CHAPTER II

REVIEW OF LITERATURE

CHAPTER 2

Various new effective drugs are required day today for the treatment of various cancers, drug-resistant bacteria, fungal infections, emerging viruses, and parasitic protozoa infections. Natural products have provided the basis for the majority of many new drugs and their continuing roles in traditional health systems of many cultures reflected the bioactive properties of a wide variety of flora (Newman and Cragg, 2016). The importance of fungi for the production of bioactive compounds was first reported by Alexander Fleming in 1928, also by the discovery of penicillin from Penicillium notatum. After that, there has been a particular focus on the discovery of natural products from fungi, especially during World War II, with large-scale production of secondary metabolites. Among common microorganisms, they represent themselves as promising sources of natural products that have the advantage of producing large quantities of bioactive compounds at a sustainable and reasonable cost by large-scale cultivation and fermentation of source organisms (Michael et al., 2001). Micro bacteria were isolated from soil samples in earlier times. But in the search for new sources of bioactive metabolites especially those that are associated with higher plants and these bacteria have been proved to be a potential source of bioactive metabolites producing a vast category of new biologically active secondary metabolites (Amal et al., 2010; Sandoval et al., 2004). In recent times, bioprospecting is a term commonly used to give clarification about the discovery of bioactive metabolites or different organisms of commercial importance from the world's biological community. The reality lies in the fact that tropical forests are rich in different and more species, compared to the temperate forests, forests surrounded by tropical regions have much higher plant and different types of micro diversity. Therefore, sampling of a particular group of bacteria from the defined area will be more revealing and fruitful in search of the undescribed bacteria for natural products. Approximately 300,000 vascular plant species have been estimated to serve as reserves of large numbers of microbes in tissue known as endophytes (Itthayakorn et al., 2007).

2.1 Endophytes: The term 'endophytes' includes all those organisms that grow inside plant tissue without causing any symptoms of the disease (Karunai-Selvi and Balagengatharathilagam, 2014). Endophytic microorganisms are therefore may be defined as those microorganisms (mostly fungi and bacteria) which spend their life in the plant tissues generally bellow the epidermal cell layers without causing any symptoms of disease (Strobel and Daisy, 2003). By some conditions, endophytes can be parasitic, and also pathogenic, which can cause symptomatic infections (Brown et al., 1998). It can be regarded as an unbalanced status of a symbiosis when the host is stressed and physiological or ecological conditions favors virulence (Muller et al., 2005; Barbara and Schulz, 2005). Endophytes of certain plant could be a pathogen for the other plants, depending on the balance between pathogenicity and endophytism of the microorganism in the different hosts. Once the host-endophyte interaction becomes imbalanced either disease results in the host plant or the plant defense machinery kills the pathogenic endophytic fungus. Whether the interaction is balanced or imbalanced depends on the general status of the partners, the virulence of the fungus, and the defenses of the host, and both virulence and defense being variable and influenced by environmental factors, nutritional status and developmental stages of the partners. Therefore, a refined balance of mutualism and commensalism is needed between plant defense responses and the nutrient demand of the endophyte (Donald et al., 2005).

It has also often been observed that some endophytes show similarity to photochemical, rightly the host's competence has changed the field of endophytic biology as well as the importance of producing secondary metabolites of endophytes. Based on different facts, the products obtained from nature are different classes, which have been discovered from different group of endophytes, they have anti-bacterial, anti-fungal, anti-malarial, anti-parasitic, anti-tuberculosis and anti-cancerous etc. Even some unusual molecules which have been isolated from these endophytes such as Antidiabetic, immunomodulators, insecticidal, herbicidal etc (Kaul et al., 2012). Some data have been presented by Newman and Cragg (2016) as follows, data taken by the United States Food and Drug Administration (USFDA) from 1981 (December 34) to January 1981 to December 2014. In which different (total) number of different approved drug entries or medical entries are reported, as indicated below.

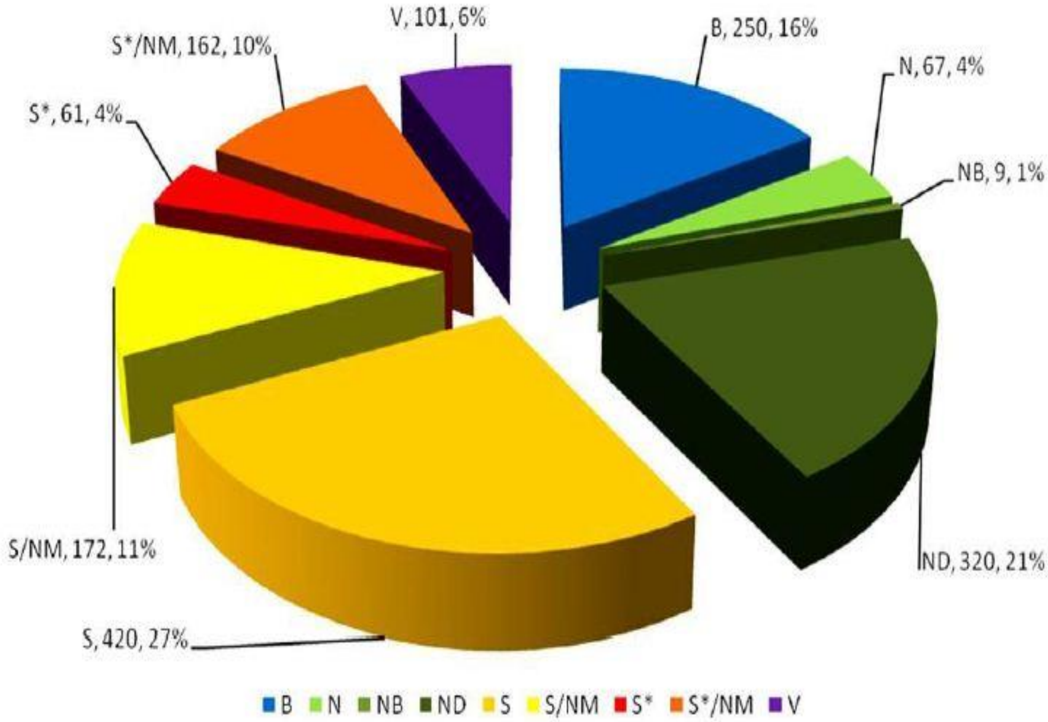


Figure 2.1: All new approved drugs (n = 1562) i.e. therapeutic agents or drug entities approved by the USFDA from the past 34 years (January 1981− December 2014). Here (B) is Biological macromolecule, (N) Unaltered natural product, (NB) Botanical drug (defined mixture), (ND) Natural product derivative, (S) Synthetic drug, (S*) Synthetic drug (NP pharmacophore), (/NM) Mimic of natural.

Plant endophytic bacteria have a special ability to produce the same or more comparable compounds from their host plants, as well as a great number of various bioactive compounds, which can be implicated in the protection of its host against pathogens and herbivores (Suryanarayanana et al., 2009; Berdy, 2005). The different role of natural products in the discovery of new different therapeutic agents can be demonstrated by the analysis of the number and sources of bioactive agents. At least 200,000 natural metabolites with various bioactive properties have been confirmed to be discovered (Selim et al., 2012). The discovery of natural products involves isolation, structural elucidation and establishing the bio-synthetic pathway of the secondary metabolites. It remains a subject of great interest to scientists, Different complexity and different biosynthesis of isolated compounds (Horwitz et al., 1993). World's first billion and most famous and the anticancer drug, taxol, was The Pacific was first separated from the Yew Tree in the early 1960s. And this drug was approved by the US Food and Drug Administration in 1992 for the treatment of ovarian cancer. This medicine has also been found effective against lung, breast, head and neck cancers and also in the advanced forms of Kaposi's sarcoma (in people with AIDS). Taxol (mitotic inhibitor) has a unique mechanism of action of breakdown of microtubule during cell division (Strobel et al., 1996). This has enforced to the search for novel sources of taxol from trees and isolation of an endophytic fungus and bacteria (Zhou et al., 2007). However, the isolation of endophytic bacteria from plants is a relatively simple process, but the identification of taxol-producing endophytic microbes is very difficult (Margret et al., 2000). Another surprising fact in terms of drug resistance is that the number of new antibacterial drugs receiving approval has decreased day by day over the years. Endophytes colonize host tissue internally, sometimes in very high numbers, identifying the symptoms of plant disease without harming the host. The endosphere consists of the endorhizosphere (tissue of internal root) and the endophyllosphere (leaf tissue and internal shoot). As shown in Figure 2.1

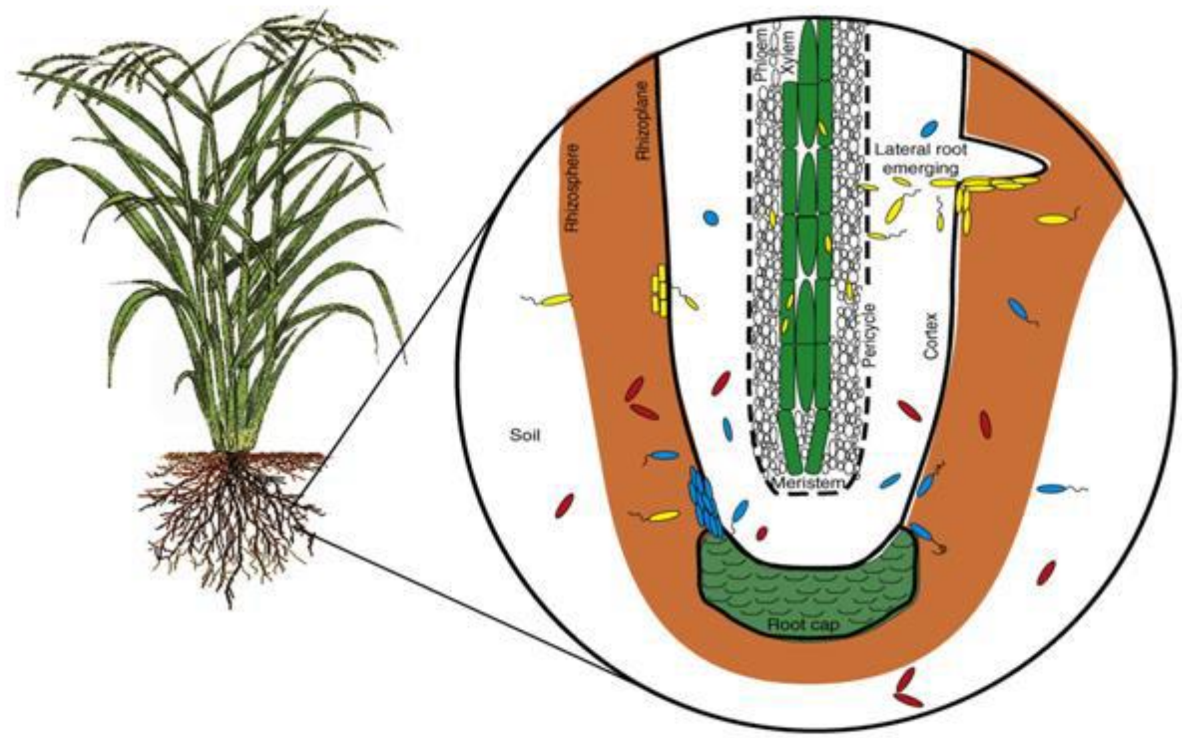


Figure 2.2: Types of endophytes and their root colonization process. It is the demonstration of the Presence of endophytes in most of the tissues of roots like cortex, phloem, rhizoplane.

2.1.1. Types of Endophytes.

Endophytes are mainly divided into three parts, which include bacterial endophytes, fungal endophytes, and Actinomycetes.

Bacterial Endophytes: Endophytic bacteria can be defined as those bacteria that colonize the plant's internal tissue, which live without leaving a negative impact on their host. For example: - *Burkholderia pickettii* in Maize (John et al., 1995) *Azorhizobium caulinodans* in rice (Rajasekaran et al., 2003). *Pseudomonas fluorescens* and *Pseudomonas putida* (Joao et al., 2006). *Bacillus* spp. in Citrus plants and *Streptomyces* in Wheat (Berg et al., 2005). Bacterial endophytes colonize an ecological indentation similar to that of phytopathogens, which makes them suitable as biocontrol agents (Sturz and Matheson, 1996). Indeed, several reports have shown that endophytic microorganisms may have the ability to control plant pathogens (Duijff et al., 1997; Hallmann et al., 1998), nematodes (Azevedo et al., 2000), and insects (Chanway, 1997). In some cases, these accelerate seed germination, and can also promote plant establishment in adverse conditions (Strobel et al., 2004) and enhance plant growth. Bacterial endophytes have been shown to stop disease development through endophyte-mediated de novo production of new compounds and antifungal metabolites. Study of the biodiversity of endophytic strains for novel bioactive metabolites may identity new drugs for efficient treatment of diseases in humans, plants and animals (Siciliano et al., 2001). Along with the production of new chemicals, many endophytes have shown an active natural capacity for xenobiotic degradation or act as vectors to present degrading symptoms. The capability of some endophytes to show resistance to heavy metals/antimicrobials and degrade organic compounds most likely stems from their exposure to diverse compounds in the plant/soil niche. This natural capability to degrade these exonobiotics is being investigated in relation to improving phytoramidation (Barac et al., 2001; Posada and Vega, 2005). The endophytic niche provides a variety of protection from the environment, for those different bacteria that can colonize and establish in planta. These bacteria generally colonize the intercellular spaces, and they have been isolated from different part of the plant including seeds (Miche and Balandreau, 2001). It has also been observed that endophytic bacteria have been also isolated from monocotyledons and dicotyledons. Species of wood trees such as from oak and pear can also be isolate from plants of veg crops such as beetroot and corn. Conventional studies on the diversity of bacterial endophytes have focused on the characterization of isolates derived from internal tissues after disinfection of plant surfaces with sodium hypochlorite or similar agents. A review by Lodewyckx et al. (2002) highlights the different methods used to isolate and characterize endophytic microorganism (bacteria and fungus) from different plant species (Meneses et al., 2011).

2.1.2. Attachment of bacterial endophytes to the surface of the host plant:

It has often been observed that the attachment or adhesion of bacterial cells to the plant surface has been considered as the first step of the colonization process. Often bacteria in the areas around the roots of plants float towards the roots, using chemotactic affection for root exudates. It is then observed that bacteria attach to the root surfaces, which is important in reaching lateral root emergence areas or potential entry sites due to potential lesions or mechanical injuries. Exopolysaccharide (EPS) synthesized by bacterial cells facilitates the attachment of bacterial cells to the root surface and can sometimes be important in the early stages of endophytic colonization. The EPS produced by endophytic bacterium *Gluconacetobacter diazotrophicus* Pal5 was reported as a necessary factor for rice root surface attachment and colonization (Meneses et al., 2017). A current colonization study in rice plants using Diazotrophicus PAL5 has shown that bacteria survive oxidative damage to cells by Exopolysaccharide. Colonization always decreases, in an EPS knockout strain of *G. diazotrophicus.* The interesting thing is this there is subtraction in the Colonization was rescued by the addition of EPS produced by the different types of the wild strain (Balsanelli et al., 20145). Another study shows that, Balsanelli et al. 2014. Carried out analysis on mutant strains of *Herbaspirillum seropedicae* and told that That EPS production decreases and at its base they conclude that EPS is not necessary for plant bacterial colonization, which implicitly indicates differences in the genes required for colonization in a variety of endophyte species (Balsanelli et al., 2010). Bacteria have some structure such as flagella, fimbriae or cell surface polysaccharide which helps the bacteria to attachment to the plant cell. According to the *Balsanelli et al.* bacterial lipopolysaccharide (LPS) is very important to the attachment and succeeding (Balsanelli et al., 2013) endophytic bacterial colonization of different plant roots. Later it is also seen that the binding of N-acetyl glucosamine of LPS with maize root lectins helps the bacteria to attachment to the root surface, as well as to improve colonization inside the root (Compant et al., 2005). Bacterial compliance and colonization of the root interior likely happen in close succession given how rapidly colonization is observed in roots after inoculation with bacterial endophytes (Vargas et al., 2012; Rangjaroen et al., 2017; Romanstchuk, 1992). The procedure of compliance of Rhizobium on legume roots, plant pathogenesis bacteria in plant leaf or on the root surfaces, And Agrobacterium on the roots of the host plant has been studied extensively in the past, and whatever the result of this study (Rodriguez-Navarro et al., 2007; Matthysse, 2014; Hardoim et al., 2015), the different mechanisms by which different endophytic microbe's attach to plant internal surfaces remain relatively unexplained.

2.1.3. Entry of Bacterial Endophytes into the Host Plant: Bacterial endophytes are also called rhizoplanes, as they initially attach to the root surface and continue to explore potential entry locations to reach internal plant tissue. And it has been observed that in roots where root hairs or excessive lateral roots emerge, as well as stems, wounds and hydathodes in shoots as the main entry point that endophytes use to enter the host plant (Compant et al., 2005). Some endophytic bacteria can be seen using these natural different imbalances in this body of the plant to reach their place i.e. the internal plant cells and tissues. Apart from this, there are some bacteria that enter the internal tissues of plants by modifying the plant cell wall using different enzymes such as cellulase, xylanase, pectinase and endoglucogenesis. This is very convenient for different bacteria penetration and also spread everywhere within different tissues of the plant (Reinhold-Hurek et al., 2006; Naveed et al., 2014). Numerous colonization studies recommended that the natural cracks that occur at the different originating sites of different lateral roots are the most common and well-known parent entry site for endophytic bacteria (Hardoim et al., 2015; Iniguez et al., 2004; Compant et al., 2005). Apart from this, there are some bacteria which enter the plant tissue for colonization through root apex and root hairs (Prieto et al., 2011; Rangel de Souza et al., 2016).

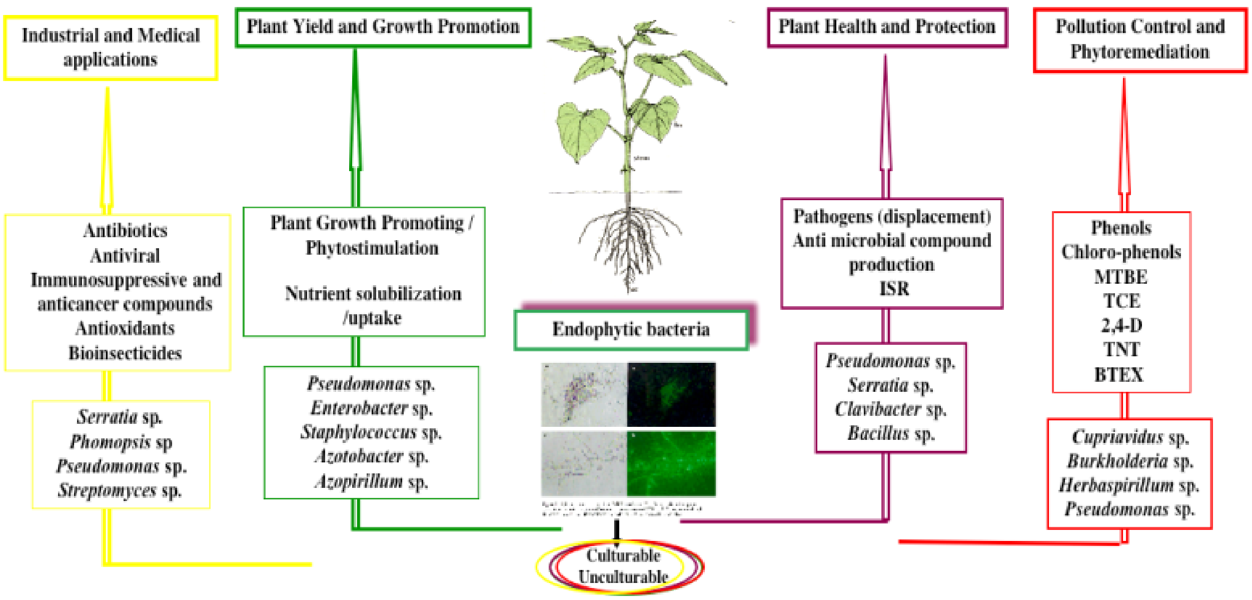


Figure 2.3: Schematic diagram of the different plant–bacterial endophyte interactions that have been studied and their applications

2.1.4. Colonization Cycle of Bacterial Endophytes in the Host Plant: Bacterial endophytes that are seen colonizing various seed parts including embryos. These are endophytes bacteria that have the ability to grow during seed germination or during the early days of seed germination (Nelson, 2017; Truyens et al., 2015). As the seed sprouts up and the plant begins to grow, the interactions between root and soil microbes can be seen. Whatever food material is present in the plant, it has been observed that it improves endophytes bacterial activity. Finally, some specialized endophytes can be seen initiating colonization from tissues, such as the stem, leaves, and early part of the root and some special endophytes bacteria that colonize can be seen to live their entire lives. There are also many bacterial endophytes that can colonize in flowers, seeds, and most likely come from the maternal endophytic community (Mitter et al., 2017; Truyens et al., 2015). Furthermore, a recent study showed that endophytes could colonize Corresponding seeds after the flowers were inoculated. Plants are constantly challenged by various biotic and abiotic conditions such as drought, heat, and biological disasters, various diseases and vegetarian attacks. And so, endophytes likely continue to manipulate their functional traits that allow them to interact rapidly with the host plant and react rapidly to minimize the consequences of adverse growth conditions (Doty, 2017; Compant et al., 2010). The presence of distinctive endophyte communities in diverse environmental situation and different stages of the host life sequence indicates that specific functional groups of bacteria are likely to be active in retort to a particular stress. In addition to vertically migrated endophytes bacteria, "Foreign different endophytes bacteria" (a new group of endophytes that have just been discovered) accrue in the plant endosphere throughout plant growth. "Different alien endophytes" that can colonize different plant parts and have the capacity to incorporate new functional traits into phytobomes by horizontal gene transfer together with different microbes.

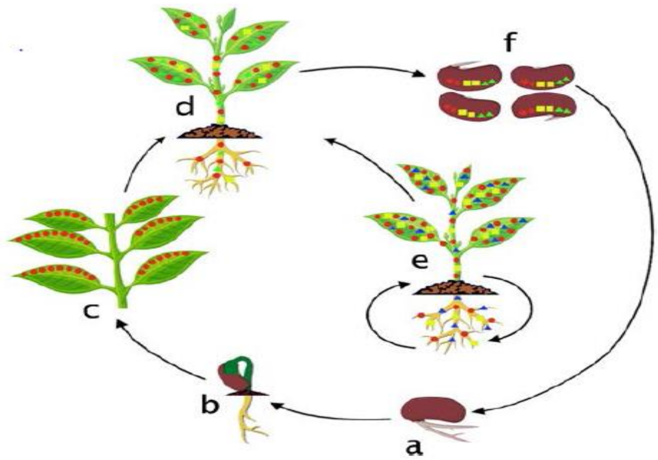


Figure 2.4: Hypothesized colonization cycle of different bacterial endophytes in the host plant. (**a**) The immobilization of different bacteria in germinating seed. (**b**) Congregate of alien endophytes from the soil in developing seedlings. (**c**) Colonized by different foreign endophytes bacteria and differently inherited endophytes bacteria. (**d**) Colonization on entire plant by various endophytes. (**e**) Differentiation of endophyte communities in the host plant in the context of various biotic and abiotic stresses. (**f**) Vertical relocates of endophytes into whole seed.

Endophyte Species	**Plant Colonized**	**Tissues Colonized**	**Native Host**	**Effect on Plant**	**References**
Pseudomonas thivervalensis	Tobacco, Black nightshade.	Root	Black nightshade	Growth improvement	Long et al., 2008
Pseudomonas sp.	Poplar	Leaf, stem, Root	Poplar	N/A	Germaine et al., 2004
Rhanella aquatilis	Hybrid poplar	N/A	Sweet potato	promote rooting	Khan and Doty, 2009
Rhizobium sp.	Wheat	Root	Poaceae family (sorghum maize, Pearl millet, and rice, wheat.)	Growth improvement	Patel and Archana, 2017
Consortium (*Herbaspirillum seropedicae, Gluconacetobacter diazotrophicus,*	Sugarcane	Shoot, Root.	Sugarcane	Growth improvement, improved N content	Oliveira et al., 2002

, Azospirillum amazonense and Burkholderia sp., Herbaspirillum rubrisubalbicans)					
Consortium (*Enterobacter asburiae, Rhizobium tropici, Acinetobacter calcoaceticus, Curtobacterium sp., Rhanella sp., Burkholderia vietnamiensis,., Sphingomonas yanoikuyae, Pseudomonas sp., Burkholderia sp)*	Hybrid poplar	N/A	Willow, Poplar	rise promoter, increased drought tolerance	Khan et al., 2016
Serratia marcescens	Rice	Leaf, stem, Root.	Rice	Growth promoter	Gyaneshwar et al., 2001
Consortium (*Pseudomonas putida, Rhanella sp., Acinetobacter sp.,*	Sweet corn	Shoot, Root.	Willow, Poplar	Growth improvement, improved CO2 assimilation	Knoth et al., 2012

Herbaspirillum sp., Burkholderia vietnamiensis, Sphingomonas spp.					
Consortium (Sphingomonas sp., Rhizobium tropici, Burkholderia sp., Acinetobacter calcoaceticus, Rhanella sp., Sphingomonas yanoikuyae, Burkholderia vietnamiensis, Pseudomonas sp.)	Rice	Root, shoot	Willow, Poplar	Growth enrichment (N-limited conditions)	Kandel et al., 2015
Consortium (Paentbacillus spp., Sphingomonas azotifigens, Pseudomonas spp.,)	Ryegrass	Root, stem, leaf	Rice ,Ryegrass	improved TFA, Growth enhancement.	Castanheira et al., 2017
Acetobacter diazotrophicus	Sugarcane	Stem	Sugarcane	N/A	Dong et al., 1994

Azospirillum sp.	Maize	N/A	Maize	Growth improvement	Riggs et al., 2001
Bacillus pumilus	Rice	Root	Rice	Growth improvement	Bacilio-Jimenez et al., 2001
Gluconacetobacter diazotrophicus	Sorghum, Wheat	Root, shoot, stem, leaf	Sugarcane	N/A	Luna et al., 2010
Enterobacter sp.	Maize	Root, stem, leaf	Maize	improved drought tolerance, Growth enrichment.	Naveed et al., 2014
Enterobacter sp.	Wheat	N/A	Tomato	Growth enhancement	Tian et al., 2017
Gluconacetobacter diazotrophicus	Rice, Sugarcane	Root, Shoot	Sugarcane	N/A	Balsanelli et al., 2014
Herbaspirillum seropedicae	Maize	Root	Maize	N/A	Liu et al., 2017
Herbaspirillum seropedicae	Sorghum maize, wheat, rice and	Root, stem, leaf	Maize	N/A	Roncato-Maccari et al., 2003
Ochrobactrum sp.	Rice	Root	Rice	N/A	Verma et al., 2004
Pseudomonas putida	Willow	Root	Hybrid poplar	concentrated phytotoxicity of phenanthrene ,Growth enrichment, decrease	Khan et al., 2014

				phytotoxicity of phenanthrene, degradation of phenanthrene	
Pseudomonas fluorescens	tobacco ,Black nightshade	Root	nightshade Black	Growth enrichment	Long et al., 2008; Stephane et al., 2010

Table 2.1: The colonization outline and growth-promoting characteristics of bacterial endophytes in different plant species are presented.

2.2 Benefit of Endophytes: allelochemicals of Endophytic bacterial are promoting plant growth and, as a result, have an advantageous effect on the host plant. These bacteria, which help the seeds to germinate, provide a kind of vigor to the plant, help the plant height increase and the nutrient material of the seedlings also increase, helping the flowers to bloom quickly. As well as increased amounts of chlorophyll and the development of stink in the plant is considered to be the result of bacteria. The beneficial effect of endophyte bacteria that is on the plant can be direct or indirect. In direct method, endophytes bacteria support to the plant growth in the nonappearance of different pathogens. Examples of direct plant growth encouragement by biofertilization which includes (a) Nitrogen fixation (b) It controls plant stress as well as stimulation of root growth, and directly promotes growth in the plant because they can reduce damage caused by pathogens (c) Phosphate and other organic and inorganic substance solubilization (d) Bioremediation (e) Phytohormone production (f) ammonia production (g) siderophore production (h) Reactive oxygen species detoxification (i) Production of antibiotics and biocidal volatiles (j) Hydrogen cyanide production.

(Lugtenberg and Kamilova, 2009). Many endophyte bacteria continue to produce secondary metabolites, which, along with their plants, benefit the human race.

2.2.1 Nitrogen Fixation: Nitrogen is a necessary element in plant growth and an important factor in plant development. It represents about 2.5% of the whole plant dry matter that enters the food chain. Even so the plant always accepts direct atmospheric nitrogen, and cannot use denitrogen gas. It takes about 80% nitrogen from the atmospheres. The remaining nitrogen plants always take the form of ammonia and nitrites. Only a few bacteria that take atmospheric nitrogen directly through a process known as biological nitrogen fixation, which is the change of atmospheric N_2 to NH_3 with the facilitate of nitrogenase, a NH_3 that can be used by plants (Franche et al., 2009).

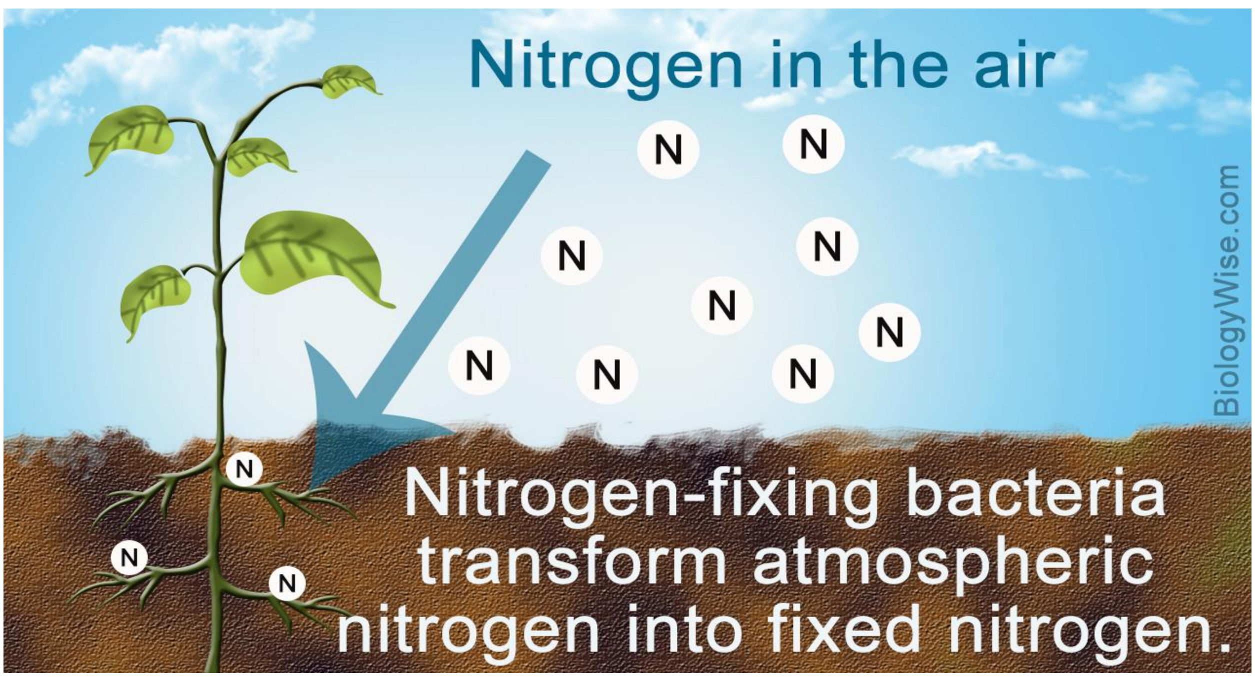


Figure 2.5: Nitrogen-fixing bacteria transform atmospheric nitrogen into fixed nitrogen.

There can be two types of nitrogen fixation, one symbiotic and nonsymbiotic. (Martinez-Viveros et al., 2010). Symbiotic nitrogen fixation is essential mechanism in which atmospheric N is fixed, but it is limited to legume plant species, only some trees and shrubs. This procedure is carried out in clear nodule structures. The most commonly used symbiotic bacteria are *Mesorhizobium, Bradyrhizobium, Sinorhizobium and Rhizobium* (Zahran, 2001). While on the other hand, nonsymbiotic biological N determination is made by free-living *diazotrophicus* and it can always be seen to stimulate the growth of non-legume plants (Antoun et al., 1998). In this way, nitrogen fixation by bacteria contributing to deceased dependence on nitrogen based biofertilizers (Bhattacharjee et al., 2008). Non-symbiotic N-fixing *rhizospheric* bacteria belonging to genera *Pseudomonas, Acetobacter, Azospirillum, Herbasprillum, Burkholderia, Azoarcus* and Enterobacter. In this case of nitrogen-fixing endophytes bacteria are hosted in a large particular cavity located on the dorsal lobe of the leaves. During its entire life cycle, symbiosis is always associated with its host and automatically nurtures the plant from generation to generation during sexual reproduction (Santi et al., 2013).

2.2.2. Endophytes improve Bioremediation: It is a special treatment that is used by natural microbes to break down or remove less toxic or non-toxic substances present in the environment. Heavy metals are a major concern for human health due to their different cytotoxicity, because this metal continues to directly impact on human health and there are various pathogens (carcinogenicity and mutagenicity). Polluted different soils are also remediated by use of plants termed as a phytoramidation. Phytoremediation is a type of eco-friendly and low-cost effective technology that is being used extensively on global labels (Glick, 2010). Metal tolerant plant–microbe associations have been the objective of meticulous consideration due to the probable of microorganisms for bio concentrated metals from polluted environment and therefore, enhancing metal uptake and improved plant growth. Synergistic use of plants and microbes has made it possible to clean the metalized soil (Jing et al., 2007). According to the Saravanan et al., 2007 that produced of 5-ketogluconic acid by endophytic diazotroph (*Gluconacetobacter diazotrophicus)*, which it dissolves different zinc sources such as ZnO, $ZnCO_3$, or Zn_3 $(PO_4)_2$, hereby production Zn available for plant use. Madhaiyan et al. (2007) says that inoculation with endophytic bacteria, *Magnaporthe oryzae* and *Burkholderia* sp. Both of these improve the growth of the plant but reduced nickel and cadmium accumulation in root of tomato plants.

2.2.3 Hydrogen Cyanide Production: Hydrogen cyanide (HCN) is a one type of volatile molecule and a secondary metabolite that has the ability to control weeds, suppresses the pathogens, and at the same time it also negatively affects the growth and development of plants ((Siddiqui et al., 2006). Bacteria secreted HCN as biocontrol compounds. There are many other bacteria that have the ability to produce Hydrogen cyanide, such as *Rhizobium, Alcaligenes, Bacillus* and *Pseudomonas*. The HCN secreted is found to be a general trait of *Pseudomonas* (87.89%) and *Bacillus* (51%) (Saharan and Nehra, 2011). Cyanogenic glucosides form a group of N-containing protective compounds other than these alkaloids, which releases toxic HCN and in particular these are members of Leguminosae Graminae and Rosaceae families. They are not toxic in themselves, are readily broken down to give off volatile poisonous substances, HCN, and it has been seen that when the plant is crushed; under normal conditions, HCN is a toxin of cellular respiration by binding to the Fe-containing heme group of cytochrome oxidase and other different respiratory enzymes.

2.2.4 Different Antibiotics Production: We can say that despite a wide variety of antibiotics, less than 1% of antimicrobial bioactive metabolites agents have medicinal or marketable value. For example, penicillin has a high therapeutic index because it is commonly observed that it does not affect human cells, while other antibiotics have not seen any effect in this way yet.

Native organisms for penicillin production, Penicillium notatum was isolated in 1926 by Alexander Fleming as a possibility contamination while culturing of other organisms. Production of antibiotics is a natural phenomenon in history, after the initial discovery of penicillin; most antibiotics were discovered as a consequence. It has been seen that more and more bacteria are developing resistance to different antibiotics daily; it has become necessary to find different antibiotics. And in addition to research and development in the production of various new antibiotics, improving the repackaging and delivery system has also become very important to maintain the efficacy of the currently produced antibiotics.

Improvements in this area are seeing the potential to add antibiotics to properly implanted devices, aerosolization of antibiotics for direct delivery, and many different antibiotics are also being combined with non-antibiotics for improve the results. The raise of antibiotic resistant strains of pathogenic bacteria has led to an improved urgency for the funding of research and development of antibiotics and an incline for production of new and best acting antibiotics. Antibiotics are defined as low-molecular-weight organic natural products made by microorganisms that are active at low concentration against other microorganisms. Mostly, endophytes bacteria are considered a main source of these antibiotics. It has often been observed that bioactive metabolites derived from endophytes and kill a wide variety of harmful disease-causing agents including bacteria, fungi, viruses, and protozoa, which cause negative effect on the humans and animals. The antibiotics produced by plant growth producing bacteria (PGPB) include: zwittermycin A, oomycin A, oligomycin A, kanosamine, phenazine-1-carboxylic acid, pyrrolnitrin, viscosinamide, pyrrolnitrin, 2, 4-diacetyl phloroglucinol (2, 4- DAPG) and xanthobaccin (wipps, 2001). Antibiotics are different products of different endophytes bacteria used as anticancer agents, immunosuppressive compounds and antidiabeties etc. Is a plant, such as *A. annua* producing antimalarial compounds? There are also some endophytes that show potent activity against protozoal diseases. Large range-spectrum antibiotics are produced by *Streptomyces* sp. strain NRRL 30562, an endophyte in *Kennedia nigriscans*. These antibiotics are called munumbicins; widely different types of biological activity occur depending on the target organism. In common, the munumbicins demonstrate activity along different gram-positive bacteria such as *Bacillus anthracis* and much multidrug-resistant *Mycobacterium tuberculosis*, *Plasmodium falciparum* as well as a number of other drug-resistant bacteria (Castillo et al., 2002). And many nonpeptideal fungal (L-783, 281) metabolites have been isolated from endophytic fungi (*Pseudomas saria* sp.) It was collected from an African rainforest close to Kinshasa in the Democratic Republic country of Congo.

This compound can be seen to act in a way that work like insulin, and like insulin, it is found not to be destroyed in the digestive system, and may be given orally. The possibility of new treatment of diabetes may arise through these results (Zhang et al., 1999). There are also some microbes (bacteria and fungus) that produce immunosuppressive drugs; Immunosuppressive drugs are currently being used to prevent allogelotype rejection in transplant patients and in the future, they could be used to treat autoimmune diseases such as rheumatoid arthritis and different insulin-dependent diabetes (Lee et al., 1995). Some paclitaxel and some of its active derivatives are produced by various endophytes and act as anticancer agents. Paclitaxel, a highly functionalized diterpenoid, is found in each of the world's yew (Taxus) species. Torreyanic acid, is a selectively cytotoxic quinone dimer (anticancer agent), was isolated from a *P. microspora* strain. This strain is originally obtained from the endangered tree T. taxifolia (Florida torreya) (Lee et al., 1996). Torreyanic acid was checked in a lot of cancer cell lines samples and it established 5 to 10 times more effectiveness in those lines that are sensitive to protein kinase C agonists and causes cell death by apoptosis. Various biopesticides which form a very small part of the pesticide sector. Market is being continuously increased these days? Several endophytes bacterial are known to have many anti-insect properties. Novel indole diterpenes, Nodulisporic acids that demonstrate effective insecticidal properties against the larvae of the blowfly, work by activating insect glutamate-gated chloride channels. Nodulisporic compounds were first isolated from an endophyte a *Nodulisporium sp.* and this species was isolated from a plant Bontia daphenoids. The two new active compounds, 5-hydroxy-2-(1'-hydroxy-5'-methyl-4' hexenyl) benzofuran and 5-hydroxy-2-(1'-oxo-5'-methyl-4' hexenyl) benzofuran, both show toxicity to spruce budworm, and the latter is also toxic to the larvae of spruce budworm. In this way, the isolation of many active compounds has been done, and many active compounds remain to be isolated. Which act as antidrug in various diseases?

The greater parts of EB do produce various kinds of antibiotics; and in fact, the endophytes bacteria are one of the untapped probable sources of new antibiotics. Ecomycins, Pseudomycins, Munumbicins, Kakadumycins are some examples of the new antibiotics produced by endophytes bacteria.

Ecomycins: The Ecomycins characterize a family of novel lipopeptides and are made up by some remarkable amino acids including homoserine and β-hydroxy aspartic acid. The Endophytic bacteria, *Pseudomonas viridiflava* is known to produce Ecomycins (Condron et al., 1998). Endophyte is a kind of plant associate bacteria, such as *fluorescent Pseudomonads*; and the identified and moderately characterized 03 antifungal lipopeptides synthesize by *P. viridiflava strain EB273* are called as Ecomycin A, B and C. out of these three molecules, Ecomycin A shows some similarities (amino acid composition) to previously reported antibiotic syringotoxin (Ballio et al., 1990). Ecomycin B and C represent an exceptional set of related lipopeptides in not possessing arginine, lysine, ornithine or diaminobutyric acid and phenylalanine which are constituents of such compounds as the syringostatins, pseudomycins, syringotoxin and syringomycins (Ballio et al., 1990; Harrison et al., 1991). Detail study was done with some bacteria; it was found that these bacteria are also producing antifungal lipopeptides as seen with the previous bacteria, such as *P. viridiflava* strain EB274 (California, USA) and EB227 (Israel) whose masses are identical to those of Ecomycins B and C. These compounds were able to inhibit the human pathogens *Cryptococcus neoformans* and *Candida albicans* as reported by Harrison et al., 1991.

Pseudomycins: Pseudomycins is a microbe and synthesizes the biochemical of a peptide group. And these peptides are separated from the Pseudomycins liquid medium and these microbes belong to a plant. It is an antifungal peptide that is related to lipopeptides and contains various amino acids such as L-diaminobutyric acid, L-chlorothreonine, and both D-and L-hydroxy aspartic acid. The *P. syringae* is a member of the *Pseudomonadaceae* family of *Proteobacteria* Phylum. Pseudomycin A, the major peptide in a family of four, pseudomycins A, B, C and D, has remarkable activity against the different human pathogen (Harrison et al., 1991). Pseudoscience A and C contains various amino acids such as serine, arginine, hydroxyaspartic acid, diaminobutyric acid and lysine. Pseudomycin D molecular weight is 2401 Dalton which is more complex than Pseudomycin A and C. However, they are establishing to be different from the formerly described antimycotics from *P. syringae*, including syringomycin, syringotoxin and syringostatins. They are successful against a variety of human as well as plant pathogenic fungi including *C. neoformans* and *C. albicans* (Harrison et al., 1991).

Munumbicins: Munumbicins that induces 04 new groups of bioactive metabolites.

Munumbicins A, B, C and D are recently described antibiotics with a broad spectrum of bioactivity against human/plant pathogenic fungi and bacteria, and different Plasmodium species. These compounds were received from a bacterium that has been accumulated as *Streptomyces* NRRL 30562 in the National Laboratory of mediPeoria USDA. This endophyte is primarily a bacteria found in the snake hole. The munumbicins show anti-properties against various gram-positive bacteria, such as *Staphylococcus aureus, Bacillus anthracis, Enterococcus faecalis* and *Streptococcus pneumonia.* And along with that, the methicillin-resistant strain of *S. aureus* (MRSA, ATCC 33591) and a vancomycin-resistant strain of *E. faecalis* (VREF, ATCC 51299) are 02 of the Gram-positive munumbicin-sensitive bacterial strains that are generally drug-resistant.

Munumbin B effectively works against various drug resistances (MDR), Mycobacterium tuberculosis bacteria and an acid-fast bacterium. However, the mainly peculiar fact is that just the MDR strain of *M. tuberculosis* was sensitive to munumbin B whereas the drug-susceptible strain of this organism was not as much of sensitive. The munumbicins C and D are of a particular interest because in addition to being efficient against Gram positive and negative bacteria, they are effective against the malarial parasite Plasmodium falciparum, the most pathogenic Plasmodium producing malaria. The munumbicin D was reported as additional powerful than chloroquine, the gold-standard antimalarial drug. (Obianime and Aprioku, 2009) moreover, munumbicins B, C and D did not cause any detectable lysis of human red blood cells.

X**iamycins:** The Xiamycins represent single of the indolosesquiterpenes isolated from Prokaryotes. They are novel pentacyclicindolosesquiterpene, named as Xiamycin-A and its methyl ester-2 synthesized from Streptomyces sp. strain GT2002/1503, an endophyte from the mangrove plant, Bruguiera gymnorrhiza. (Dinge et al., 2010) Interestingly, Xiamycin-A exhibits selective anti-HIV activity. (Dinge et al., 2011) 03 novel indolo sesquiterpenes, Xiamycin B (1b), Indosespene (2), and Sespenine (3), along with the known Xiamycin A (1a) from the culture broth of Streptomyces sp. strain HKI0595, a bacterial endophyte of the extensive mangrove tree, Kandelia candel has been reported by (Dinge et al., 2011) Their research obtaining suggest that these Xiamycins do have reasonable to strong antimicrobial bioactivities against numerous bacteria and fungus, together with methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*.

Kakadumycins: Kakadumycins is a peptide produced by endophytes (Streptomyces NRRL30566) that are isolated by the Grevillea tree (Grevillea pteridifolia, Synonym: Grevillea chrysodendron R. Br). (Castillo et al., 2003) Kakadumycin A is chemically associated to echinomycin, an extra Streptomyces derived quinoxaline antibiotic, a probable anticancer drug. (Katagiri et al., 1975; Waring and Wakelin, 1974) Kakadumycin A has antibacterial activity parallel to Munumbicins and it is also efficient against *P. falciparum*.

Name of the endophyte	Antibiotics isolated	Reported activities	Source of the Endophyte	Chemical Nature	Reference
Streptomyces sp. Strain (NRRL - 30566)	Kakadumycin	Gram positive and negative bacteria	Greevillea pteridifolia	Peptide with quinoxaline rings	Miller et al., 1998
Streptomyces sp. strain (NRRL-30562)	Munumbicin A, B, C and D	Gram positive and negative bacteria	Kennedia nigriscans	Peptide	(Castill.et al., 2002; Castillo et al., 2006)
Streptomyces sp. GT 2002/1503	Xiamycin A	Anti HIV	*Bruguiera gymnorrhiza*	Pentacyclicindolosesquiterpine	Waring and Wakelin, 1974
Pseudomonas viridiflava EB 273	Ecomycine B, C	Antifun al	*Lactuca sativa*	Lipopeptides	Miller et al., 1998
Streptomyces sp. HK 10595	Xiamycin B, Indosespine and Sespenine	Antibacterial	*Kennedelia candel*	Pentacyclicindolosesquiterpine	Ding et al ., 2011

Table 2.2: List of novel antibiotics produced by endophytic bacteria and their reported activities.

2.2.5 Production of some Volatile Antibiotics by Endophytes: *Muscodar albus* is a new endophytic fungus that has been isolated from small organs of Cinnamomum zeylanicum (cinnamom tree) (Worapong *et al.,* 2002). This *xylariaceaous* (non-spore-forming) fungus efficiently inhibits and kills certain other fungi and bacteria by producing a mixture of volatile compounds. Identification of most of the volatile compounds is done through different techniques such as, HPLC, gas chromatography-mass spectrometry, NMR and FTIR. Each of the five classes of volatile compounds produced by the fungus was detected in contrast to different bacteria and fungi, but not one of them was fatal to these bacteria and fungi. However, these metabolites, acting collectively, served as an inhibitor for various plant and human pathogenic fungi and bacteria. The mainly efficient class of inhibitory compounds was the esters of which isoamyl acetate was the highest biologically active compounds.

Considering the mycofumation effect of the M album, America is going to stop using methyl bromide in its agriculture very soon and its use will be illegal. Mycofusion-wide use has been started for various treatments of soil, seeds and plants at a very big level. In fact, these microbes are already treating or decontamination human waste. By using *M. albus* as a screening tool, it has been possible to isolate other fungi, thereby producing metabolites of a good quality. *Muscodor rosans* have been isolated from trees growing in the northern region of Australia; this plant has been seen to growing twice a year. This fungus is immediately as useful in causing inhibition and death of test microbes in the laboratory as M. albus (Worapong et al., 2001). In addition, for the initial time, one more muscodor species, a *Gliocladium sp.* was discovered to be a volatile antibiotic producer. Metabolites of these microbes are completely different from metabolites of *M. albus* or *M. roseus*. The bioactivity metabolites of the volatile compounds of *Gliocladium sp*. is not as good quality or comprehensive as those of the *Muscoldor spp.* (Stinson et al., 2003).

Muscodol albus is endophytes that are isolated from Cinnamomum zeylanicum and it endophytes produces volatile organic compounds in culture and its range of antimicrobial activity is wide. Using *M. albus* as a selection tool, scientists want to isolate different microbes with different culturally and biochemically properties that can form highly active metabolites. And some isolates have also been done, for example *Terminalia prostrata* (nanka bakarra), *Grevillea pterifolia* (fern-leafed grevillea), and *Kennedia Nigriscans* (snake vine). In bioassays with a variety of test micro-organisms, 41 including fungi and bacteria, every isolate obsessed biological activity but the range of activity were vast.

Compounds	**Biological activity**	**Endophytic bacteria**	**References**
Bacillomycin	Antifungal, Hemolytic	*B. subtilis, B. amyloliquefaciens*	Aranda et al. 2005
Nystatin	Anti-fungal	*Streptomyces noursei*	Fjaervik and Zotchev, 2005
KB425796-A	Anti-fungal	*Paenibacillus* sp. 530603	Kai et al., 2013
Bacilysocin	Anti-fungal	*B. subtilis* 168	Tamehiro et al., 2002
Ecomycin	Anti-fungal	*Pseudomonas viridiflava*	Miller et al., 1998
Harmaomycin	Antibacterial	*Streptomyces* sp.	Bae et al., 2015
Munumbicin	Antibacterial	*Streptomyces* NRRL 30562	Castillo et al., 2002

Tetracyclin	Antibacterial	*Streptomyces remosus* and *S. aureofaciens*	Mark et al. (2001)
Bacteriocins	Antibacterial	*B. subtilis*	Sansinenea and Ortiz, 2011
Amicoumacin	Antibacterial, anti-inflammatory	*B. subtilis*	Pinchuka et al. (2002)
Treponemycin	Anti-tuberculous	*Streptomyces Strain MS-6-6*	Mahmoud et al., 2015
Camptothecine	Anti-cancer	*Lysinibacillus sp. and B. cereus*	Singh et al., 2013
Androprostamines	Anti-prostate cancer	*Streptomyces sp. MK932-CF8*	Yamazaki et al., 2015
Doxorubicin	Treatment of Breast cancers and tumors	*Streptomyces sp.*	Brayfield, 2013
Indolocarbazoles	Anti-cancer	*Streptomyces sp.*	Dong et al., 2014
Anthracyclin	Antitumor	*Streptomyces sp. YIM66403*	Wei et al., 2015
Monensin	Prevent coccidiosis	*Streptomyces cinnamonensis*	Lowicki and Nski, 2013

Daptomycin	Bacterial infections of skin and underlying tissues	*Streptomyces roseoporous*	Miao, 2005
Saadamycin	Anti-dermatophyte	*Streptomyces sp. Hedaya48*	Gendy and Bondkly., 2010
Mytomycin C	Chemotherapeutic agent	*Streptomyces caespitosus and S. lavendulae*	Danshiitsoodol et al., 2006
Albaflavenol B	As sesquiterpene	*Streptomyces sp.*	Raju et al., 2015
Saadamycin	Anti-dermatophyte	*Streptomyces sp. Hedaya48*	Gendy and Bondkly, 2010
b-exotoxin	Insecticidal	*B. thuringiensis*	Espinasse et al., 2002
Strepturidin	Immunotherapy	*Strepturidin Streptomyces albus DSM 40763*	Pesic et al., 2014
Xiamycin	Anti-HIV activity	*Streptomyces sp.*	Ding et al., 2010
Thaxtomin A	Cellulose synthesis inhibitor	*Streptomyces scabies*	Francis et al., 2015

Table 2.3: List of some Volatile Antibiotics produce by Endophyte microbes.

2.2.6 Control of Plant Stress (Increment of Drought Resistance): Stress can be considered as a major factor in any environmental change that has different types of effects on the plant by affecting the biochemical and physiological response to such different changes, and may on occasions lead to damage or injury. The main causes of plant stresses are climate (likes: wind, hail, hot, dry, low relative humidity and cold), some biofactors such as (bacteria, nematode, insects, and fungus) and some phenologic stages (flowering, ripening and budding) or crop management (phytotoxicity by pesticide and root, shoot damage) etc. Plants never grow under optimum conditions during their life cycle, but suffer many adverse conditions that cause different types of stress, and prevent them from going to a maximum growth and development rate. Endophytes bacteria help reduce this stress. It has often been observed that plants that contain endophytes have higher biomass percentage (17% more total, than the without contain endophytes plants).

Below low water, endophyte-infected plants produced 23% extra root biomass, but just 15% additional shoot biomass than disinfected plants. When there is a shortage of water, it is often observed that disinfected plants have 29% more leaf content than endophyte-infected plants. The systemic endophyte Neotyphodium coenophialum in long fescue grass (Lolium arundinaceum) can increase host growth and survival relative to unirrigated plants when drought occurs (Kannadan and Rudgers, 2008). Endophyte-mediated plants reductions in root diameter, increased root hair length, and a decrease in root production near the soil surface can increase plant water absorption and always reduce the likelihood of drought.

2.2.7. Industrial enzymes production by endophytes: Different endophytic microorganisms (bacteria or fungi) that is predominantly symbiotic of the plant. Throughout their lifetime, all these endophytic microorganisms try to stay connected to the plant forever. And this association that starts from seed germination to fruit development. These are the Endophyte distributors, these spermosphere (in seeds), anthosphere (in flowers), carposphere (in fruits), rhizosphere (roots) and phylloplane (in leaves). This idea is given by Clay and Holah (1999). Various researchers (Sessitsch et al. 2012; Saikkonen et al. 2004; Lindow and Brandl 2003) He explained the role of unique endophytes, bacteria and fungi with the plant, All these microbes continue to provide mankind with an alternative resource, or facilitate the distribution or production of various active metabolites from biological forms, such as phytohormones, nutrients, enzymes, minerals, biochemical chemicals (Schulz et al. 2002). Fungi and bacteria have the ability to produce different types of enzymes. Which are oxidoreductases, lyasese, transferases and hydrolases (Traving et al. 2015). These enzymes that target different types of macromolecules and micro molecules' such as phosphate, lignin, carbohydrates, proteins and sugar-based polymers to break into movable product throughout the cells and to carry on heterotopic metabolism (Boer et al. 2005; Strong and Claus 2011; Sinsabaugh 1994). Hallmann et al. (1997) are saying that Endophytes can produce enzymes, and at the same time help the hosts initiate the symbiosis process. Apart from establishing cooperation with the host, these endophytes also use external and internal hydrolyses to combat pathogenic infection of bacterial plants (Leo et al. 2016; Tan and Zou 2001). Many such ecological roles have been allotted to endophytic bacteria and fungi, which also play their roles well. Many endophytic bacteria can be seen as a new form of gene, biochemical and protein compound, which can be further improved by industrial processes. There is a gene named Agla that is cloned from a citrus endophytic Bacillus strain. Angle encodes beta-1, 4-endoglucanase under in vitro conditions that are capable of hydrolyzing cellulose. An incubation period of more than 45–55 °C is a feature for further applications of the enzyme in biological processes, in which needs of higher temperatures (Lima et al., 2005). Cho et al. (2007) reported that interference of quorum sensing and virulence of the rice pathogen Burkholderia glumae by an engineered endophytic bacterium. Of these, 44 endophytic are derived from the surface-sterilized root of rice. KJ006 were selected for more study because it given that the most important virulence thing of Burkholderia glumae is prohibited in a population dependent way (quorum sensing) N-acyl-homoserine lactonase (aii A) gene beginning Bacillus thuringiensis was introduced into Burkholderia sp., It regulate the seedling rot and grain rot of rice which caused by Burkholderia glumae. The results of the engineered strain KJ006 (pKPE-aiiA) not only prevented quorum-sensitive signals from being produced by Burkholder Gluma, but also worked the disease of rice seedling rot due to Burkholder Glume. Such types of results point to the different engineered bacterial endophyte with aii a gene may be used as a best biological control agent against pathogenic Burkholderia glumae. According to Cho *et al.* (2007) Different roots of plants that contain different communities of endophytic bacteria, and do not originate any negative impact on the plant. Despite being everywhere in the root, stem, leaf, flower, and seed of the plant, the bacteria do not put any negative impact on the plant, but only helps in the development of the plant in every way. Endophytic bacterial strains have been isolated and identified

From a range of plants such as wheat (Triticum aesitivum), cucumber (Cucumis sativa), tomato (Lycopersicum esculentum), corn (Zea mays), pea (P. sativum), oat (Avena sativa), potato (Solanum tuberosum), radish (Raphanus sativus), soybean (Glycine max) and lettuce (Lactuca serriola). And many bacterial endophytes have also been isolated from different medicinal plants and there is also some new strain. Their name is, *Enterobacter, Flavobacterium, Rhizobium, Erwinia, Serratia*

Genuses, Arthrobacter, Alcaligenes Bacillus, Flavobacterium Pseudomonas and Aeromonas (Gray and Smith 2005). There are some endophyte bacteria that are known to produce various products, such as asparaginase, cellulases, amylase, protease, pectinase, phytase, esterase, protease, lipase etc (Carrim et al. 2006; Sturz et al. 2000). In such investigative studies based on agar plate detection methods, examined that Lavandula dentate harbored more than 32 endophytic bacterial strains. In phyllosphereic a part of the Lavandula dentate, the endophytic bacteria produced high quantities and quality of plant cell wall-degrading enzymes. As also evidenced by Verma et al. (2001) who have reported great number of endophytic bacteria from diazotrophs plant and their growth regulation by producing pectinase and cellulase.

There are broad arrays of source studies performance that production of these enzymes by endophytic bacteria is detected from different bio-instruments **(Table 2.4).**

Different Species	Detection method	Enzyme produced	References
Pseudomonas sp.	Spectrophotometer, NMR	Exo-b-agarase	Gupta et al. (2013)
Bacillus licheniformis, Bacillus pyogenes, Bacillus coagulans, Corynebacterium renale, Pseudomonas stutzeri, megaterium, Actinomyces, Staphylococcus sp., Bacillus sp. Bacillus circulans.	Agar medium	Protease, Amylase, Lipase, esterase.	Carrim et al. (2006)
B. amyloliquefaciens *Bacillus sp.*	Spectrophotometer Spectrophotometer	Phytase L-asparaginase	Idriss et al. (2002) Ebrahiminezhad et al. (2011)
pumilus, Bacillus, Bacillus sp., Bacillus licheniformis Bacillus Clausii.	Agar medium	Cellulose, Amylase, protease, lipase	Kannan et al. (2015)

Bacillus amyloliquefaciens	Spectrophotometer	Phytase	Idriss et al. (2002)
Paenibacillus polymyxa	SDS Page, Agar medium,	Fibrinolytic enzymes	Lu et al. (2007)
Pantoea, *Massilia*, *Kosakonia*, *Pseudorhodoferax*, *Caulobacter*, *Sphingomonas*, *Burkholderia*, *Rhizobium*, *Curtobacterium*, *Microbacterium*, *Mucilaginibacter*, *Chitinophaga*, *Massilia*, *Kosakonia*, *Bacillus*.	Endoglucanase, ACC deaminase, Protease	Agar medium	Chimwamurombe et al. (2016)
Macrococcus, *Pseudomonas hibiscicola*,	Agar diffusion method	Amylase, Cellulase, Xylanase, Pectinase	Akinsanya et al. (2016)

Senitriformus, Bacillus licheniformis, Paenibacillus, Bacillus Pseudomycoides.	M9 medium	L-asparaginase	Joshi and Kulkarni (2016)
Bacillus Amyloliquefaciens *Bacillus subtilis*	Colorimetric Method MALD-TOF-MS SDS-PAGE,	Exopolysaccharides YbdN protein	Chen et al. (2013) Jamal and Mudarris (2010)
Bacillus subtilis, Serratia marcescens, Bacillus siamensis Bacillus Methylotrophicus.	Spectrophotometer	L-asparaginase	Nongkhlaw and Joshi (2015)

Paenibacillus polymyxa, Bacillus sp.	Agar diffusion method	Pectinase, Cellulase, xylanase.	Cho et al. (2007)

Table 2.4: List of Enzyme produced by different endophytes microbes.

Endophytic microbes produced production of ligninases obtained from Brazilian mangrove ecosystem. Yang et al. (2011) according to Alcaligenes faecalis produced cellulases and xylanases with high capability to breakdown to cellulosic substrate in a coculture system.

2.2.8. Enzymes Quantification: Extracellular enzymes are measured qualitatively and quantitatively from sophisticated advanced spectrophotometric, well dish diffusion and minimum inhibitory concentration (MIC) methods to agar plate-based methods. The ability of endophytic microbes to produce enzymes is also designed for their ability to increase or reproduce in a particular type of media. Overall, various endophytic microbes are isolated from the hard surface of plants through proper sterilization, by using antifungal for bacteria and antibacterial drugs for fungal. When once all the isolation process is over, and once pure bacterial isolate is obtained, we identify it in different ways. For example, 16S rRNA sequencing or internal transcribed spacer (ITS), using PCR amplification, Sanger sequencing, BLASTn, and finally a detailed phylogenetic Analysis. An isolated endophyte is allowed to grow in a special compositional media to allow bacteria to produce different types of enzymes while growing; these enzymes can be detected by agar plate (initial screening) or by detailed spectrophotometric methods (UV / Vis or fluorescence). Currently, fluorogenic substrates such as 4-methylumbelliferone (MUB) are being used for soil or marine enzyme analysis on a much larger scale (Khan et al. 2016; Wallenstein et al. 2008).

However, different techniques should be adopted not only to assess the amount of analysis of different enzymes but also to combine such studies with molecular and genomic functions to validate their findings and processes.

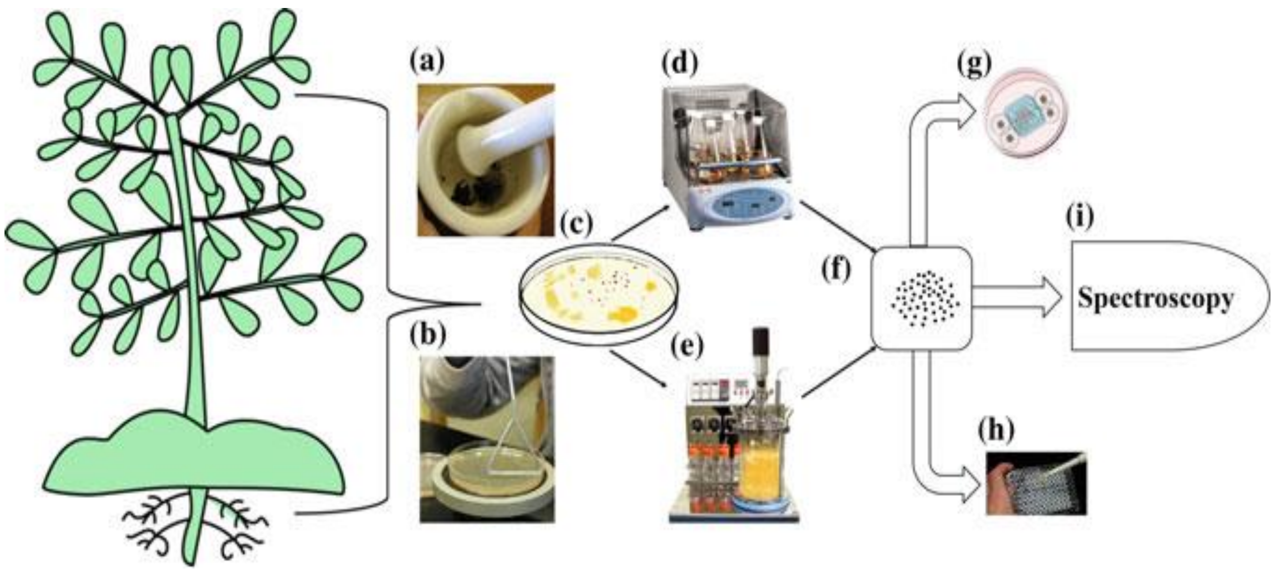


Figure 2.6: Diagrammatic image of the isolation, fermentation and quantification of enzymes. (a) Cursing and grinding of plant piece (b) isolating of endophytes bacteria from piece (c, d) some pure culture development (d) fermentation process (e, f, g, h) isolation of different enzymes and (i) quantification through high level different chromatographic techniques.

For the last two and a half decades, the scientific community is familiar with what the main role of endophytic microbes is in agriculture, ecology, biotechnology and industry, and is constantly engaged in the research of thesis, and there are still a lot of unexplained mysteries left from microorganisms unique in ecological form that make primary active metabolites (different enzyme) in particular that still require special attention. These can be used in Enzymology-based enzyme fermentation industries, whatever endophytes are isolated from plants living in highly contrasting environments, and they have a greater ability to produce different enzymes. Many scientists have been experimented on monoculture, while coculture and other aspects such as the growth of media and the signaling pathway of quorum sensing of endophytes are yet to be developed (White and Torres, 2010). Methods such as fluorescence spectrophotometer, near-infra red (NIR), and FTIR-based methods further improve enzyme analysis. The endophytic biomasses are particularly significant in determining their capability and alternative to a vital reserve for biofuel production. The molecular and genomic bases of these endophytic resources for enzymes production also require cross-validation (Xiong et al., 2013).

2.2.9 Products from Endophytes bacteria as Antioxidants: Two compound pesticide and isopesticide have been obtained by culturing of *P. microspora*, which this compound shows a high-level antioxidant property. *P. microspora,* an endophyte isolated from a combretaceaous plant. Isopectacin's antioxidant activity was previously suspected because its structure was very similar to flavonoids. By Electron spin resonance spectroscopy measurements inveterate this antioxidant activity; the compound is able to scavenge superoxide and hydroxyl free radicals in

Solution (Strobel, 2002). Pestacin was later shown to be the same fluid that naturally occurs as a racemic mixture and exhibits a powerful antioxidant property.

Projected antioxidant activity of pestacin arose primarily via elevage of a usually reactive C−H bond and to a lesser extent, though O−H abstraction. The antioxidant activity of pestacin is at least 1 order of magnitude superior to that of trolox a vitamin E derivative (Harper et al., 2003).

2.2.10 Products from Endophytes bacteria as Anticancer Compounds: Cancer is said to be a kind of controlled growth of the cell in which the cell gets growth without any contact inhibition, and there is nothing called mortality in it and which can result in death if not controlled (American Cancer Society, 2009). And cancer is considered the leading cause of death all over the world. Whose number is 6.7 percent (about 13% of all deaths) of the total population of the world (WHO Mortality., 2009). The anticancer drugs display nonspecific toxicity to proliferating regular cells, possess enormous side effects, and are not effective against many forms of cancer (Gangadevi and Muthumary, 2008; Pasut and Veronese., 2009).

Thus, the therapy of cancer has been improved primarily due to diagnosis enhancements which allow earlier and more exact treatments (Pasut and Veronese., 2009). There is some evidence through which some bioactive metabolites are produced from endophytes which can be used to make some new drugs for the treatment of cancer. Many natural products from various plants, microorganisms and sea sources have been identified as anticancer agents. And it is being used in various cancer treatments (Firakova et al., 2007). The anticancer properties of several secondary metabolites from endophytes have been investigated recently. Following, approximately examples of the possible of endophytes on the production of anticancer agents are mentioned. The diterpenoid “Taxol” (also known in the literature as paclitaxel) have generated more attention and interest than any other novel drug since its discovery, this is probably due to its unique mode compared to other anticancer agents (Firakova et al., 2007; Gangadevi and Muthumary, 2008). These anti-cancerous metabolites reduce the growth and spread of cancer cells by manipulating the cell's division. FDA (Food and Drug Administration) has proved that Taxol is an important drug for breast, ovarian and lung cancer which is used in such cancers (Cremasco and Hritzko, 2009). Taxol ($C_{47}H_{51}NO_{14}$) was firstly isolated from the bark of trees belonging to Taxus family

(Taxus brevifolia), its most common source (Wani et al., 1971). Tax-producing plants meet rare and at the same time produce a lot of toxol production. Due to which its price is very high in the market. Besides, in the context of environmental degradation, the use of plant source as unique option has limited the supply of this drug due to the destructive collection of yew trees (Guo et al., 2008).

After the discovery of Taxol, a research paper about its properties was published after a long time (Peltier et al., 2006). The isolation of Taxol-producing endophyte *Taxomyces andreanae* has provided a substitute approach to obtain an inexpensive and extra obtainable product via microorganism fermentation (Stierle et al., 1993). After that, Taxol has also been found in a number of different genera of fungal endophytes either associated or not to yews, such as Wollemia nobilis (Strobel et al., 1997); Taxodium distichum (Li et al., 1996); Bartalinia robillardoides (Gangadevi et al., 2008); Pestalotiopsis terminaliae (Gangadevi and Muthumary, 2009); Botryodiplodia the obromae (Pandi et al., 2010); Phyllosticta spinarum (Kumaran et al., 2008).

If talking about another anti-cancerous drug, then that other drug is alkaloids “Camptothecin” ($C_{20}H_{16}N_2O_4$), a strong anti-neoplastic agent which was initially isolated from the wood of Camptotheca acuminata Decaisne (Nyssaceae) in China (Wall et al., 1996).

Camptothecin and 10-hydroxycamptothecin are 02 significant ancestors for the synthesis of the clinically valuable anticancer drugs, irinotecan and topotecan (Uma et al., 2008). In field of medical science, the unmodified Camptothecin is less commonly used due to some drawbacks such as low solubility and high toxicity in aqueous solvents (Li et al., 2009; Kehrer et al., 2001). And on the other hand, some captorhinid derivatives retain their properties and in other cases maintain different profit properties without any causing over drawbacks (Kusari et al., 2009; Jew et al., 1999). So, it is necessary to develop approaches for isolation, mixture separation, and production of Camptothecin and its analogues from new endophytic bacterial sources. Microbial products and its two derivatives (9-methoxycamptothecin and 10 hydroxycamptothecin) are already reported. Which has been used in the treatment of various diseases in the medical field and will continue to happen in the future. Because it is a natural product which has less adverse effect on health.

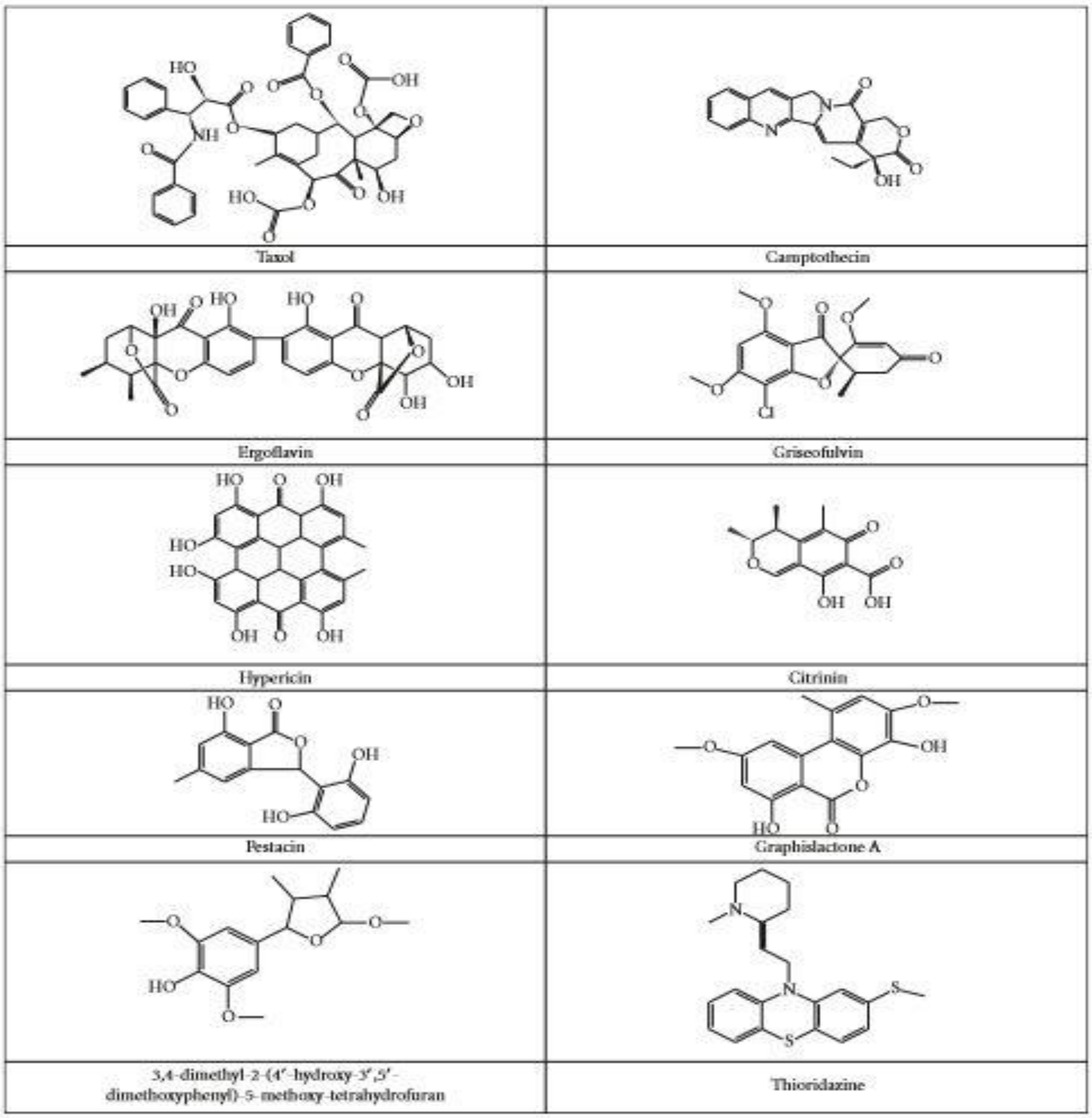


Figure 2.7: Some structures obtained from endophytic microorganisms.

More than a few reports have described other Camptothecin (or analogues) producing endophytes microorganism (Shweta et al., 2010; Puri et al., 2005). Since then, endophytes have Been involved in several studies purposing novel approaches for new drug design. “Ergoflavin” ($C_{30}H_{26}O_{14}$), a dimeric xanthene attached at position 2, related to the compound category called ergochromes and was described as a newly synthesized anticancer agent isolated from an endophytic microorganism surviving on the leaves of a traditional Indian medicinal plant ex. Mimusops elengi (Sapotaceae) (Deshmukh et al., 2009). And when we talk about another compound, it “Secalonic acid D” ($C_{32}H_{30}O_{14}$), a mycotoxin also related to ergochrome class, is known to have great anticancer activities.

It was synthesized from the mangrove endophytic microbes and detected high cytotoxicity on K-562 and HL-60 cells lines by inducing leukemia cell line apoptosis (Zhang et al., 2009). Phenylpropanoids” have much interest for medicinal use as antimicrobial, anticancer, antioxidant, immunosuppressive and anti-inflammatory properties (Korkina et al., 2007). Although phenylpropanoids, which belong to the largest group of bioactive metabolites produced by microbes, the report shows the production of a compound that has been medically described through various reports. *Penicillium brasilianum* endophytes found in the root of the Melia azad plant. And this endophyte promotes phenylpropanoid amides biosynthesis (Fill et al., 2010). Similarly, 02 monolignol glucosides, syringing and coniferin, it is not only produced by plants but is also synthesized by microbes, but were also recognized by the endophytic *Xylariaceae* species as chemical indications during the establishment of bacterial-plant interactions (Chapela et al., 1991). Koshino and coworkers detected two phenylpropanoids and lignin from stromata of Epichloe typhina on Phleum pretense (Koshino et al., 1988). Lignans” are deferent types of anticancer mediators synthesized as secondary metabolites by the shikimic acid pathway and performed different biological functions, works that make many lines of research interesting (Gordaliza et al., 2004). Although their molecular backbone consists only of two phenylpropane units (C6-C3), lignans which has a large structure and biodiversity seen to a great extent. “Podophyllotoxin” ($C_{22}H_{22}O_8$) and analogs are clinically applicable mostly due to their cytotoxicity and antiviral properties and are appreciated as the precursor to valuable anticancer medicines like teniposide, etopophos phosphate and etoposide (Kusari et al., 2009; Kour et al., 2008). The different tetralin lignans ex, podophyllotoxin, are naturally important synthesized by Podophyllum sp., alternative source shave been investigated to avoid endangered plant. And many different study showed a new bacterial as well as fungal endophyte, Trametes hirsute, that different produces podophyllotoxin and other associated aryltetralin lignans with effective anticancer and properties (Puri et al., 2006). Novel discovered microbial sources of Podophyllotoxin were described from the endophytic microbes such as, *Aspergillus fumigatus Fresenius* isolated from Juniperus communis L. Horstmann (Kusari et al., 2009), *Phialocephala fortinii* isolated from Podophyllum peltatum (Eyberger et al., 2006), and *Fusarium oxysporum* from Juniperus recurva (Kour et al., 2008). Lastly, talk about some different types of anti-cancer compound that is obtained from endophytes microbes. such as “cytoskyrins” (Brady et al., 2000), “phomoxanthones A” and “B” (Isaka et al., 2001), “photinides A-F” (Ding et al., 2009), “rubrofusarin B” (Song et al., 2004), and “(+) epiepoxydon (Klemke et al., 2004).

Various endophytes are obtained from medicinal plants (*Murraya koenegi*i, *Catharanthus roseus*, *Withania somnifera*, *Aloe vera*, and *Oscimum sanctum*), producing L-asparaginase which produces novel anti-cancer drugs in the pharmaceutical industry (Joshi *et al.* 2012). Jian and Tang (2014) have reported the overall number of natural product entries for cancer approved by the United

States Food and Drug Administration (US-FDA) from last 03 decades (33 years) via January 1981 to December 2013 as indicated graphically as below.

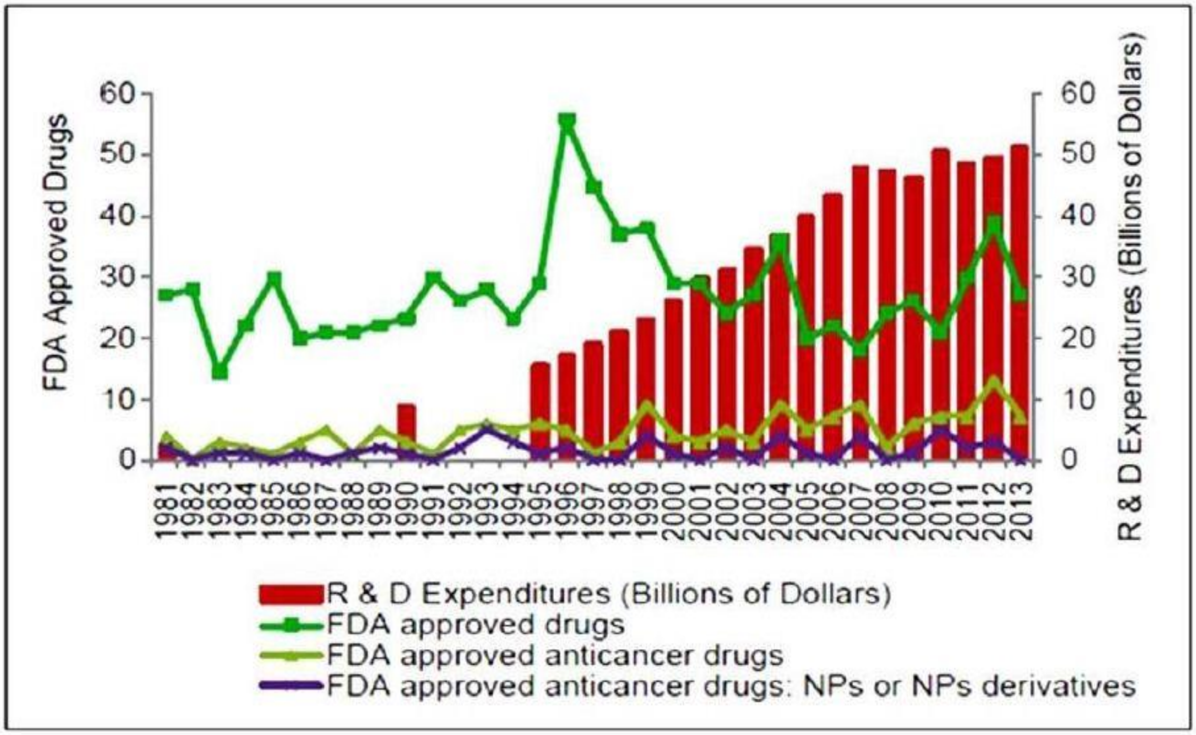


Figure 2.8: Different bioactive metabolites produced in different years. **Source: (1)** Pharmaceutical research and Manufacturers of America. Ph-RMA, Annual Membership Survey. 1996-2014; **(2)** *Natural Reviews Drug Discovery*, 2014, 13: 85-89; **(3)** *J Nat Prod.* 2012, 75, 311-335; **(4)** *Front Chem.* 2014, 2, 20.

2.2.11 Products from Endophytes bacteria as Antimicrobial Compounds: Metabolites behavior antibiotic activity can be determining as low-molecular-weight organic natural substances made by microorganisms that are active at little concentrations against other microorganisms (Gu et al., 2008). It is a concept that by producing secondary metabolites, pathogens can develop a strong immunity mechanism in resistance to invasive microbes (Tan et al., 2001). To date a large number of antimicrobial compounds isolated from endophytes have been reported through studies that belong to several structural classes such as, flavonoids, steroids, phenols, peptides, quinines, alkaloids, and terpenoids (Yu et al., 2010). The discovery of innovative new antimicrobial metabolites by endophytes is a significant substitute to overcome the increasing levels of drug resistance by plant and human pathogens, the insufficient quantity of effective antibiotics against various bacterial species, and limited novel antimicrobial compounds in growth, possibly due to relatively unfavorable returns on investment (Yu et al., 2010; Song, 2008). The anti-microbial compound is not only used as a medicine, but it is also used to control the food spoilage microbes, so as to preserve the food which makes it a concern in the food chain for the world. Has happened, and it is getting increased day by day (Liu et al., 2008).

Various bioactive metabolites, including anti-fungal compounds, was isolated from the genus *Xylaria* exist in different plant hosts, like sordaricin with different microbial (anti-fungal) activity contrary *Candida albicans* (Pongcharoen et al., 2008) mellisol, and 1,8- dihydroxynaphthol 1-O-a-glucopyranoside with activity against herpes simplex virus-type 1 (Pittayakhajonwut et al., 2005) multiplolides A and B with activity against *Candida albicans* and other fungus (Boonphong et al., 2001). The bioactive metabolite synthesized from the culture extracts of the endophytic microbes *Xylaria sp. YX-28* isolated from Ginkgo Biloba L. was identified as "7-amino-4-methylcoumarin (Liu et al., 2008). The compound obtainable broad-spectrum inhibitory activity against numerous food-borne and food spoilage microorganisms including *E. coli, S. typhia, A. niger, S. aureus, A. hydrophila, Yersinia sp., V. anguillarum, Shigella sp., V. parahaemolyticus, C. albicans, P. expansum, S. typhimurium* and *S. enteritidis* especially to *A. hydrophila A. niger* and was recommended to be used as natural antimicrobial in food (Liu et al., 2008). There is a different type of endophyte that has been identified in recent times, named *Xylaria sp*. It is also derived from the Abies holophylla plant. Which produces a particular type of metabolism (griseofulvin; $C_{17}H_{17}ClO_6$).

And these metabolites are used in the treatment of various human and animals' mycotic diseases. These metabolites are Spiro benzofuran antifungal antibiotic (Park et al., 2005). Four anti-bacterial aliphatic compounds were characterized from stromata of *E. typhina* on P. pratense (Koshino et al., 1989). Two new ester metabolites isolated from an endophyte of the eastern larch presented antimicrobial bioactive. One compound was toxic to spruce budworm (Choristoneura fumiferana Clem.) larvae, and the other may serve as potent antibacterial agent against Vibrio salmonicida, Pseudomonas aeruginosa, and Staphylococcus aureus.

2.2.12 Endophytes as biofertilizers: Different man-made fertilizers (especially N and P fertilizers) are used in different crop production to increase the productivity of the crop so as to provide food to the growing population. Those interested in large-scale crop production are continuously using a wide variety of pesticides, fungicides and bactericides, regardless of their harmful effects on the ground and surface waters. Pesticides, fungicides not only affect the environment, but also affect our upcoming generation. Due to which different types of new diseases are also giving rise. Some new and alternative methods should also be used to deal with these various problems, using endophytes as biofertilizers is the best solution among all these methods. Endophytes neither affect soil fertility nor exert a negative effect on the atmosphere. kasugamycin and polyoxins are being used as bio pesticides; spinosyns, *Bacillus thuringiensis* crystals and nikkomycin are used as bio-insecticides; bioherbicides (bialaphos) find application as bioherbicides; ivermectin and doramectin as anti-helminthic and endectocides against worms, lice, ticks, and mites; ruminant growth promoters in the form of coccidiostats; plant hormones like gibberellins as growth regulators are the most common application (Demain, 1995).

2.3. Secondary bioactive metabolites: Metabolism can be seen as the sum of all types of biochemical reactions carried out by an organism**.** Different medicinal plants and various types of microbes which are natural resources for secondary bioactive metabolites production. Due to which different types of drugs are developed (Pichersky and Gang, 2000). Approximately 60% of the world's population and about 80% of the population of developing countries depend on traditional medicine (Secondary metabolites) for their primary health and care (Aharoni, A. et al. 2004)). There are about 4500 plant species in India, out of which about 1700 species are of high plants, which includes many flowering plants which are used in traditional medicine. There are many microbial products that do not have any clear function in the life of living organisms such as antibiotics, alkaloids, toxins, gibberellins, carotenoids, pigments, etc., which we know by the name of secondary metabolites. Not all microbes produce this type of metabolites. Their types of formation are generally reserved during logarithmic growth and are depressed during the suboptimal or stationary growth phases. Production or synthesis of secondary metabolite is controlled by regulatory mechanisms and different media control parameters. Which is still understood today? If seen, metabolites are a type of intermediate products, which are usually quite small and fine organic chemicals. Every cell in which there is a division produces cells metabolites. It has often been observed that secondary metabolites, which are only incidentally present in microbes, do not have any significant role in organisms. And at the same time are not paramount to the life of the organism. Though secondary metabolites are resultant of primary metabolism, they do not construct fundamental molecular skeleton of the organism. No change can be seen in the life of an organism due to its absence, but the living entity is disturbed. Which is a perfectly opposite situation for the secondary metabolism? (Tiwari and Rana, 2015). The dissimilarity between primary and secondary metabolite is unclear since several of the intermediates in primary metabolism is overlapping with the intermediates of secondary metabolites (Verpoorte et al., 2000). Amino acids, which are products of one type of primary bioactive metabolites if seen secondary metabolites are also products of primary metabolites in a way. Sterols are also a type of secondary metabolite, and are also an essential part of a cell's various structural structures. The mosaic character of an intermediate indicates universal biochemical pathway being common by primary and secondary metabolism (Yeoman and Yeoman, 1996).

According to Owen and Hundley (2004), Endophytes bacteria act as a chemical synthesizer inside the plant. Bacteria living in various medicinal plants contain a large amount of bioactive secondary metabolites, and these metabolites are used in the treatment of various diseases (Tejesvi et al., 2007; Kumar et al., 2014; Singh and Dubey, 2018). From time to time, researchers are trying to get and identify various bioactive metabolites from different endophytes. Fermentation of endophytic bacteria with probable for bioactive compound formation has some advantages like dependable and reproducible output. These endophytes are grown in fermenters on a greater scale, so that the bioactive compound can be properly made available to all people and thus commercially exploited to use different biosynthetic methods, under different culture conditions. Changes should be kept in mind so that we can produce various new compound and its derivatives (Zhao et al., 2013; Strobel et al., 2004; Goutam et al., 2014). Newman and Cragne have presented 34 years of data obtained by the Food and Drug Administration (US-FDA) of the United States, which reports the total number of drug entries (natural products) or therapeutic entries. This data is from January 1981 to December 2014.

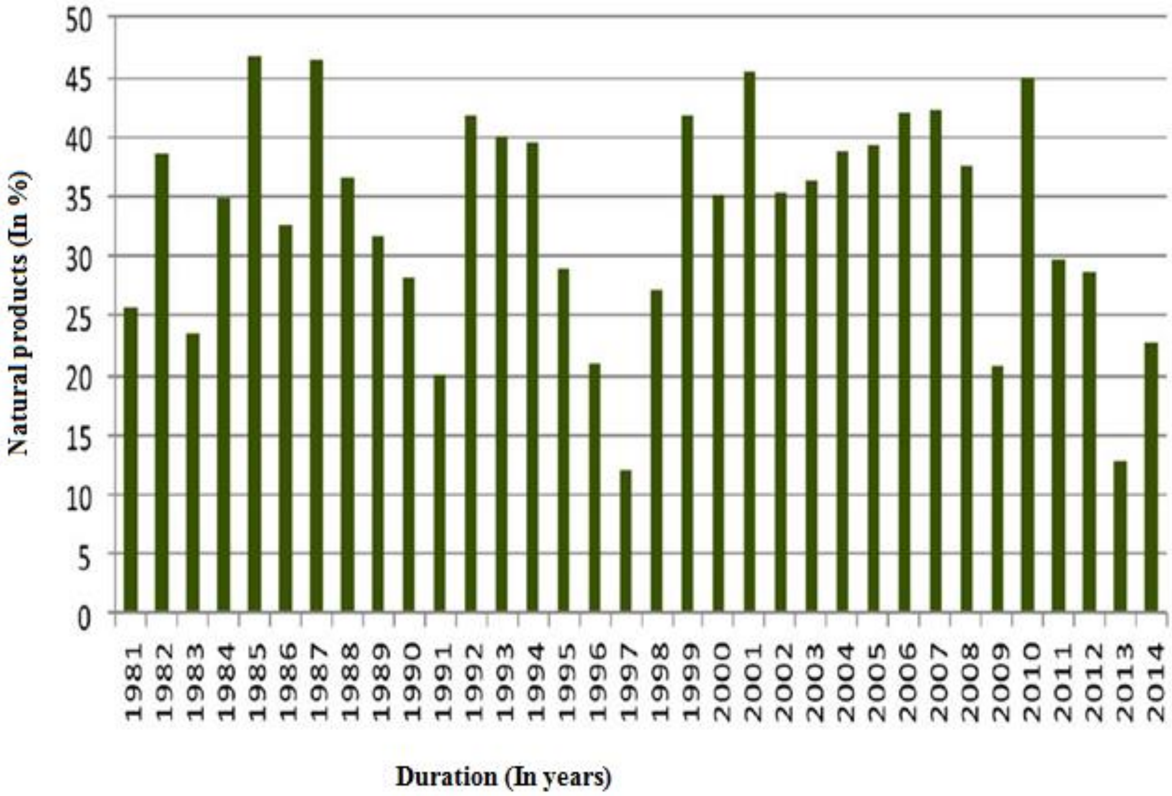


Figure 2.9: Percentage of Natural products (N) i.e. therapeutic agents or drug entities approved by the USFDA from the past 34 years (January 1981− December 2014). **Source:** *J Nat Prod.* 2016**,**

There is vitality and a delicate equilibrium between the activities of the primary and secondary metabolism being influenced by development, tissue differentiation and development of the cell or body, and also peripheral pressures.

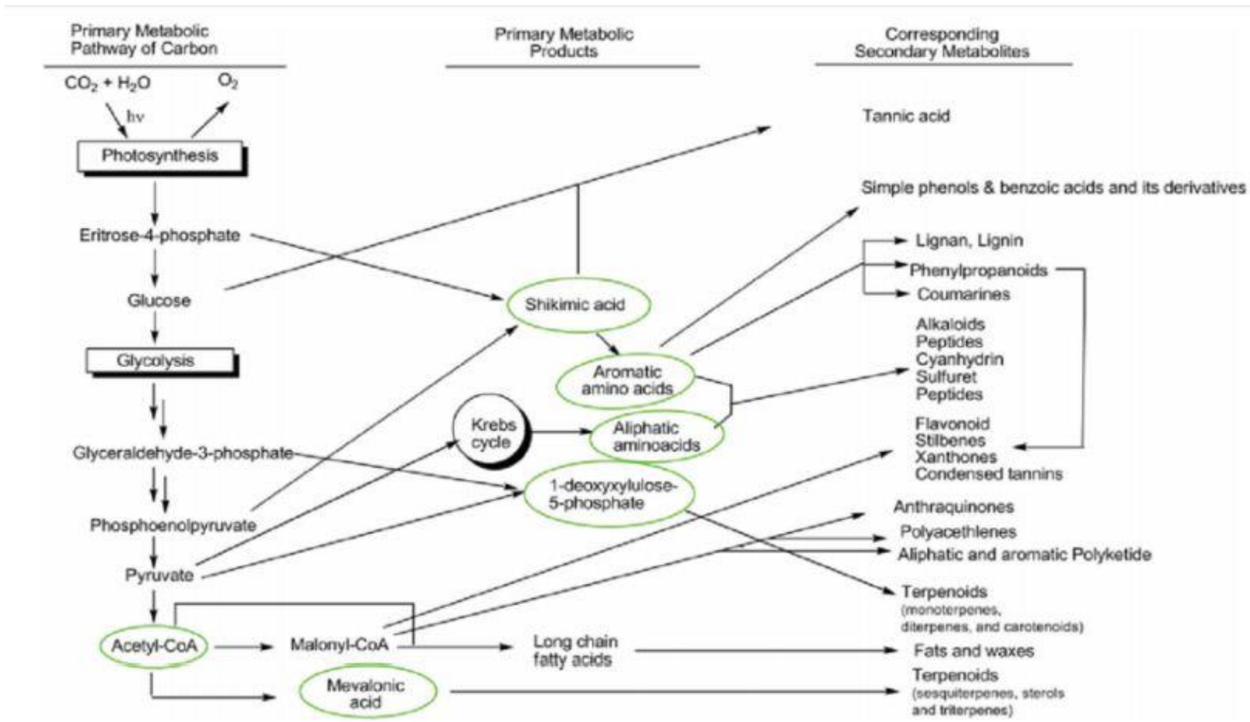


Figure 2.10: Schematic diagram representing unification of secondary and primary metabolism.

Therefore, secondary metabolites can also be defined as a heterogeneous group of natural metabolic products. This cannot be said to be necessary for different vegetative growth of organisms. They are known as various compounds, which show the adverse conditions. Such as symbiosis, metal and water transport, competition, self-defense, ecological interactions, signaling molecules and acting as defensive compounds. The main reason for secretions of secondary metabolism is to improve the health of mankind. It produces various compounds such as, (Antitumor agents, anticancerous, antiparasitic agents, antimalarial, enzyme inhibitors, growth enhancer of plants and animals, antibiotics and immunomodulators).

It also increases health nutrition such as (Nutraceutical and pigments), improve agricultural productivity likes (pheromones, insecticides, pesticides, effectors of ecological competition and symbiosis), and one definitely affects economics in our society positively and is a definite source of antibiotics.

2.3.1. Primary and Secondary metabolism: Many enzyme-rich chemical processes take place in organisms, which are called metabolism to keep life maintained? Primary bioactive metabolites are synthesized by primary metabolic pathway, and there are different types of compounds like (sugars, amino acids and citric acid). On the other hand, molecules such as nucleic acids, proteins, peptides, and polysaccharides that are reported as universal building blocks of life, and vary from one organism to another, but have common functions (Tan, B.C. et al. 1997).

Secondary metabolites have multiple functionality and bioactivity because of presence of different functional group (Schwartz et al., 1997; Queshi and Porter, 1981). Synthesis of secondary metabolites takes place using primary metabolites as intermediates. The function of secondary metabolites in microbes is quite limited (Banthorpe et al., 1972). Synthesis of secondary metabolites is not a random process. But some specific cells, organs and organelles produce it (Mahmoud and Croteau, 2002). Many enzymes participate in the chemical synthesis process for the biosynthesis of secondary metabolites and their metabolism is involved in morphological as well as biochemical patterns.

2.3.2. Sources of secondary metabolites: According to the history of microbial biotechnology, we have also received many products such as foods, beverages, pesticides and antibiotics using different microorganisms. In the nineteenth century, after the production of penicillin, the use of microbes became incremental, which increased day by day, its result was that microbes started being exploited on a large scale. After the production of the 1940, s Pencilinium, Louis Pasture began research on microorganisms by exploiting microorganisms, which brought a revolution in the field of microbiology ((Demain and Fang, 2000).

Until 1970 just two groups of naturally occurring β -lactam antibiotics, cephalosporins and penicillins were identified. With the beginning of new screening and isolation techniques, different variety of β-Lactam-containing molecules (Wells et al., 1992) and other types of antibiotics have been recognized.

Isolation of different types of microbes started with the emergence of new diseases and at the same time the production of novel antibiotics has increased quickly, the last three decades. With the screening of new antibiotics, the discovery of low-molecular-weight secondary metabolites with different biological activities continues day by day. On the other hand, secondary metabolites have some different properties, such as plant growth stimulators, insecticides, immunosuppressant's herbicides, enzyme inhibitors and antihelminthics. The bacteria that are isolated for the production of bioactive metabolites have two strategies. The first strategy is to isolate the known secondary metabolites to evaluate the biological activities not mentioned in the literature and the second one is to isolate new secondary metabolites with various biological activities. Most bioactive metabolites are used in medical practice, which we call antibiotics.

The major source of secondary bioactive metabolites are various microbes and plants.

Source	Bioactive	Antibiotics	All known compound
Plant kingdom	150,000–200,000	~25,000	600,000–700,000
Natural product	200,000–250,000	25,000–30,000	Over one million
Algae, lichen	1500–2000	~1000	3000–5000
Microbes	22,000–23,000	~17,000	Over 50,000
Animal kingdom	50,000–100,000	~5000	300,000–400,000
Higher plants	~100,00	10,000–12,000	500,000–600,000
Invertebrates	NA	~500	~100,00
Protozoa	100–200	~50	Several hundreds
Insects/ worms/ etc.	800–1000	150–200	8000–10,00
Marine animals	7000–8000	3000–4000	20,000–25,000
Vertebrates (mammals, fishes, amphibians, etc.)	50,000–70,000	~1000	200,000–250,000
Invertebrates	NA	~500	~100,000

Table 2.5: Approximate number of known natural metabolites.

Some bioactive metabolites such as Adriamycin, bleomycin, daunomycin and mithramycin used as antitumor compounds.

Some different and other properties of secondary metabolites are found such as vasodilatories, anesthetics, anti-inflammatory, immunosuppressant's (cyclosporine-A and tacrolimus), hemolytics, anabolism, anticoagulants and hypocholesterolemics (statin) (Bentley, 1997). In the last 50 years, more than 23000 bioactive metabolites have been discovered, of which 1600 are antibiotics, this is being used by humans in their primary health (Janos Berdy, 2005). Bioactive metabolites consist of a variety of specific and complex chemical structures, with fascinating array of diverse and unique functional groups.

Studies on secondary bioactive metabolites exposed the fact that the microbial secondary metabolites have inimitable molecular skeleton which is not present in the chemical libraries which makes the chemist not capable to synthesize more than 40% of the metabolites (Feher and Schmidt, 2003). Fungus and Actinomycetes which are a fibrous/filamentous microorganism known for producing highly bioactive metabolites, some of these are Streptomyces species that produce more than 180 bioactive metabolites (Demain and Fang, 2000). It has often been observed that the genus Bacillus group of bacteria produces the most secondary metabolites. Different types of antibiotics such as bacillomycin, moenomycin, bacilli, and dipicidine are produced by Bacillus species (Zweerink and Edison, 1987; Patel *et al.*, 1995). Apart from *Bacillus*, *Myxobacterium* is one more genus which produces large number of antibiotics. Approximately 80% of isolated *myxobacteria* that are produce antibiotics with different properties, many of them exhibiting different antifungal activity properties (Dolak et al., 1983).

Secondary bioactive metabolites have some important functions as follows:

- Secondary bioactive metabolites act as competitive weapons against insects, some plant and microorganisms.
- It acts like a metal transport agent.
- Bioactive metabolites also act as differential effects.
- It acts as a communication agent between organisms and
- Secondary Bioactive metabolites acts like reproductive agent.

Apart from this, if it talks about something else, it involves spore formation (not mandatory) and interference in germination. the secondary metabolites are used for variety of biological activities like antimicrobial and antiparasitic agents, enzyme inhibitors and antitumor agent, immunosuppressive agents, etc.

2.3.3 Secondary metabolites work as a Hydrolytic enzymes and quorum sensing inhibitors:

The association among the endophytes and their host plants may extend from dormant phyto-pathogenesis for the mutualistic symbiosis if seen, endophytic bacteria/fungi have been producing various types of hydrolytic enzymes, through which they infect other organisms and plant cells. This is their kind of defense system, in order to resist any pathogenic invasions as well as to acquire the essential nutrition from their host, such as cellulases, pectinase, protease, xylanases and amylases (Firakova et al., 2007). Such enzymes have been used to make a variety of industrial products (biofuel, detergents, food and pharmaceutical), which are used for human welfare (De-Veras et al., 2018). The soybean (Glycine max L.) is one of the mainly grown leguminous crops universal for the high oil and protein content of its grain. It is mainly used for human and animal nutrition and it is also use for production of bioethanol biopolymers, biodiesel, disinfectants, lubricants, soap, some medicine and cosmetics (Waqas et al., 2015). It indicates major source of good quality of protein and little content in saturated fat and a high amount of different dietary fiber. It is value mentioning that its isoflavone content makes it significant among other legumes (Asif et al., 2013). Up to date, there are few reported studies on the endophytic microbes (endophytes) related with soybean. There are a number of endophytic microbes' species studied till date like; *Haplosporangium sp. Fusarium sporotrichioide, Cercospora sp., Papulospora sp., Phomopsis sp. Lasidiplodia sp., Papulospora sp. Fusarium graminearum Scopulariopsis costantini, Scopulariopsis sp. Trichoderma sp.* and *Fusarium semitectum* have been reported to demonstrate pectinase activity xylanase activity, amylolytic activity, Cellulase activity and Protease activity, (Rajesh et al., 2013).

2.3.4 Different Antithrombotic Activity of secondary bioactive metabolites: If we see today's date, then heart disease and thrombosis are the main cause of death of human race all over the world, which is being increased day by day. Thrombus-added ischemic stroke or myocardial infarction is the major reason of cardiovascular diseases (CVD) associated death. Thrombus development is a critical event in the pathophysiology of atherosclerotic cardiovascular diseases (Fares, 2013). Thrombus deposition due to an irregular coagulation process is often observed in arteries or veins and may result in decease of blood flow. Some *Fusarium sp.* CPCC 480097 *has* been reported to produce antithrombotic metabolites. The fibrinolytic activity of the purified antithrombotic agent from *Fusarium sp.* was estimated by a modified fibrin plate method. Centipede Scolopendra subspinipes mutilans L. Koch (microbial) is a medicinal listed in the Korean Herbal Pharmacopoeia and Chinese Pharmacopoeia used in various heart-related diseases. Such as hematuria, epileptic, seizures, tetanus and pain. Antiplatelet agents are therefore considered as a key tool in the treatment and/or prevention of cardiovascular thrombotic diseases. This drug is produced from microbes obtained or isolated from various natural medium (Cai et al., 2013; Ma et al., 2015). A type of peptides and proteins are found in microbial toxin that shows an antithrombotic activation. This is an antithrombotic compound (Kong et al., 2014; Kong et al., 2013; Liu et al., 2012). There are only some little chemical studies on the secondary metabolites from microbes, where the quinoline, alkaloids are characterized as representative small-molecule metabolites from microbes.

2.3.5 Different Antiviral activity of secondary bioactive metabolites: Biomolecules are being isolated from endophytes as a large number of antiviral agents, and we still have limited reports for the number of these compounds and however, the previously isolated compounds reported have been identified as anti-viral after promising results and provide an alternative means. The main limitation to antiviral compound discovery is most probably related to the absence of antiviral screening system in most of the compound discovery programs.

In a report two novel compounds cytonic acid A (89) ($C_{32}H_{36}O_{10}$) and B (90) ($C_{32}H_{36}O_{10}$) have been isolated from *Cytonaema sp*. These compounds are reported to work as novel protease inhibitors for human cytomegalovirus. Isolated an important antiviral compound Pestalotheol-C from the microbial endophytes *Pestalotiopsistheae* of an unidentified tree on Jianfeng Mountain, China. The isolated compound expressed anti-HIV properties (Sanjana Kaul, 2012). In recent times, various new antiviral compounds are needed worldwide to solve drug resistance problems, just as microbes resistant increasing agaised to various drugs.

The resistance of human disease to well-known (commercial) antibiotics is increasing quickly in some recent times, so discovering novel and different agents are necessary required for management those lesions. In recent times, Endophytes are not well-studied microorganisms, yet they remain to be studied a lot, gaining immense importance due to their various biological activities and high levels of structural and functional diversity as well as properties of bioactive compounds (Tan and Zou, 2001; Strobel et al., 2004). They are measured as a highly source of novel and natural metabolites for exploitation in pharmaceutical, Nutraceutical, medicine, industry and agriculture. The medicinal plants are measured as one of the main reservoirs of endophytic bioactive metabolites for the use in the different medicinal applications (Selim et al., 2016; Selim et al., 2015; Bacon and White, 2000).

2.3.6 Antitubercular activities of secondary bioactive metabolites: According to the World Health Organization (WHO), currently 50 million people are infected with various diseases and 1500 people die of tuberculosis salt disease every hour. Only in India its number is very high. After mycobacterium tuberculosis generates and spreads resistant to many drugs, the discovery of new anti-mycobacterial agents is very important. The information based on medicinal plants as repository for bacterial endophytes with metabolites containing biologically active compounds against various human pathogenic diseases for potential use in modern medicine have turned the attention of researcher to focus on endophytes. Endophytic fungi from Garcinia sp. are good source for exploring the possibility of new anti-mycobacterial drugs. Phomoxanthone A and B isolated from the endophytic microbes Phomopsis sp. from Garcinia sp. in Thailand exhibits significant activity against *M. tuberculosis* (Isaka et al., 2001). *Phomopsis sp*. isolated from Garcinia dulcis produced new metabolites as Phomoenamide and Phomonitroester inhibiting *M. tuberculosis* (Rukachaisirikul et al., 2012). Tenuazonic acid ($C_{10}H_{15}NO_3$) was isolated by bioassay guided fractionation of dichloromethane extract of Alternaria alternate. It was found to be active against *Mycobacterium tuberculosis* at MIC of 250 µg/ml concentrations (Sonaimuthu et al., 2011). *Diaporthe sp.* isolated from Pandanus amaryllifolius leaves produced two new benzopyranones diaportheone A and B. Both compounds inhibited the growth of virulent strains of *Mycobacterium tuberculosis (*Bungihan et al., 2011).

2.3.7 Different cytotoxicity activity of secondary bioactive metabolites: Cytotoxicity is the feature of being toxic to different type of cells. Examples of toxic agents are low molecular, small and different types of chemical substance. Treating cells with a cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis.

A polymorphonuclear leukocyte (PMNs) are the most common effector cells that actively kill invading microorganisms and tumor cells and helps the host cell to survive and protect it from invading microorganisms. According to an author (K. M.) and his contemporaries reported that a number of antitumor immunomodulators like TAK, a linear fl-i, 3-D-glucan from Alcaligenes faecalis var. rnyxogenes IFO 13140, induced potent tumoricidal activity of PMN in vitro, and showed that hydrogen peroxide is a direct cytolytic mediator in the cytotoxicity (Morikawa et al., 1985). But here PMN is an important component in monitoring and protection systems for a broad spectrum of hosts' defenses and is a widespread and inappropriate response that leads to hypotension reactions that help inhibit invasive microbial infections. It has often been observed that the attraction of an active PMN can cause damage to undesirable tissue, even in inflammatory processes (Roit et al., 1989). Nature has a lot of herbal medicine (as secondary metabolites) which is responsible for exhibiting anti-inflammatory effects and they are used from time to time in all these activities. Endophytic microbes *Chaetomium sp.* isolated from Salvia officinal's produced bioactive secondry metabolites which were reported to display anti-proliferative activity against L5178Y mouse lymphoma cell line using the micro culture tetrazolium (MTT) assay (Abdessamad et al., 2009). In another study, Bioactive Metabolites from the Endophytic Fungus *Stemphylium globuliferum* displayed strong kinase inhibitor properties against Aurora-B, FLT3 kinases and CDK4/CycD1 which were the most susceptible to this natural bioactive metabolite (Abdessamad Debbab et al., 2009).

Scientists have started to believe that the plant is a mine for microorganisms called Reservoir of Endophytes. And from this, such microbes can be obtained that produce metabolites that are of a kind of cytotoxic nature (Schulz et al., 2002; Strobel et al., 2004).

2.3.8 Different Bioactivities of Microbial Secondary Metabolites: It has always been the most unclear field in the entire field of microorganism and biochemistry, there has always been a desire of the scientist to know the true definition of the secondary metabolites and the actual position and function.

Secondary metabolites produced from different microorganisms demonstrate different types of biological behavior. In nature, microbial secondary metabolites are important for various organisms by performing the following functions such as sex hormones, ionophores, competitive weapons against other bacteria, fungi, amoebae and insects. The different secondary metabolites produced from different microorganisms with unbelievable range of chemical structures results in the multipurpose biological activities. The scope of the discovery of various active metabolites has been greatly increased in today's date. The exploitation of microorganisms has been greatly increased due to bioactive metabolites its use in antitumor (doxorubicin) and agricultural antibiotics, (antiparasitic avermectin, feed additive monensin and herbicide glufosinate) (Maplestone et al., 1992). If seen, secondary metabolites produced from microorganisms are mainly used in the treatment of various diseases in agriculture and medicine.

<u>Microbial sources</u> **(I)Bacteria:**	**Compound**	**Natural Bioactivities**	**References**
Bacillus silvestris	Bacillistatins 1 and 2	Anticancerous	Pettit et al., 2009
Bacillus subtilis	Bacilosarcin A	Plant growth regulator	Azumi et al., 2008
Rivularia sp.	Bis-bromoindoles	Anticancer	Baker, 1984
Bacillus laterosporus	Basilikamides A& B	Antifungal	Barsby et al., 2002
(II) Actinomycetes:			
Streptomyces sp.	Komodoquinone A	Neuritogenic activities	Itoh et al., 2003
Streptomyces sp.	Trioxacarcins	Antibacterial, Anticancer and Antimalarial	Maskey et al., 2004
Podophyllum peltatum	Podophyllotoxin	Anticancer	Canel et al., 2000
Micromonospora sp.	Diazepinomicin (ECO-4601)	Antibacterial, Anticancer and Anti-inflammatory	Charan et al., 2004
Actinomadura sp.	Chandrananimycins	Antibacterial,	Maskey et al., 2003

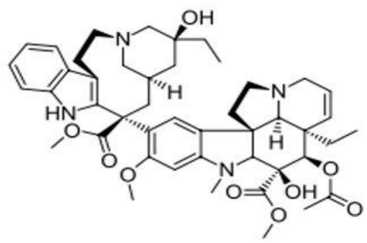
		Anticancer, and antialagl	
(III) Fungus			
Trichoderma virens	Trichodermamide B	Anti-microbial	Garo et al., 2003
Microsporum sp.	Physcion	Cytotoxic activities	Wijesekara et al., 2014
Aspergillus funigatus	fumagilline	Anti-angiogenetic	Lee et al., 2007; Lu et al., 2006
Aspergillus versicolor	Aspergillitine	Antibacterial	Lin et al., 2003
Aspergillus wentii	Wentiquinone -C	Antioxidant	Li et al., 2014
Aspergillus carneus	Carneamides- A, C	Cytotoxic activities	Zhuravleva et al., 2012
(IV) Algae			
Ecklonia cava	Dieckol	Postprandial Hyperglycemia-Lowering effect	Lee et al., 2010
Ishige foliacea	Octaphlorethol	Glucose uptake effect in skeletal muscle	Lee et al., 2012
(V) Sponges			
Haliclona exigua	Bis-1-oxaquinolizidine	Antifouling activity	Limna et al., 2009

Cryptotethya crypta	Aabinose-nucleosides	Anticancer	Joseph and Sujatha, 2011
Neopetrosia sp	Renieramycin-A	Antitumor	Nakao et al., 2004
Luffariella variabilis	Manoalide	Anti-inflammatory	Joseph and Sujatha, 2011

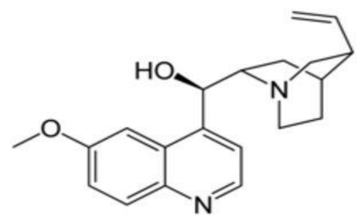
Table 2.6: List of some important secondary metabolites produced by microbes.

Secondary metabolites are classified based on chemical composition (containing nitrogen or not), chemical structure (for example, having rings, containing a sugar), biosynthesis pathways (e.g., phenylpropanoid, which produces tannins) and solubility in various solvents. Despite all these, the secondary metabolites are mainly divided into three parts (A- alkaloids, B-Terpenes and C-Phenolics).

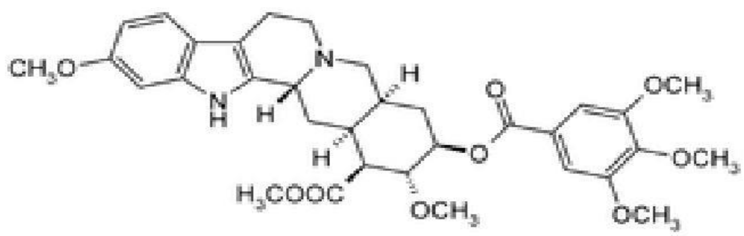
(a) Alkaloids: Alkaloids are derivative of amino acid. So far, about 10000 Alkaloids has been discovered from more than 300 different sources (Raffauf, 1996). Alkaloids are made up of one or more carbon rings, which mostly contain nitrogen content. The position of nitrogen in carbon rings depends on the type of Alkaloids and resource of alkaloid. The History of Medical Applications of alkaloids is very old, and the first alkaloid synthesis was done in the 19th century and they immediately found application in clinical practice (Hesse, 2002). In today's date, many different types of alkaloids are still being used in medical science that too in the treatment of various diseases and Gold is used as various salts. Some examples include vinblastine [Figure 2.11 (a)] which has antitumor property (Jordan & Leslie, 2004); quinine [Figure 2.11(b)] which has antimalarial and antipyretics properties (Reyburn et. al, 2009); and reserpine [Figure 2.11 (c)] which can be used to treat high blood pressure (Moser, 1987).



Vinblastine (a)



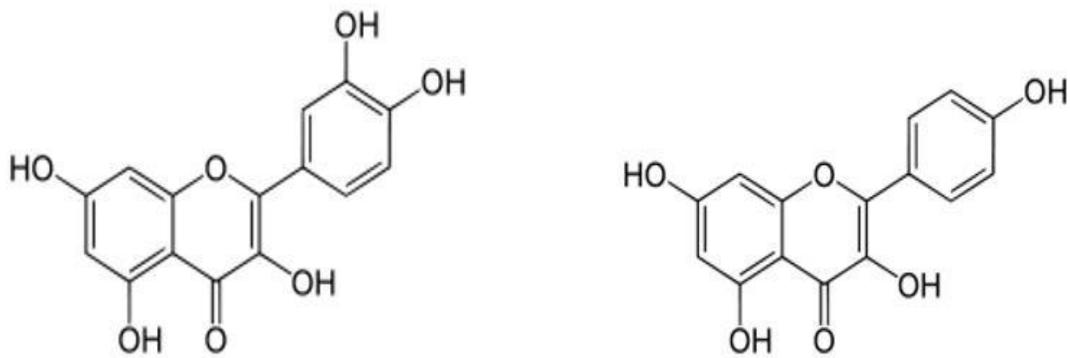
Quinine (b)



Reserpine (c)

Figure 2.11: Structures of alkaloids vinblastine (a), quinine (b) and reserpine (c)

(b) Phenolic compounds: Phenols are mainly found by plant and microbes, are the most abundant secondary metabolites of plants. Today, more than 4000 phenol compounds have been discovered and ranging from lower molecules such as phenolic acids to extremely polymerized substances such as tannins. Phenol is primarily synthesized from the shikimic acid pathway product (Knaggs, 2001). A common character of phenolic compounds is the existence of at least one hydroxyl-substituted aromatic ring system. There are different types of compound in phenol which are as follows tannins, phenolic acid and the less common lignans stilbenes and stilbenes. Maximum phenol which is related to flavonoids (kaempferol, anthocyanin, chalcones, flavones and quercetin). The structures of quercetin and kaempferol are shown in Figure 2.12. Flavonoid is most commonly used as an antioxidant in cases of various diseases. According to who reported natural phenol is used as an antifungal, antibacterial, antiviral (Perez, 2003), anti-inflammatory (Santos et.al., 2006) and vasodilatory actions (Padilla et. al., 2005).



Quercetin (a) Kaempferol (b)

Figure 2.12: Structures of flavonoids quercetin (a) and kaempferol (b).

(c) Terpenes: Terpenes are hydrocarbons resulting from the combination of numerous 05 carbon isoprene units. Terpenoids can be expected as modified terpenes in which methyl groups have been removed, or oxygen atoms are supplementary. Terpenes can be divided based on the number of isoprene units such as triterpenoids (6 isoprene units), hemiterpenoid (1 isoprene unit), monoterpenoids (2 isoprene units), diterpenoids (4 isoprene units), sesterterpenoids (5 isoprene units), polyterpenoid (>7 isoprene units), sesquiterpenoids (3 isoprene units), and tetraterpenoids (7 isoprene units). Terpenes Synthesis can be done through the mevalonic acid pathway (Newman & Chappell, 1999). or 2-C methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway) (Barbara et. al., 2007). terpenes are mainly used in the case of fragrance and flavoring agents. Prenol [Figure 2.13(a)] and α-bisabolol [Figure 2.13(b)] are used in fragrance due to fruity odor and sweet floral aroma correspondingly. Terpenoids roll as pharmaceutical agents such as antibacterial, antifungal and antineoplasia are still under investigation.

Figure 2.13: Structures of terpenoids (a) prenol and α-bisabolol (b).

2.3.9 Some other secondary metabolites (Alkaloid group): Indole derivatives of Alkaloid group, 6-isoprenylindole-3-corboxylic acid showing Antifungal activity has been isolated from the endophyte *Colletotrichum sp.* showing growth inhibition property against phytopathogenic fungi; *Phytophthora capsici*, *Rhizoctonia cerealis*, and *Gaeumannomyces graminis var. tritici* (Lu et al. 2000). Another isolate of *Phomopsis sp.,* isolated from *Gossypium hirsutum* has yielded antifungals compounds, epoxycytochalasin H, cytochalasin N, and cytochalasin H in chloroform: methanol extract of it. And they were found active against Sclerotinia sclerotiorum, Bipolaris maydi, Fusarium oxysporum, Pyrocidine A and antimicrobial compound reported from Acremonium zeae, an endophytic fungus isolated from maize (Wicklow et al. 2005). Chaetomium globosum an endophytic fungus of Ginkgo biloba is reported to reservoir of antifungal metabolite gliotoxin (Li et al. 2011). Cryptocandin an antifungal compound was isolated from

Cryptosporiopsis cf. quercina an endophytic fungus of Tripterigeum wilfordii and this was found active against plant pathogenic fungi Sclerotinia sclerotiorum and Botrytis cinerea (Strobel et al. 1999). graminis var. tritici and *Rhizoctonia cerealis*. 1-N-methyl albonoursin, an antibiotic alkaloid, was reported from endophyte isolated as a *Streptomyces* from perennial ryegrass and having morphological similarity to *Acremonium* (Gurney and Mantle 1993). Antifungal mycotoxin fumigaclavine C and fumitremorgin C were isolated from *Aspergillus fumigatus*, an endophytic fungus of *Cynodon dactylon* (Cole et al. 1977).

Terpenoids and steroids: A number of antifungal and antibacterial compounds have been reported to be of Terpenoids and steroids in nature, some impotent are in listed in table 2.7.

Host plant	**Endophytic Microbes**	**Biological activity**	**Functional metabolite Reported**	**References**
Phleum pretense	*Epichloe typhina*	Antifungal	Sesquiterpenes-Chokols A-G	Koshino et al., 1998
Juniperus communis	*Hormonemasp.*	Antifungal	Enfumafungin	Pelaez et al., 2000
Artimisiaannua	*Colletotrichum sp.*	Antifungal (Phytophthora capsici,	3b-hydroxyergosta-5-ene (29); 3-oxoergosta-	Lu et al., 2000

		Helminthosporium sativum, Rhizoctonia cerealisand Gaeumannomyces graminis var. tritici	4, 6,8 (14), 22-tetraene (30); 3b, 5adihydroxy- 6b-acetoxyergosta-7, 22-diene (31) and 3b, 5adihydroxy- 6b- phenylacetoxyergosta- 7, 22-diene (32)	
Mallushalliana	*Alternaria brassicicola*	Antifungal	Cerevisterol	Guet et al., 2009
Cassia spectabilis (Leguminosae)	*Phomopsis cassiae*	Antifungal	Cadinanesesquiterpenes	Silva et al. 2006
Piper aduncum	*Xylaria sp.*	Antifungal	Phomenone Phaseolinone	Silva et al., 2010
Arisaema erubescens	*Phoma sp.*	Antibacterial and Antifungal	b-sitostero	Wang et al., 2007

Table 2.7: Metabolites of endophytic microbes having antifungal activity Terpenoids and steroids Groups.

2.3.10 Phytohormones and defense enzymes: Phytohormones are a kind of signaling molecule, which controls plant growth and cellular activities. They play critical roles in controlling plant responses to several stresses at very low concentrations. When bacteria and plants interact, different plant hormones and their regulation occur in the form of results (Lopez et al. 2008; Glick 2012). IAA is the common, naturally occurring, plant hormone of the auxin's category. The endophytic *Bacillus pumilis* (ECL4), *Pseudomonas putida* (ECL5), *B. cereus* (ECL1), *Bacillus* sp. (ECL3), *Clavibacter michiganensis* (ECL6) and *B. thuringiensis* (ECL2) isolated from *C. longa* L. produced IAA (Javid et al. 2011; Kumar et al. 2016). *Pseudomonas*, *Agrobacterium* and *Bacillus* isolated from the root of *Cassia tora* synthesized phytohormones and solubilized tricalcium phosphate (Kumar et al. 2015). Endophytic *Artherobacter* EZB-4 and *Bacillus* EZB-8 isolated by the pepper plant (*Capsicum annum* L.) produced IAA and increased the plant biomass. These endophytic bacteria also accure a significant reduction in up or down-regulation of the stress inducible genes (*CaACCO* and *CaLTPI*) compared to the gene expression in non-inoculated plants. Both the strains reduced osmotic and drought stresses (Sziderics et al. 2007). *Achromobacter piechaudi* E6S simplified the plant growth by producing IAA, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and solubilizing phosphate (Ma et al. 2016) Abscisic acid is said to be a kind of plant stress hormone. It is responsive to various stress such as water, salts and cold temperature etc. Drought is a kind of stress reducing crop product throughout the world. Which is very dangerous for the crop cycle? It is the biggest obstacle to environmental damage which hinders crop productivity more than any other stress (Farooq et al. 2012; Lambers et al. 2008).

2.3.11 Inspired modulation by endophytic bacteria to produce secondary metabolites: Different bioactive metabolites can be produced using different delivery techniques depending on the plant requirement and different developmental stages of endophytic bacteria. The seed treatment and foliar spray with different bacterial endophytes seem to be reasonable because of minor charge. If it is said that the knowledge of how bacterial endophytes enter the plant and how to limit the colonized plants, much knowledge has been gained. Endophytic plant growth promoting bacteria (PGPB) used as microbial inoculants improved the synthesis of biologically active compounds in the host plant. Gold contain large number Phenolics compound such as, sesquiterpenoid and curcuminoids. Inoculation of turmeric rhizomes with endophytic Azotobactor chroococcum CL13

Enhanced the production of curcumin (Kumar et al. 2014). Bacterial prodigiosins and their artificial derivatives are effective pro-apoptotic agents against various different cancer cell lines, with several cellular targets including multi-drug resistant cells with modest or non-toxicity towards normal cell lines. *S. marcescens KC-1*, an endophytic bacterial strain, produce red color agent prodigiosins (Darshan and Manonmani 2015; Khanam and Chandra 2015).

Those endophytic produce secondary metabolites that may be more widespread in aromatic and medicinal plants. Different quality and quantity of taste in strawberries are also affected by *methylobacteria* associated with plants (Verginer et al., 2010). *Stenotrophomonas maltophilia* (N5-18) delivered through foliar spray considerably improved the photosynthetic effectiveness, whole alkaloid and morphine content coupled with reduce in the baine content in Opium poppy (Pappaver sominiferum). The increase in capsulated biomass concentration and alkaloid different content was followed by the subsequent increase in productivity of Opium poppy (P. sominiferum) (Bonilla et al. 2014).

2.3.12 Bioactive compounds produced by microbes are more convenient than plants: Secondary metabolites produced by endophytes microbes demonstrate strong physical activity of the plant. At the same time, it has been observed that there is evidence of their production being prone to various problems including varying quality and inadequate levels of productivity. In control environmental condition microorganisms produce primary and secondary metabolites, as well as assuring their maximum efficiency in uniform and high quality (Sato and Kumagai 2013). The relationship of plant with endophytic microorganisms has been proved as a source of materials and products with maximum medicinal potential as compared to the plants alone. Various secondary metabolites and enzymes synthesized by microorganisms such as bioactive compounds can be found extensively in the form of food and its dosages, pharmaceuticals (Subbulakshmi et al. 2012; Mitsuhashi 2014; Wendisch 2014), Nutraceutica l, biopesticides (Waldron et al. 2001; Yoon et al. 2004) and detergents (Shaligram and Singhal 2010), as well as in the manufacturing process of these industrial products (Kirk et al. 2002; Merino and Cherry 2007) and biofuels (Geddes et al. 2011). Metabolites and enzymes production methods have been enhanced since the time of their initial importance was realized. *Lactobacillus plantarum* and *B. subtilis* enhanced the content of bioactive compounds in kidney bean extracts after fermentation (Limona et al. 2015).

2.3.13 Role of endophytes in Agriculture: Endophytes are used in many ways in agriculture. Based on various research and its results, it can be said that endophytes do not cause disease or any significant morphological changes for any plant. The mixed microbial community of endophytes has been playing an important and unique role in the functioning of the agrosystem, which not only increases the productivity of the crop but also the yield of the soil. Bacillus and Pseudomonas are common germs often found in agricultural diseases known for ring diseases (Seghers et al., 2004). Presence of many endophytic species depends frequently on plant species and bacteria genotype and biotic and abiotic environmental factors. Endophytic population depends upon several factors like the different tissue type of plants, season of isolation in a single host plant species. It has often been observed that endophytic species mostly belong to the β and γ protobacteria subgroups and are closely related to epiphytic species.