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ENDOPHYTES: A REVIEW

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ABSTRACT
India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants with nearly about 70% of the medicinal plants found in tropical forests in Eastern and Western Ghats and Himalayas. Medicinal plants are the "backbone" of traditional medicine. Endophytes are an endosymbiotic group of microorganisms that colonize in plants and microbes that can be readily isolated from any microbial or plant growth medium. They act as reservoirs of novel bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties. While plant sources are being extensively explored for new chemical entities for therapeutic purposes, endophytic microbes also constitute an important source for drug discovery. Ironically, in today's world the microorganisms are producing more beneficial components compared to the plants. This review aims to comprehend the contribution and uses of endophytes as an impending source and other possible medicinal use.

KEYWORDS: Endophytes, medicinal plants, plant enzymes, metabolites, endophytic bacteria.

INTRODUCTION
The survey report of World Health Organization indicated that almost 80% of the world's population especially in developing countries depends on traditional medicines which involve the use of plant extracts for primary health care.[1] The therapeutic properties possessed by the plants are utilized to their fullest in many parts of the world.[2] However, in many cases it was found that the medicinal compounds extracted from these plants were actually being produced by the micro-organisms residing in the tissues of the host plants.[3] These microorganisms belong to diverse species of bacteria and fungi, called as endophytes. The class of microscopic organisms called endophytes are those bacteria that can be isolated from surface-disinfected plant tissue or extracted from within the plant, and that do not visibly harm the plant.[4] Ironically, in today's world the microorganisms are producing more beneficial components compared to the plants.

Medicinal Plants
Medicinal plants are the "backbone" of traditional medicine.[5] It has considerable significancefor its attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs due to its rich ingredients.[6] India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants with nearly about 70% of the medicinal plants found in tropical forests in Eastern and Western Ghats and Himalayas.[7]

Phytochemicals
Medicinal plants have bioactive compounds which are used for curing various human diseases and an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids which exhibit vital pharmacological activities.[8,9] The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs.[10]

Primary and Secondary Metabolites
The term metabolite is usually restricted to small molecules with various functions, including fuel, structure, and signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own (usually as a cofactor to an enzyme), defense, and interactions with other organisms.[11] A primary metabolite is directly involved in normal "growth", development and reproduction. A secondary metabolite is not directly involved in those processes, but usually has an important ecological function.[12] Examples are antibiotics and pigments such as resins and terpenes etc. Secondary metabolites can be classified

based on the chemical composition, chemical structure, biosynthetic pathway or their solubility in various solvents. Secondary metabolites can be divided into three large categories, namely alkaloids, terpenes and phenolics.[13]

Microbes as Alternative Approaches

World is encompassing accelerated loss of wild medicinal plant species; one third of the medicinal plant species are threatened with extinction from over harvesting and natural anthropogenic habitat destruction.[14] To circumvent this many biological systems like bacteria, fungi, yeast, cyanobacteria, actinomycetes and plants have been used. But the best one appears to be the use of plants.[15] Furthermore, the feasibility of access to plant bioactive compounds is challenged by the low levels at which these products accumulate in native medicinal plants, the long growth periods required for plant maturation, and the difficulty in their recovery from other plant-derived metabolites.[16] Therefore, it is important to find alternative approaches to produce the medicinal plant-derived biologically active compounds, in particularly those derived from endangered or difficult-to-cultivate plant species, to meet the medical demand. This can be achieved by exploiting the ability of endophytic bacteria residing in plants to produce the same or similar bioactive compounds as their hosts.[17,18]

Endophytes

Several microbes inhabit the plant species, some may be beneficial, some neutral. Each plant species could possess thousands of microbes, categorized as epiphytes (microbial inhabitants of the rhizosphere and phyllosphere; those near or on plant tissue) or endophytes (microbes residing within plant tissues in leaves, roots or stems), depending on their area of colonization in the plant species.[19]

Occurrence of Endophytes

Endophytes are an endosymbiotic group of microorganisms – often bacteria or fungi – that colonize the inter- and/or intracellular locations of plants. For these organisms, all or part of their life cycle occurs within their hosts, without causing any apparent symptoms of disease. They are ubiquitous in nature and exhibit complex interactions with their hosts, which involve mutualism, antagonism and rarely parasitism.[20] Endophytic bacteria seem to be distributedin most plant species and have been isolated from roots, leaves, and stems, and a few from flowers, fruits, and seeds.[21] As per many researchers, the definition of endophytes could be suitable for the hypothesis that they live inside the plant species, where the environment is more stable compared to the soil, where the plant grows. However, there are also some endophytes that only appear in the plant during part of their lifecycle. Thus, the endophytic community is made up of organisms from distinct origins, with those with larger genomes likely to live in variable environments, such as soils, while those with smaller genomes are likely to exist in the stable environment and are vertically transmitted.[22]

Action of Endophytes

The population of endophytes in a plant species is highly variable and depends on various components, such as host species, host developmental stage, inoculum density and environmental condition.[23] Endophytes enter plant tissue primarily through the root zone; however, aerial portions of plants such as flowers, stems and cotyledons, may also be used for entry.[24] Specifically the bacteria enter tissues via germinating radicals secondary roots, stomata or as a result of foliar damage.[25] Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant.[26] The endophytes gain shelter, nutrition, and dissemination via host, and can contribute to host fitness enhancements including protection against insect and vertebrate herbivores, enhancements against drought tolerance and nutrient status, and improved growth particularly of the roots.[4] Microbial endophytes mainly bacteria and fungi, are detected after surface sterilization of a plant part and are assumed to originate from the leaves, the roots surrounding environment and the aerial portions of plants.Endophytes are known to enhance host growth and nutrient gain.[27]

Effect of Climate

Endophytic population varies from species to species, within the same species it varies in climatic conditions. They found that matured leaves of teak and rain tree had greater number of genera and species, with highercolonization frequency, than those in the young leaves and their occurrence in leaves increased during rainy season.[28] The endophytic population and frequency tended to differ among sampling dates for all the organs studied, namely, young leaves, petiole, and twigs ofGingko biloba L. They proved that the occurrence of Phyllosticta sp. in both leaves and petioles was first detected in August and peaked in October with none in the month of May. Phomopsis sp. was detected in twigs throughout the growing season. These results suggest that the distribution of the two dominant endophytic organisms was organ-specific and differed within seasons.[29]

Types of Endophytes

Endophytes are classified mainly into three types i.e. Bacterial endophytes, Fungal endophytes and actinomycetes. Endophytes are associated with plants in various forms, including bacteria (actinomycetes or mycoplasma) or fungi that have been colonized inside the plant tissues. More than 200 genera from 16 phyla of bacterial species have been reported to be associated with endophytes and among them, most of the species belong to the phyla Actinobacteria, Proteobacteria, and Firmicutes.[30] The diversity of endophytic bacteria ranges from gram-positive to gram-negative bacteria, such as *Achromobacter*, *Acinetobacter*, *Agrobacterium*,

Bacillus, Brevibacterium, Microbacterium, Pseudomonas, Xanthomonas etc.[31]

Bacterial Endophytes

Bacteria that are present within plants known as bacterial endophytes. For example:-*Azorhizobium caulinodans* in rice,[32] *Burkholderia pickettii* in Maize, *Enterobacter sakazakii* in Soyabean,[33] *Pseudomonas fluorescens* and *Pseudomonas putida*,[34] Bacillus spp. in Citrus plants[35] and Streptomyces in Wheat[38] are some of the examples.

Fungal Endophytes

Fungal micro-organisms that are found inside plant tissues. For example:-Pestalotiopsis versicolor and Pestalotiopsisneglecta are the fungal endophytes live inside Taxus cuspidate.[36] Similarly, *Alternaria tenuissima* and *Guignardia vaccinii* are endophytes that persist inside *Ocimum sanctum* and *Sapindus detergens* respectively.[37]

Actinomycetes Endophytes

Actinomycetes that are found inside plant tissue are known as Actinomycete endophytes. For example:- Streptomyces actinomycetes that live inside Wheat. Irrespective of the type of endophyte, it needs to be surface sterilized for further study or research which is done by surface sterilization techniques. More than 300 endophytic actinobacteria and bacteria belonging to the genera *Streptomyces, Nocardiopsis, Brevibacterium, Microbacterium, Tsukamurella, Arthrobacter, Brachybacterium, Nocardia, Rhodococcus, Kocuria, Nocardioides*, and *Pseudonocardia* were isolated from different tissues of *Dracaena cochinchinensis* Lour. (a traditional Chinese medicine known as dragon's blood). Of these, 17 strains having antimicrobial and anthracyclines-producing activities also showed anti-fungal and cytotoxic activities against two human cancer cell lines, MCF 7 and HepG2.[23,38]

Surface Sterilization Techniques

In order to get rid of exophytic bacteria and other unwanted particles on the surface of the sample, itis taken care of by surface sterilization. Two methods were considered as base to design and formulate new protocols of surface sterilization. The collected plants were briefly washed under running tap water to remove the soil debris and further subjected to simple and new surface sterilization by subsequent soaking them in series of solutions as follows: sterile distilled water for 1 min, 2% of Sodium hypochlorite containing 0.1% of Tween 20 solution for 3 minutes, ethanol 70% for 1 min, and finally washed in sterile distilled water twice for 3 times and the excess moisture was blotted with a sterile filter paper. The last washing water was plated onto bacterial culture media of Nutrient agar respectively.[4] The success of surface sterilization method was confirmed by the absence of any microbial growth onto the cultural media from the plating of last washing water. Surface sterilization was also performed by using the conventional method i.e. by immersing the leaf tissues in 70% ethanol for 5 minutes and then plating them in the culture media.[18]

Isolation of Endophytes

The endophytic niche offers protection from the environment for those bacteria that can colonize and establish in planta. These bacteria generally colonize the intercellular spaces, and they have been isolated from all plant compartments including seeds.[39] Endophytic bacteria have been isolated from both monocotyledonous and dicotyledonous plants, ranging from woody tree species, such as oak and pear, to herbaceous crop plants such as sugar beet and maize. Classical studies on the diversity of bacterial endophytes have focused on characterization of isolates obtained from internal tissues following disinfection of plant surfaces with sodium hypochlorite or similar.[40] Surface sterilized samples were crushed in sterile distilled water using a sterile mortar and pestle and the aliquots were serially diluted and then streaked on nutrient agar plates. The incubation period is for 48hrs. This is followed by screening process.

Screening of Endophytic Microbes

Identification of endophytic bacteria: When bacteria have visibly grown, they are often still mixed. The identification of a microbe depends upon the isolation of an individual colony, as biochemical testing of a microbe to determine its different physiological features depends on a pure culture. The identification of endophyitc is done by its morphological structures, biochemical and molecular characterization.[6]

Morphological identification: Itincludes the Gram staining with the raw sample before incubation or staining freshly grown colony material helps to determine if a colony consists of uniformly appearing bacteria or is mixed, and the color, and shape of bacteria allow a first classification based on morphology.[41]

Biochemical identification: It involves a set of agars in vials to separate motile from non-motile bacteria. other test are used to findt the ability of a bacterium to produce particular end products and the following test includes IMViCtest, triple sugar iron agar test, catalase test, oxidase test, starch hydrolysis test, nitrate reduction test etc.[03]

Molecular identification: Molecular techniques are major tools for the analysis of microorganisms from food and other biological substances. The techniques provide ways to screen for a broad range of agents in a single test[42] for differentiation of species, strain identification and definition of strain relatedness from various samples. Molecular methods varies with respect to discriminatory power, reproducibility, ease of use, and ease of interpretation.[43] The molecular identification of isolated endophytic bacteria can be done using 16S RNA sequencing and other methods which includes MALDI-

TOF etc. This offers precise identification of bacteria up to subspecies level which is most suitable method.[44]

Applications

No research is successful with the feasibility of the product or organism which leads to application point of view.

Pharmaceutical and Drug Discovery: Bacterial endophytes have several potential applications in pharmaceutical and drug discovery due to the rich source of bioactive compounds in them.[45] Endophytic microorganisms play an important role in this search for natural bioactive compounds, with potential use in the health sector and in drug discovery.[46] It improves the understanding of endophytes and address the need for new and useful compounds necessary to combat various pathogens associated with human health and other possible medicinal uses.[47]

Endophytic microorganisms produce low-molecular weight secondary metabolites that include antimicrobial compounds, phytohormones, or their precursors, vitamins like B12 and B1[48], biopro-tectants.[49] Several secondary metabolites are alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols and phenols.

Agricultural Applications: Endophytes associated with ethnomedicinalplants serve as a potential source of natural products forapplication in oxidative stress and as new bioactive agents.[58] The antimicrobial agents counteract the multi-drug resistance (MDR) in pathogenicmicrobes. Many microorganisms of agricultural concernhave also acquired resistance to the commonly usedantimicrobial compounds and the interest in naturalmethods of pathogen control through new, eco-friendlyagents has been increasing day by day. Endophytic bacteria and fungi can promote plant growth and yield and can act as biocontrol agents.[50] Endophytes also produce extracellular hydrolyases such as cellulases, proteinase, lipases and esterases to establish resistance against plant invasions.[51] Endophytic bacteria associated with Hypericumperforatum and Ziziphoracapitata belong to Arthrobacter, Achromobacter, Bacillus, Enterobacter, Erwinia, Pseudomonas, Pantoea, Serratia, and Stenotrophomonas. H.perforatum with antibacterial activity supported colonization of more bacteria with antagonistic activity, as compared to Z.capitata. Theses isolates were able to control tomato root rot caused by F.oxysporum.[52]

Phytoremediation: In addition, it has been shown that they have the potential to remove soil contaminants by enhancing phytoremediation and may play a role in soil fertility through phosphate solubilization and nitrogen fixation.[53] There are many reports which show that in a microbe-plant relationship, endophytes contribute substances that possess various types of bioactivity, such as antimicrobial and antifungal. Exploitation of such endophyte - plant interactions can result in the promotion of plant health and can play a significant role in low input sustainable agriculture applications.[54]

Therapeutic Role of Endophytes: Recently, many known as well as new endophytic bioactivemetabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, antiinflammatory, antioxidant, and immunosuppressive agents etc., have been identified.[55]

- **Anti-cancerous compounds:** Several bioactive compounds produced by endophytes havebeen identified as anti-cancer agents.[53] Ginseng (Panax ginseng) is known for its ginsenosides that have anti-cancerous property. The transformed *Paenibacillus polymyxa*, an endophytic bacterium of Ginseng leaf, showed high ginsenoside concentration. This endophytic bacterial strain on inoculation to Ginseng plants through foliar applications combined with irrigation enhanced plant growth and the concentration of ginsenosides.[56]

- **Antibiotics:** Antibiotics are natural compounds produced by microorganisms as secondary metabolites to kill or inhibit other microorganisms. The majority of endophytic bacteria produce different kinds of antibiotics like ecomycin, pseudomycins and kakadumycins are some of the novel antibiotics produced by endophytic bacteria.[57] Pseudomonas viridiflava, an epiphyte or endophyte of the leaves of many grasses, produced ecomycin, which is used for the treatment of respiratory and urinary tract infections, skin, eye and gut infections.[47]

- **Antioxidants:** Among the endophytes, endophytic fungus Aspergillus sp. from Trigonellafoenum-graecum seeds demonstrated the highest both total phenolic content in term of gallic acid equivalent and antioxidant activity for DPPH.[58] Endophytic bacterial communities form Aloe vera showed 75 to 80% DPPH scavenging activity due to the bioactive principles present in the species of bacteria in the plant.[59] A range of 40 different endophytic fungi were isolated from 10 different medicinal plants from Palolo, Central Sulawesi which shows the abundance of antioxidant properties in medicinal plants rich in endophytic fungi or bacteria.[60]

- **Imunosuppressive or anti-inflammtory:** Immunosuppressive drugs are used today to prevent allograft rejection in transplant patients, and in the future they could be used to treat autoimmune diseases such a rheumatoid arthritis and insulin-dependent diabetes. The endophytic fungus Fusarium subglutinans, isolated from T. wilfordii, produces the immunosuppressive but noncytotoxic diterpene pyrones subglutinol A and B.[61] Still, the lack of toxicity associated with subglutinols A and B

suggests that they should be explored in greater detail. The Microbiology Department at Sandoz Ltd. developed a computer-aided evaluation program to screen and evaluate fungi for bioactivity. The program can recognize and eliminate from study common fungi producing known compounds and thereby direct attention to the evaluation of rare samples, which are more likely to produce metabolites with novel bioactivity. This approach resulted in the discovery of the fungus *Tolypocladium inflatum*, from which cyclosporine, a hugely beneficial immunosuppressant, was isolated.[62]

Industrial applications: Endophytes are an increasingly important area of research in many fields because of their chemical diversity and their ability to produce many novel secondary metabolites that can be utilized for fuel, medicine, restoration and agriculture. It is their chemical diversity that sparks profound interest in these organisms.[63] Microorganisms are important to enzymatic production processes because of their high production capability, low cost and susceptibility to genetic manipulation. There is strong biotechnological interest in microbial enzymes in several fields including food processing, detergent and textile manufacturing, agricultural and pharmaceutical research, medical therapy and molecular biology.[64] Enzymes are potential biocatalysts for a large number of reactions. Microorganisms represent a viable alternative source of enzymes, as they may be cultured in large quantities within short time frames by fermentation, are biochemically diverse, and are amenable to genetic manipulation.[65] Several enzymes were produced by endophytic bacterial species which includes amylase, protease, lipase, cellulase, pectinase etc.

1. **Amylase enzyme:** Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. amylases have been widely used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization. These enzymes account for about 30% of the world's enzyme production.[66]
2. **Protease enzyme:** A protease (also termed peptidase or proteinase) is any enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. They have diverse application in wide variety of industries like biscuit manufacturing, brewing industries. Proteolytic enzymes are very important in digestion as they breakdown the peptide bonds in the protein foods to liberate the amino acids needed by the body. Additionally, proteolytic enzymes have been used for a long time in various forms of therapy. Their use in medicine is notable based on several clinical studies indicating their benefits in oncology, inflammatory conditions, blood rheology control and immune regulation.[67]
3. **Lipase enzyme:** Lipases (triacylglycerol acylhydrolases) are a class of hydrolase which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil–water interface. In addition, lipases catalyze the hydrolysis and transesterification of other esters as well as the synthesis of esters and exhibit enantioselective properties. The ability of lipases to perform very specific chemical transformation (biotransformation) has make them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries.[68]
4. **Pectinase enzyme:** Pectinases are the enzymes that are capable of hydrolyzing the Pectin polysaccharide into smaller fragments. All the peels of the fruits are made of pectin layer which can be easily digested by the microbial pectinases so as to extract the juices of the fruits. Therefore, pectinase enzymes are commonly used in processes involving the degradation of plant materials, such as speeding up the extraction of fruit.[69]
5. **Cellulase enzyme:** Cellulose is the most abundant biomass on Earth. It is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the biosphere. Cellulose is commonly degraded by an enzyme called cellulose.[70] This enzyme is produced by several microorganisms. Bacteria which have high growth rate as compared to fungi have good potential to be used in cellulase production. However, the application of bacteria in producing cellulase is not widely used. The cellulolytic property of some bacterial genera such as Cellulomonas, Cellvibrio, Pseudomonas sp. Bacillus, and Micrococcus, was also reported. Enzyme production is closely controlled in microorganisms and for improving its productivity these controls can be ameliorated.[71]

Ecological Role of Endophytes: Endophytic bacteria may accompaniment certain metabolic properties, such as promoting plant growth, controlling soil-borne pathogens, or helping host plant to defeat stress responses to environmental abuse.[72] Furthermore, the interactions between plants and bacteria help plants to settle in ecosystem restoration processes.[73] These interactions may increase the ability of plants to utilize nutrients from the soil by increasing root development, nitrate uptake or solubilizing phosphorus, and to control soil-borne pathogens.[74] Endophytes improve the plant's ability to tolerate various types of abiotic and biotic stresses, and enhance the resistance of plants to insects and pests. They produce phytohormones and other bioactive compounds of biotechnological interest (enzymes and pharmaceutical drugs).[75]

Bioplastics: Bioplastics are biomaterials that are receiving increasing commercial interest. It was first described a bioplastic, poly-3-hydroxybutyrate (PHB) produced by *Bacillus megaterium*. They are polyesters, produced by a range of microorganisms cultured under different nutrient and environmental conditions.[76] The most widely produced microbial bioplastics are poly-3-hydroxyalkanoate (PHA) and PHB. Genomic analysis indicates that many species of bacteria have the potential to produce bioplastics.[77]

Future Prospects

As our understanding of endophytic bacteria continues to grow, the potential to exploit their unique characteristics of bioactive compound synthesis alone or with plants is also increasing day by day. The plant benefits enhanced by combined application of beneficial microorganisms in the form of bio-fertilizer have become an alternative tool for organic farming. Exploitation of endophytic bacteria as a plant growth-promoting agent further necessitates our ability to understand and utilize bacterial endophytes in agriculture under integrated bio-fertilizer technology programme. How endophytes modulate the physiology of plant and its metabolism and how they use the intermediary substances of primary and secondary metabolism as nutrition and precursor to produce either novel compounds or enhance the existing important secondary metabolites are still largely unknown.[78,79]

CONCLUSION

Due to the swift dwindling of rich endangered species of medicinal plants globally, awareness due to the fact that the herbal drugs are expensive and cannot meet the demands of the world requirement, microbes play a paramount role to replace plant sources. Large scale harvesting of medicinal plants has become a major threat to biodiversity. As an alternative to these problems, endophyte seems as a potential source of therapeutic agent. Earlier investigation supports that the metabolites produced by endophytes in culture is a potential source of bioactive agent. Considering all above mentioned aspects, the focus of the present study is on reiterating the importance of isolation of microbial endophytes for their natural products which has a wide spectrum of applications.

REFERENCES

1. Patwardhan B, Vaidya ADB, Chorghade M. Ayurveda and natural products-Drug discovery. Curr Sci, 2004; 86(6):789–99.
2. Stone JK, Bacon CW, White Jr JF. An overview of endophytic microbes: endophytism defined. Microbial Endophytes, 2000; 7: 17-44.
3. Murugesan T, Palaniswamy R. Isolation and screening of protease producing bacteria from different market soils. Int J Res BioSci, 2018; 7(3): 35-40.
4. Ryan RP, Germaine K, Franks A., Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett, 2008; 278(1): 1-9.
5. Ahamed M, Khan MM, Siddiqui MK, AlSalhi MS, Alrokayan SA. Green synthesis, characterization and evaluation of biocompatibility of silver nanoparticles. Physica E Low Dimens Syst Nanostruct, 2011; 43(6): 1266-71.
6. Singh R, Medicinal plants- A review. J Plant Sci, 2015; 3(1-1): 50-55.
7. Mittermeier RA, Myers N, Mittermeier CG, Robles G. Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions. CEMEX, SA, Agrupación Sierra Madre, SC, 1999.
8. Nostro A, Germano MP, Dangelo V, Marino A, and Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol, 2000; 30(5): 379-384.
9. Mahato SB, Sen S. Advances in triterpenoid research. Phytochem, 1997; 44(7): 1185-236.
10. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem, 2013; 2(4): 1-4.
11. Palaniswamy R, Raghunathan P. Phytochemical analysis of *Majorana hortensis* leaves. J Pharma Res, 2018; 7(5): 70-72.
12. Zhao J, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol, 2005; 23(4): 283-333.
13. Khamwan S, Boonlue S, Jogloy S, Mongkolthanaruk W. Characterization of endophytic bacteria and their response to plant growth promotion in *Helianthus tuberosus* L. Biocatal Agric Biotechnol, 2018; 13: 153-9.
14. Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med, 2016; 11(1): 37.
15. Ganesh Babu MM, Gunasekaran P, Extracellular synthesis of crystalline silver nano particles and its characterization. Mater Lett, 2013; 90: 162-164.
16. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv, 2015; 33(8): 1582-1614.
17. Rai A, Saito K, Yamazaki M. Integrated omics analysis of specialized metabolism in medicinal plants. The Plant J, 2017; 90(4): 764-87.
18. Zin NM, Sarmin NI, Ghadin N, Basri DF, Sidik NM, Hess WM, and Strobel GA. Bioactive endophytic streptomycetes from the Malay Peninsula. FEMS Microbiol Letters, 2007; 274(1): 83-88.
19. Oldroyd, GED, Murray JD, Poole PS, Downie JA. The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet, 2011; 45: 119–144.
20. Miliute I, Buzaite O, Baniulis D, Stanys V. Bacterial endophytes in agricultural crops and their role in

stress tolerance: a review. *Zemdirbyste-Agriculture*, 2015; 102(4): 465-478.
21. Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, der Lelie DV. Endophytic bacteria and their potential applications. Critic Rev Plant Sci, 2002; 21(6): 583-606.
22. Mitter B, Brader G, Afzal M, Compant S, Naveed M, Trognitz F, Sessitsch A. Advances in elucidating beneficial interactions between plants, soil, and bacteria. In: Donald, L.S. (Ed.), Adv. Agron, 2013; 381– 445.
23. Dudeja SS, Giri R. Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non-legumes. Afr J Micrbiol Res, 2014; 8: 1562–1572.
24. Gayathri P, Muralikrishnan V. Bioprospecting of endophytic bacteria from mangrove bananas and sugarcane plants for their PGPR activity. Eur J Biol, 2013; 21(4): 456-469.
25. Gayathri S, Saravanan D, Radhakrishnan M, Balagurunathan R and Kathiresan K. Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt marsh plant species. Indian J of Biotechnol, 2010; 9(4): 397-402.
26. Hallmann J, HaUmann A Q, Mahaffee W F, Kloepper J W. Bacterial endophytes in agricultural crops. Can. J. Microbiol, 1997; 43(10): 895-914.
27. Schulz B, Boyle C, Draeger S, Römmert AK, and Krohn K. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res, 2002; 106: 996–1004.
28. Chareprasert S, Piapukiew J, Thienhirun S, Whalley AJS, and Sihanonth P.Endophytic fungi of teak leaves Tectona grandis L. and rain tree leaves Samanea saman Merr. World J Microbiol Biotechnol, 2006; 22(5): 481–486.
29. Hallmann, J. Plant interactions with endophytic bacteria CABI Publishing, New York, 2001; 87-119.
30. Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M. Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie Van Leeuwenhoek, 2015; 108: 267–289.
31. Sun H, He Y, Xiao Q, Ye R, Tian Y. Isolation, characterization, and antimicrobial activity of endophytic bacteria from *Polygonum cuspidatum*, Afr J Microbiol Res, 2013; 7: 1496–1504.
32. Engelhard V, Nieuwenhove C, Holm L, Kulasooriya SA, Vlassak K. Establishment of *Azorhizobium caulinodans* in the rhizosphere of wetland rice (*Oryza sativa L.*). Biology and fertility of soils, 2000; 31(2): 143-9.
33. Kuklinsky-Sobral, J, Araújo W L, Mendes R, Geraldi I O, Pizzirani-Kleiner AA, and Azevedo JL. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol, 2004; 6(12): 1244-1251.
34. Duan K, Dammel C, Stein J, Rabin H, and Surette MG. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. Mol Microbiol, 2005; 50(5): 1477-1491.
35. Suman TY, Radhika, Rajasree SR, Kanchana A, Elizabeth SB. Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using *Morinda citrifolia* root extract. Colloids Surf B Biointerfaces, 2013; 106: 74-78.
36. Coombs JT, Franco CM. Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol, 2003; 69(9): 5603-5608.
37. Kumaran RS, Hur BK. Screening of species of the endophytic fungus Phomopsis for the production of the anticancer drug taxol. Biotechnol Appl Biochem, 2010; 4(1): 21-30.
38. Bhagat, Kalra A, Darokar MP, Chandra M, Aggarwal N, Singh AK, Khanuja SP. Endophytic bacteria from *Ocimum sanctum* and their yield enhancing capabilities. Curr Microbiol, 2012; 60(3): 167-71.
39. Posada F, Vega FE. Inoculation and colonization of coffee seedlings (*Coffea arabica L.*) with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycoscience, 2006; 47(5): 284-9.
40. Miché L, Balandreau J. Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. Appl Environ Microbiol, 2001; 67(7): 3046-52.
41. Hung PQ, Annapurna K. Isolation and characterization of endophytic bacteria in soybean (Glycine sp.). Omonrice, 2004; 12: 92-101.
42. Field D, Wills C. Abundant microsatellite polymorphism in Saccharomyces cerevisiae, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. Proc Natl Acad. Sci, 1998; 95: 1647-1652.
43. Lasker BA. Evaluation of performance of four genotypic methods for studying the genetic epidemiology of *Aspergillus fumigatus* isolates. J Clin Microbiol, 2002; 40: 2886-2892.
44. Liaquat F, Eltem R. Identification and characterization of endophytic bacteria isolated from *in vitro* cultures of peach and pear rootstocks. Biotech, 2016; 6: 120, 119-125.
45. Strobel, GA. Endophytes as sources of bioactive products. Microb Infect, 2003; 5(6): 535-544.
46. Gao Y, Lu Q, Pu Zang P, Li X, Ji Q, He Z *et al*., An endophytic bacterium isolated from Panax ginseng CA Meyer enhances growth, reduces morbidity and stimulates ginsenoside biosynthesis. Phytochem Lett, 2015; 11: 132–138.
47. Lam KS, New aspects of natural products in drug discovery. Trends Microbiol, 2007; 15: 279-289.
48. Praptiwi MR, Wulansari D, Fathoni A, Agusta A. Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. J Appl Pharmaceut Sci, 2018; 8(08): 069-74.

49. Ivanova EG, Fedorov DN, Doronina NV, Trotsenko YA. Production of vitamin B12 in aerobic methylotrophic bacteria. Microbiol, 2006; 75: 494–496.
50. Mercado BJ, Bakker PA. Interaction between plants and beneficial Pseudomonas sp., exploiting bacterial traits for crop protection. Antonie Leeuwenhok, 2007; 92: 367–389.
51. Nongkhlaw FMW, Joshi S R. Investigation on the bioactivity of culturable endophytic and epiphytic bacteria associated with ethnomedicinal plants. J Infect Dev Ctries, 2015; 9: 954–961.
52. Strobel GA. Harnessing endophytes for industrial microbiology. Curr Opp Microbiol, 2006; 9: 240–244.
53. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Nat Prod Rep, 2001; 18: 448–459.
54. Egamberdieva D, Wirth S, Behrendt U, Ahmad P, Berg G. Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. Front Microbiol, 2017; 8: 199.
55. Jung HJ, Yonghyo K, Hyang BL, Kwon HJ. Antiangiogenic activity of the lipophilic antimicrobial peptides from an endo- phytic bacterial strain isolated from Red Pepper leaf. Mol Cells, 2015; 38: 273–278.
56. Korkina LG. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell Mol Biol, 2007; 53: 15–25.
57. Firakova S, Sturdikova M, Muckova M .Bioactive secondary metabolites produced by microorganisms associated with plants. Biologia, 2007; 62: 251–257.
58. Christina A, Christapher V, Bhore SJ. Endophytic bacteria as a source of novel antibiotics: an overview. Pharmacogn Rev, 2013; 7: 11–16.
59. Sathiyaseelan K, Stella D. Isolation, identification and antimicrobial activity of marine actinomycetes isolated from Parangipettai. Recent Res Sci Technol, 2011; 3: 74–78.
60. Khiralla A, Mohamed L, Thomas J, Mignard B, Spina R, Yagi S. A pilot study on antioxidant potential of endophytic fungi from some Sudanese medicinal plants, Asian Pacific J of Trop Med, 2015; 8(9): 701-704.
61. Akinsanya MA, Goh JK, Lim SP, nadting ASY. Diversity, antimicrobial and antioxidant activities of cultureable bacterial endophyte communities in *Aloe vera*. FEMS Microbiol Lett, 2015; 362(23): 184-186.
62. Praptiwi, Raunsai M, Wulansari D, Fathoni A, Agusta A. Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plant. J Appl Pharmaceut Sci, 2018; 8(8): 69-74.
63. Borel JF and Kis ZL. Transplant. Proc., 23: 1867–1874.
64. Lee J, Lobkovsky E, Pliam NB, Strobel GA, Clardy J. Isolation of endophytes. J Organic Chem, 1995; 60: 7076–7077.
65. Rai M, Rathod D, Agarkar G, Dar M, Brestic M, Marostica JMR. Fungal growth promotor endophytes: a pragmatic approach towards sustainable food and agriculture. Symbiosis, 2014; 62: 63–79.
66. Reinhold-Hurek B, Hurek T. Reassessment of the taxonomic structure of the diazotrophic genus *Azoarcus sensulato* and description of three new genera and new species, *Azovibrio restrictus* gen. nov., sp. nov., *Azo spiraoryzae* gen. nov., sp. nov. and *Azonexus fungiphilus* gen. nov., sp. nov. Int J Syst Evol Microbiol, 2000; 50(2): 649-59.
67. Anbu P, Annadurai G, Hur B. Production of alkaline protease from a newly isolated Exiguobacterium profundum BKP23 evaluated using the response surface methodology. Biologia, 2013; 68(2): 186-193.
68. Burhan A, Nisa U, Gokhan C, Omer C, Ashabil A, Osman G. Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkalophilic Bacillus sp. isolate ANT-6. Process. Biochem, 2016; 38: 1397–1403.
69. Naidu SK. Characterization and purification of protease enzyme. J Appl Pharmaceut Sci, 2011; 01(03): 107-12.
70. Park H, Lee K, Chi Y, Jeong S. Effects of methanol on the catalytic properties of porcine pancreatic lipase. J Microbiol Biotechnol, 2005; 15(2): 296–301.
71. Beulah D, Sunitha EM, Srilakshmi T. Production , purification and assay of pectinase enzyme from *Aspergillus niger*. Helix, 2015; 1: 673-U45.
72. Tomme P, Warren RA, Gilkes NR. Cellulose hydrolysis by bacteria and fungi. Adv Microbial Physiol, 1995; 37: 1-81.
73. Nakamura K, Kppamura K. Isolation and identification of crystalline cellulose hydrolysing bacterium and its enzymatic properties. J Ferment Tech, 1982; 60(4): 343–348.
74. Santoyo G, Moreno-Hagelseib G, Carmen M, Glick B. Plant Growth Promoting Bacterial Endophytes. Microbiol Res, 2016; 183: 92-99.
75. Whipps J M. Microbial interactions and biocontrol in the rhizosphere. J Exp Bot., 2001; 52: 487511.
76. Vijayalakshmi R, Kairunnisa K, Sivvaswamy SN, Dharan SS, Natarajan S. Enzyme production and antimicrobial activity of endophytic bacteria isolated from medicinal plants. Indian J Sci Technol, 2016; 9(14).
77. McInroy JA, Kloepper J W. Survey of indigenous bacterial endophytes from cotton and sweet corn. Plant and soil, 1995; 173(2): 337-342.
78. Kalia VC, Chauhan A & Bhattacharyya G. Genomic databases yield novel bioplastic producers. Nat Biotechnol, 2003; 21: 845–846.
79. Thenmozhi M, Krishnan K. Anti-Aspergillus activity of Streptmyces sp. VITSTK7 isolated from bay of Bengal coast of Puducherry, India. J Nat Environ Sci, 2011; 2: 1–8.