which is further centrifuged and filtered to obtain crude extract. Mostly, Hexane, ether, chloroform, acetonitrile, benzene, ethanol and distilled water is used in different proportion [3,5,7,11,13,14,16,13,23,35,55].

Purification is the physical separation of specific substances from contaminating substances. Purification includes mainly filtration, centrifugation, crystallization, distillation, chromatography; electrophoresis etc. While structure elucidation of endophyte derived bioactive compounds determination of chemical composition by NMR, mass spectroscopy, Crystallography, UV-visible spectroscopy etc. [1,2,3,5,7,11,13,14,16,13,17,23,35,55].

Table 8. The use of different solvents extraction for the recovery of endophyte derived bioactive compounds.

Solvents used	Raw Material	Compound of interest	References
Ethanol, methanol, n- hexane	Liquid Wickerham medium, solid rice medium	Alternariol, altearіol methyl ether, stemphylperylenol, bostrycin, Tenuazonic acid, indole-3, carbaldehyde, Cyclo (Threonylisoleucinyl), Aloesol, Deoxybostrycin, Equisetin, Citrinin.	[21]
Chloroform, methanol, n- hexane	Mycological medium	Taxol	[36]
n- hexane, chloroform, ethyl acetate, ethanol, methanol, butanol, petroleum ether	L.B & S.C broth	Alkaloids, phenolic compounds	[24,42]
Water	Plant extract	Triethylene glycol	[22]
Ethanol	PDB	Naphthoquinones	[2,44,45]
Chloroform, ethyl acetate	Minimal medium	Flavonoids, Saponins, Alkaloids,	[43]
n- hexane, chloroform, ethyl acetate, ethanol, methanol	NB	Pyrrolo, methyl-2-o-methyla-arabino pyranoside, Propionylfilicinic, benzene, Carboxylic acid.	[30,59,60]
Ethanol, methanol	Plant extract	Flavonoids, Saponins, Tannins, Terpenoids	[35,57]

Table 9. Different purification and structural elucidation techniques for the recovery of endophyte derived bioactive compounds

Methodology		Compounds of interest	References
Purification	Structure elucidation		
TLC, HPLC, SGC	NMR, Crystallography	Steriods, ergosterol, cerevisterol	[31,44]
TLC, HPLC	GC-MS, NMR	Taxol, Taxane III	[45,61]
TLC, VLC, LC, HPLC	Ms, ESI-MS, LC-MS, HRMS, NMR	Alternariol, altearial methyl ether, stemphylperylenol, bostrycin, tenuazonic acid, indole-3, carbaldehyde, cyclo (Threonylisoleucinyl), aloesol, deoxybostrycin, equisetin, citrinin	[21]
TLC, VLC, HPLC	GC-MS, NMR	Shamiminol	[15]
TLC, CC, HPLC	NMR, ESI-MS	Cytochalasin J, cytochalasin H, 5- epialtenuene alternariol monomethylether alternariol, cytosporone C	[4,5]
TLC, HPLC	NMR	Resaveratrol	[2,58]
HPLC	ESI-MS, NMR, TOF-MS	Gold nanoparticles	[34,42]
TLC, CC, HPLC	GC-MS, NMR	Teadenol A	[61]
TLC,UV-VS	GC-MS, NMR	Alkaloids	[22,41]

8. NOVEL FUNGAL ENDOPHYTES VERSES NOVEL BIOACTIVE COMPOUNDS

Discovering novel bioactive compounds from undiscovered endophytes is current trend. Not all endophytes are culturable [47,48] and these may produce useful bioactive metabolites. There are numerous techniques to detect unculturable fungi and these include whole DNA analysis monitored by DNA cloning, DGGE or T-RLFP [34,41,42,43]. Therefore, apart from isolating culturable endophytes from different taxonomic groups of plants and plants growing in different habitats, shotgun metagenomics for endophyte community analysis and function-based screening of their metagenomics libraries could be used to harness the unculturable and truly cryptic endophytes from environmental samples for drug production. Such a metagenomics approach has been quite rewarding with soil samples [4,5,24,50]. Metabolomics of endophyte infected and endophyte free plant hosts could reveal intersections in secondary metabolite paths that may be pushed into synthesizing novel chemical species or lead compounds another possibility of manipulating these chemo diverse organisms [50,51,60]. Additionally, other novel techniques such as radiochemical labelling can be used for detecting products of genes with low expressions among endophytes (Lodge et al. 1996). In addition, the biological potential of fungal secondary metabolites could also be fully realized by the application of combinatorial techniques [23,46,47,51].

In fungal endophytes, genes coding for enzymes of secondary metabolic pathways usually occur as gene clusters being positioned in the same locus and co-expressed [23]. These gene clusters are known to evolve swiftly through multiple rearrangements, duplication and losses, and are capable of interspecific feast through horizontal gene transfer [25,27,40]. It is important to screen fungal species for their secondary metabolite assortment under different growing conditions; culture parameters such as composition of growth medium, aeration, pH and the

presence of certain enzyme inhibitors change vividly the secondary metabolite profile and even induce the synthesis of several new metabolites [8,9,21,31,35,44].

As far as drug discovery is concerned, screening of libraries created by combinatorial combination once appeared to be more hopeful than natural products screening [13,58]. Though combinatorial synthesis can churn out molecules in enormous numbers, endophytic fungi can still be a good source of novel drugs and natural product-based scaffolds for combinatorial synthesis and libraries [56]. This is because the synthetic capability of endophytes, like in other organisms, has been fine tuned by natural selection over millions of years. Smith et al. (2008) united sequence analysis with bioassay procedures to explore the endophyte diversity of the tropics. Their results suggest that tropical plants harbour a substantial portion of undiscovered endophytes that may be vested with novel biochemical diversity. Hence, the need for the inclusion of fungal endophytes in natural products discovery programmes. Testing endophytes isolated from different tissues of plant hosts and from plants, growing in unusual and less studied habitats will be more productive. We suggest a global initiative involving fungal taxonomists, ecologists, and natural product chemists to evolve systematic and rapid screens for endophytic fungi by scheming considered bioassays that would indicate the production of novel bioactive compounds [11-14,16,23,25,35,46,55].

9. CONCLUSION

Isolation of fungal endophytes from medicinal and other plants may result in methods to produce biologically active agents for biological exploitation on a large commercial scale, as they are easily cultured in laboratory and fermenter instead of harvesting plants and affecting the eco-friendly biodiversity.

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10. REFERENCES

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