Sys Rev Pharm. 2018;9(1):22-30
A multifaceted Review journal in the field of Pharmacy

Review Article

Nitrogen Containing Secondary Metabolites from Endophytes of Medicinal Plants and their Biological/Pharmacological Activities-A Review

Smita Kishor Puri [1*], Prasanna Vasantrao Habbu[1], Preeti Venkatrao Kulkarni[2], Venkatrao Hanumantrao Kulkarni[2]
[1]Postgraduate, Department of Pharmacognosy, SET's College of Pharmacy, S. R. Nagar, Dharwad 580002, Karnataka, INDIA.
[2]Postgraduate, Department of Pharmacology, SET's College of Pharmacy, S. R. Nagar, Dharwad 580002, Karnataka, INDIA.

ABSTRACT

Endophytes are the microbes that live inside of a plant and colonise the intercellular spaces of the plant tissues. Nitrogen containing compounds have an important role in research through decades as a history in the development of organic synthesis. Nitrogen is a part of all living cells and an important component of all proteins, enzymes, nucleic acids, cofactors, signalling molecules, numerous plant secondary products and necessary metabolic processes involved in the synthesis and transfer of energy. These compounds also occur in a diverse natural products and drugs showing importance in many aspects of life and commercial processes. A nitrogenous secondary metabolite obtained by endophytes originate from various biosynthetic pathways belonging to different structural groups such as alkaloids, amines, peptides, etc. These secondary metabolites show promising potentiality and benefits in safety and human health. This review covers diversity of nitrogenous secondary metabolites isolated from endophytes associated with medicinal plants possessing biological/pharmacological activities such as anticancer, antimicrobial, antifungal, antiviral, antimalarial, etc in the plant tissues from years 2008-2016.

Key words: Nitrogen, Endophytic fungi, Anticancer, Antifungal, Antibacterial, Antimalarial.

Correspondence:
Ms. Smita K Puri
Research Scholar, Department of Pharmacognosy & Phytochemistry, SET's College of Pharmacy, S. R. Nagar, Dharwad 580002, Karnataka, INDIA.
Phone no: 9986033439
E-mail id: smitamadagundi@gmail.com
DOI : 10.5530/srp.2018.1.5

INTRODUCTION

Nitrogen is an important component of living cells present in nucleic acids, proteins, enzymes, cofactors, signalling molecules, numerous plant secondary products and necessary metabolic processes involved in the synthesis and transfer of energy. Plants require nitrogen throughout their development. Plants containing large amount of nitrogen produces nitrogenous secondary metabolites forming a part of their structure. Plants obtain nitrogen from roots by nitrogen fixing bacteria and deterioration of dead tissues by micro-organisms, thus promoting plant growth.[1] Nitrogen combines chemically with oxygen or hydrogen to form various nitrogenous compounds which can be used by plants. The organic nitrogenous compounds are formed from inorganic nitrogen compounds present in the environment by nitrogen assimilation. These nitrogenous compounds are added to the soil as fertilizers in the form of ammonium (NH_4^+) and nitrate (NO_3^+). Nitrogenous metabolites are widely distributed throughout the plant kingdom.

Nitrogen is a major constituent in almost drugs used in medicine. Nitrogen containing compounds have an important role in research through decades as a history in the development of organic synthesis. Nitrogen containing medicinal compounds has been used since centuries, as they form the basis for many drugs such as taxol, campothecin, vincristine, etc. These compounds occur in a diverse natural products and drugs showing importance in many aspects of life and commercial processes, from the industrial production of fertilizers to the building blocks of life.[2]

Endophyte is a microorganism that lives inside of a plant and colonise the intercellular spaces of the plant tissues. They include organisms such as fungi, bacteria and actinomycetes which spend its lifespan inside cellular tissues for healthy host without producing any symptoms.[3] They are chemical synthesizers producing novel compounds that show numerous pharmacological and biological activities.[4] Mutualistic relation between endophytes and host plants results in beneficiary for both,[5] thus mutual relationship, between taxonomy and ecology are being studied. Majority of the secondary metabolites already discovered in plants, but micro-organisms are also capable of producing more than 20000 biologically active compounds, influencing the performance and survival of other organisms.[6] Many of these microbes producing active secondary metabolites are involved in a host endophyte relationship and thus exhibit various biological activities as antibiotic, antitumor, anti-inflammatory, antioxidant, etc. Numerous secondary metabolites produced by various endophytes possess unique structures belonging to alkaloids, glycosides, benzopyranones, flavonoids, phenolic acids, quinones, steroids, xanthones, terpenoids, tetralones and others and bioactivities, potentially useful.[7] Alkaloids, non-protein amino acids, cyanogenetic glycosides, amines, glucosinolates, alkamides, lecithins, peptides and polypeptides are important group of secondary metabolites having nitrogen produced by endophytes.

Secondary nitrogen is a commonly used in endophyte/host and plant interactions for host plant growth, uptake by endophytes and demand for synthesis of highly nitrogen containing compounds. Many of these compounds are required for the growth of the plants in the environment. Taxol, Campothecin, Vincristine, Phomoenamide, Aspergillusol A, Helvolic acid are some of the nitrogenous secondary metabolites obtained from the different endophytes exhibiting various pharmacological activities.

Many attempts were made to isolate and identify various secondary metabolites from endophytes to produce inexhaustible supply of bioactive compounds which are used commercially. Changes in the conditions of the culture can be explored by optimizing various biosynthetic pathways leading to the production of derivatives and analogues of novel compounds.[8] The increasing awareness of the secondary metabolites of medicinal plants can be influenced by endophytic infection. These endophytes may produce them on their own as that of plant or they may also alter the metabolite produced by the plant. Hence, there can be variability in the type of bioactive compounds which shows the possibilities to produce some novel type of medicines that may give results against some incurable diseases.[9] In recent studies, attention has been given to the biotransformation process of endophytes which acts as biocatalysts in the chemical transformation of natural products and drugs are due to their ability to modify chemical structures with a high degree of stereo specificity and to produce known or novel enzymes that increase the production of compounds of interest.[10]

This review article covers detailed information about nitrogen containing secondary metabolites isolated from endophytic microbes associated with medicinal plants during the years 2008-2016. Chemical diversity of these compounds and their potential biological/ pharmacological activities are also discussed.

NITROGEN CONTAINING ANTICANCER METABOLITES

Cancer is an uncontrolled growth of abnormal cells and invasion into normal tissue of various organs. In 2007, it was estimated that 7.6 million people were killed around the world due to cancer. By 2050, the annual death is may rise to 17.5 million, due to unavoidable reasons such as population growth, aging, lifestyle, environmental factors and carcinogenic agents. The existing drugs for treatment of cancer shows no specific toxicity to proliferating normal cells, produce various side effects and ineffective against many types of cancer.[11] No availability of new drugs or technologies for treating the disease, the cure of cancer has been increased by diagnosing earlier and more precise treatments.[12] Some of the examples of anticancer drugs obtained from medicinal plants are Campothecin from *Campotheca acuminate* (Nyssaceae), vinblastine, vincristine from *Catharanthus roseus* (Apocynaceae), burseran from *Bursera microphylla* (Burseraceae), colchicines, demecolcine from *Colchicum luteum* (Liliaceae). Recently several secondary metabolites from endophytes showing anticancer properties also have been investigated. Following are some examples of the nitrogen containing secondary metabolites from endophytes cited, on the production of anticancer agents and are shown in Table 1.

Vincristine (1) was the first reported metabolite of the endophytic fungus Alternaria sp. from the phloem of *Catharanthus roseus*. Compound 1 was also produced from an endophytic *Fusarium oxysporum* from the pholem of *Catharanthus roseus* (Apocynaceae). It is used as chemotherapeutic agent in acute nephroblastoma and lymphoblastic leukemia. The primary action of compound 1 is by interfering with microtubule formation and mitotic spindle dynamics, disruption of intracellular transport, decreasing tumour blood flow, with consequence of anti-angiogenesis.[13] New modified dipeptides, Trichodermamides A-C (2-4), were obtained from the endophytic fungus *Eupenicillium* sp. from bark of *Glochidion ferdinandi* (Phyllanthaceae). Compound 2 showed cytotoxic activity against the human colorectal carcinoma HCT116 and human lung carcinoma A-549 with IC_{50} values of 0.68 and 4.28 μg/ml, respectively.[14] Six new tetramic acid derivatives, Penicillenols A_1, A_2, B_1, B_2, C_1, and C_2 (5-10) were identified from *Penicillium* sp. GQ-7, an endophytic fungus associated with *Aegiceras corniculatum* (Primulaceae). All the compounds were screened for their cytotoxic effects on four cell lines by the MTT method. Penicillenols A_1 and B_1 showed cytotoxicities against HL-60 cell line with IC_{50} values of 0.76 μM and 3.20 μM.[15] Cycloaspeptide A (11) was isolated for the first time from the endophytic fungus *Penicillium janczewskii KM* Zalessky, from the plant *Prumnopitys andina* (Podocarpaceae). It exhibited low cytotoxicity towards human lung fibroblasts with $IC_{50} \geq 1000$ μM.[16] Penicidones A-C (12-14) were isolated from the culture of Penicillium species, an endophytic fungal strain residing in the stem of *Quercus variabilis* (Fagaceae). Compounds 12-14 exhibited in vitro cytotoxicity against four human cancer cell lines SW1116, K562, KB and Hela indicating that they are moderately cytotoxic with IC_{50} values between 21.1 and 90.8 μM.[17] An ergoline alkaloid, 9 deacetoxy fumigaclavine (15) was isolated from an endophytic fungus, *Aspergillus gumigatus* obtained from the stem of cynodon dactylon (Poaceae). It showed cytotoxicity against K562 cell lines with an IC_{50} of 3.1 mM.[18] Two indole alkaloids fumitremorgins B (16) and C (17) were produced by the endophytic fungus *Alternaria* sp. FL25 from *Ficus carica* (Moraceae).[19] Cochliodinol (18) and isocochliodinol (19) were isolated from an endophytic fungus *Chaetomium* species which resides in the stem of *Salvia officinalis* (Lamiaceae). Compound 18 showed potent cytotoxicity with an EC_{50} of 7.0 mg/ml.[20] Campothecin (20) a pentacyclic quinoline alkaloid was isolated from endophytic fungi *entrophosphora* associated with the inner bark of the plant *Nothapodytes foeatida* (Icacinaceae). This is followed by the isolation of campothecin from seed endophyte *Neurospora crusa* of *Campothecta accumalata* (Cornaceae). Campothecin and its two analogues viz 9 methoxy campothecin (21) and 10 hydroxy campothecin (22) were found to be isolated from an endophytic fungus, *Fusarium solane* of *campthecta accumalata*.[21] The mechanism involved in cytotoxocity was found to be by inhibition of DNA topo isomerase I enzyme.[22] They were tested for cytotoxic activity against humans in lung cancer (A549), liver cancer (hep 2), ovarion cancer (OVCAR-5) cell lines. Commonly used anticancer drug, Taxol (23) (Paclitaxel), a diterpene taxane, was isolated from the bark of Pacific yew tree *Taxus brevifolia* for the first time. It showed activity against a series of human solid tumor xenografts including CX-1 colon and MX-1 breast xenografts. Taxol was also isolated from the fungal endophyte *A. alternata* var. *monosporus* obtained from the inner bark of *Taxus yunnanensis* (Taxaceae). Compound 23 was also produced by an endophytic fungus, *Fusarium redolens*, isolated from Himalayan yew.[23] Till now, 20 genera of endophytic fungi were screened to produce paclitaxel. Some of them are *Alternaria* alternate TPF6 from *Taxus chinensis* var. mairei, *Aspergillus fumigatus* EPTP-1 *Podocarpus* sp,[24] *Aspergillus niger* var. taxi HD86-9 *Taxus cuspidate*,[25] *Botryodiplodia theobromae* BT115 *Taxus baccata*,[26] *Botrytis* sp. XT_2 *Taxus chinensis* var. mairei. Four new quinazolinone alkaloids, Aniquinazolines A-D (24-27), were isolated and identified from the culture of *Aspergillus nidulans MA-143*, an endophytic fungus obtained from the leaves of marine mangrove plant *Rhizophora stylosa* (Rhizophoraceae). They exhibited potent brine shrimp toxicity with LD_{50} values of 1.27, 2.11, 4.95 and 3.42 μM, respectively.[27] The structures of potent anticancer metabolites from endophytes are depicted in Figure 1.

NITROGEN CONTAINING ANTI-BACTERIAL METABOLITES

Infectious diseases caused by microbes are major health problems in society. The impact is large in developing countries due to the unavailability of medicines and widespread drug resistance. The incidence of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards studies on the potential antimicrobial activity of plant derived substances. The increasing problem of microbial resistance has become severe and the search for the use of antimicrobial drugs in the future is still uncertain. Antimicrobial resistance has been a major health issue and still presents threat to health care system globally. Studies have shown that microbes have developed resistance to antibiotics through various molecular mechanisms such as prevention of access to drug targets and modification of the drug.[28] Antimicrobial metabolites are low-molecular-weight organic compounds made by microorganisms to protect plant from outer invade, that are active at low concentrations against other microorganisms, and are the most bioactive natural products isolated from endophytes.[8] Search for new antimicrobial agents is needed due to the infections and diseases are global problems causing due to drug-resistant pathogens. Endophytes, by producing the secondary metabolites show resistance mechanism to overcome pathogenic invasion. Hence, studies demonstrated isolation of large number of antimicrobial compounds from endophytes, belonging to several structural classes like alkaloids, peptides, quinines and terpenoids. Asperfumoid (28) a new alkaloid, was isolated from an endophytic fungus *Aspergillus fumigatus* CY018, associated with *Cynodon dactylon* (Poaceae). Compound 28 inhibited the growth of *Candida albicans* (CA) with MIC value of 75 µg/ml. It was also isolated from the endophytic fungus *Penicillium* species, from the leaves of *Hopea hainanensis* (Dipterocarpaceae) as it inhibited the growth of CA with IC_{50} values of 20 and 25 µg/ml respectively.[29] A bioactive compound, 7-amino-4-methyl coumarin (29) was isolated from endophyte *Xylaria* sp isolated from *Ginkgo biloba* (Ginkgoaceae). Compound 29 showed strong antibacterial and antifungal activities *in vitro* against *Staphylococcus aureus* with MIC value of 16 µg/ml. It was the first coumarin metabolite to show antimicrobial activity.[30] Phomoenamide (30) and Phomonitroester (31) were obtained by an endophytic fungi *Phomopsis* species (PSU-D15), isolated from leaves of *Garcinia dulcis* (Cluciaseae). Compound 30 showed anti-microbial activities against *Mycobacterium* tuberculosis (MT) H37Ra with a MIC value of 6.25 µg/ml.[31] Two new metabolites, Cyclo (Pro-Thr) (32) and cyclo (Pro-Tyr) (33) produced by the fermentation broth of endophytic fungus *Penicillium* species isolated from mangrove plant *Acrostichum aureurm* showing antibacterial activity.[32] Polyketide amino acids derived antibiotics, Pyrrocidines A (34) and B (35), were obtained from endophytic fungi *Acremonium zeae* residing in Zea maize (maize kernals). They exhibited antimicrobial activity against microbial pathogens causing seedling blights and stalk rots. Compound 34 showed anti-bacterial activity against B*acillus mojavensis* and *Pseudomonas fluorescens* (PF) with MIC values of 1-2 µg/ml.[33] Three new indolosesquiterpenes namely Xiamycin B (36), Indosespene (37) and Sespenine (38) were isolated from the culture broth of *Streptomyces* sp. HKI0595, a bacterial endophyte isolated from mangrove tree, *Kandelia candel* (Rhizophoraceae). Both exhibited antimicrobial activities against several Gram-positive and Gram-negative bacteria reveling multi-resistance[34]. Tenuazonic acid (39) was isolated from *Alternaria alternata* SVJM015, residing in the leaves of *Indigofera enneaphylla* (Poaceae). It showed activity against MT with MIC value for 250 µg/ml.[35] (3,1″-didehydro-3[2″(3″,3″-dimethylprop-2-enyl)-3″-indolylmethylene]-6-methyl piperazine-2,5-dione) (40) is tryp-alanine derived cyclic dipeptide containing an indole and a diketopiperazine moiety which was isolated from the culture medium of *Penicillium chrysogenum*, an endophytic fungus of the mangrove plant *Porteresia coarctata* (Poaceae). Compound 40 showed significant antibacterial activity against *Vibrio cholera* (VC) (MCM B-322), a pathogen causing cholera in humans.[36] Four ansa macrolides or ansamycins, Divergolides A–D (41-44), were isolated from a bacterial endophyte *Streptomyces* sp. HKI0576 of the mangrove tree *Bruguiera gymnorrhizain* (Rhizophoraceae). Compound 41 showed the strongest activity against *Mycobacterium vaccae* (MV) with IC_{50} values ranging from 1.0-2.0 µM.[37] The structures of potent antibacterial metabolites are depicted in Figure 2.

NITROGEN CONTAINING ANTIFUNGAL METABOLITES

The functioning of invasive fungal infections increases during cancer chemotherapy, organ transplantation and allogeneic bone marrow transplantation. The use of synthetic antifungal agents against plant and human diseases has created awareness among the researchers worldwide, and thus much of the research areas are focused on development of novel, potent and green principle based antifungal agents using medicinal plants, owing to their different mode of action, different target sites with minimized side effects than the conventional available drugs.[38] However, the availability of antifungal agents for the treatment of various life threatening fungal infections is less so the new drugs should be made available in the market; the development of protection to antifungal drugs has become increasingly superficial, especially in patients with long term treatment. Microbial natural products have been an alternative natural cause for the isolation of unique molecules for various therapeutic applications.[39] Many nitrogen containing metabolites from endophytes are reported to possess antifungal activity.

Curvularides A–E (45-49), are the five-hybrid peptide-polyketides, which were obtained from the endophytic fungus *Curvularia geniculata*, isolated from the limbs of *Catunaregam tomentosa* (Rubiaceae). Compound 45 showed most significant antifungal activity against Candia albicans than other compounds.[40] 3-methylcarbazole (50) and 1-methoxy-3-methylcarbazole (51) were obtained from *Streptomyces* sp. LJK109 from *Alpinia galanga* (Zingiberaceae). Both the compounds showed antifungal activities with MIC of 30 to 240 µg/ml.[41] A chlorinated benzophenone alkaloid, Pestalachloride A (52) was obtained from *Pestalotiopsis adusta*, an endophytic fungus of the unknown Xinglong Chinese tree stem. The Compound 52 showed antifungal activity against the plant pathogens such as *Gibberella zeae* (*anamorph F. graminearum*), *Verticillium* alboatrum and *Fusarium culmorum*.[42] A new compound KL-4 (53) was isolated from the fungal endophytic *Aspergillus* species of seeds of Gloriosa superba (Colchicaceae) and was subjected to antimicrobial and anticancer activities. It showed broad spectrum as antifungal with IC_{50} value of 30 µg/ml.[43] Chaetoglobosin A (54), Chaetoglobosin D (55), Chaetoglobosin G (56), Chaetoglobosin R (57) were isolated from fungal endophyte *Chaetomium globosum* No. 04 inhabited in medicinal plant *Ginkgo biloba* (Ginkgoaceae). These antifungal metabolites were active against the phytopathogenic fungi *Rhizopus stolonifer* (RS) and *Coniothyrium diplodiella* (CD)0.[44] 12 β-hydroxy-13α-methoxy verruculogen TR-2 (58) and 3-hydroxy fumiquinazoline A (59), were isolated from the fermentation broth of *Aspergillus fumigatus* LN-4, an endophytic fungus isolated from the stem bark of *Melia azedarach* (Meliaceae). Compounds 58 and 59 exhibited antifungal activities against some phytopathogenic fungi (*Botrytis cinerea, Alternaria solani, Alternaria alternata, Colletotrichum gloeosporioides, Fusarium solani, Fusarium oxysporum f.* sp. *niveum, Fusarium oxysporum f.* sp. *vasinfectum,* and *Gibberella saubinettii)*. Compound 58 exhibited antifungal activities with MIC values of 6.25–50 µg/ml.[45] All the compounds showed good antifungal activities as shown in Table 1 and the structures are listed in Figure 3.

NITROGEN CONTAINING ANTIVIRAL METABOLITES

The emergence of resistance and multi-resistance against available drugs, the side effects and high cost of current therapies as well as the Hepatitis Immuno Virus/AIDS epidemic and AIDS associated opportunistic infections, such as cytomegalovirus and polyoma virus, made the development of novel antiviral drugs a central priority. Antiviral metabolites are the fascinating compounds from endophytes for inhibition of viruses. Some of these metabolites found to be promising against H_1N_1, influenza and other virus infections (Table 1).

Xiamycin A (60), a novel indole sesquiterpene, produced by a bacterial endophyte *Streptomyces* sp. GT2002/1503, isolated from the stem of mangrove plant *Bruguiera gymnorrhiza* (Rhizophoraceae). Compound 60 exhibited moderate antiviral activities against HIV. It specifically blocked CCR5 (R5) tropic HIV-1 while it showed no effect on CXCR4 (X4) tropic HIV-1.[46] Tricyclic sesquiterpenoids, Brasilamides A-D (61-64) were isolated from cultures of the plant endophytic fungus *Para coniothyrium brasiliense*. Verkley (M3–3341), isolated from branches of *Acer truncatum* (Sapindaceae). Compound 61-64 showed modest inhibitory effects on HIV-1 replication in C8166 cells.[47] Eight isoindolone de-

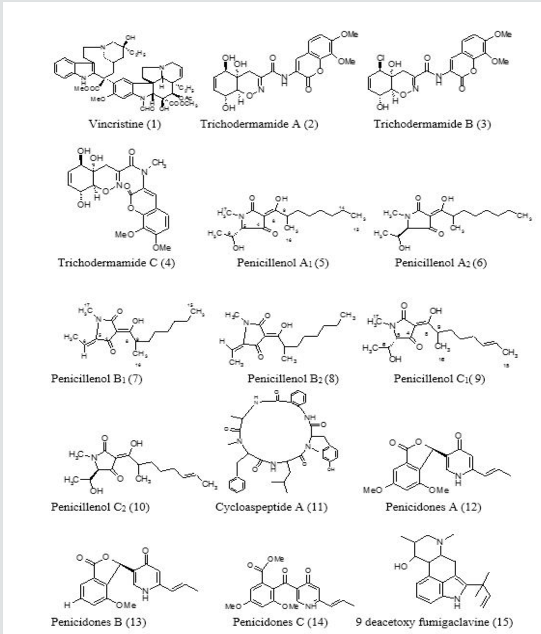


Figure 1a: Nitrogen containing anticancer metabolites from plant endophytes.

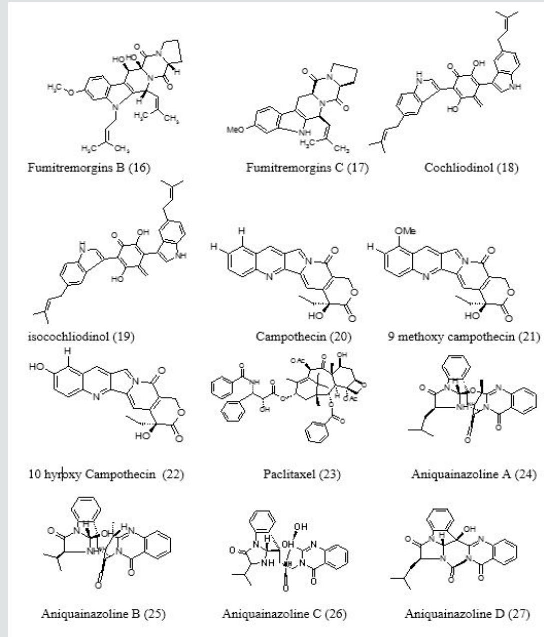


Figure 1b: Nitrogen containing anticancer metabolites from plant endophytes

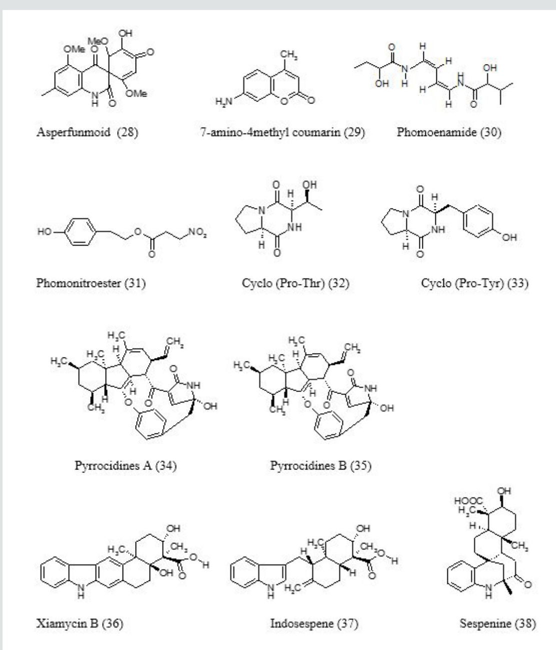


Fig 2a: Nitrogen containing anti-bacterial metabolites from plant endophytes.

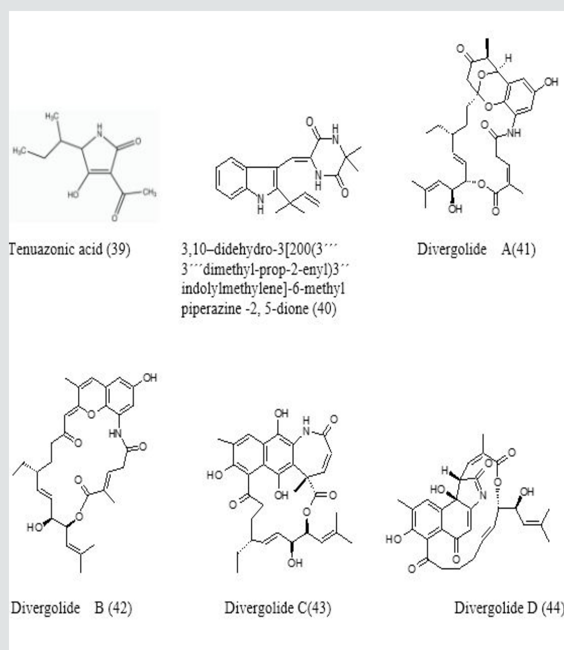


Figure 2b: Nitrogen containing anti-bacterial metabolites from plant endophytes.

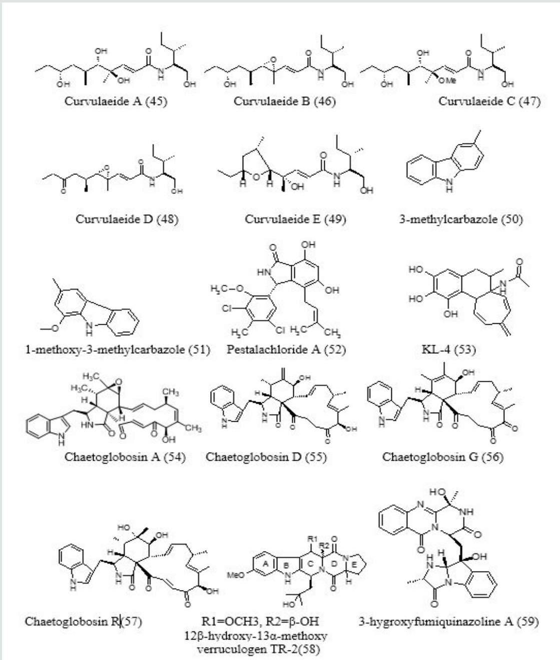


Figure 3: Nitrogen containing Antifungal metabolites from plant endophytes.

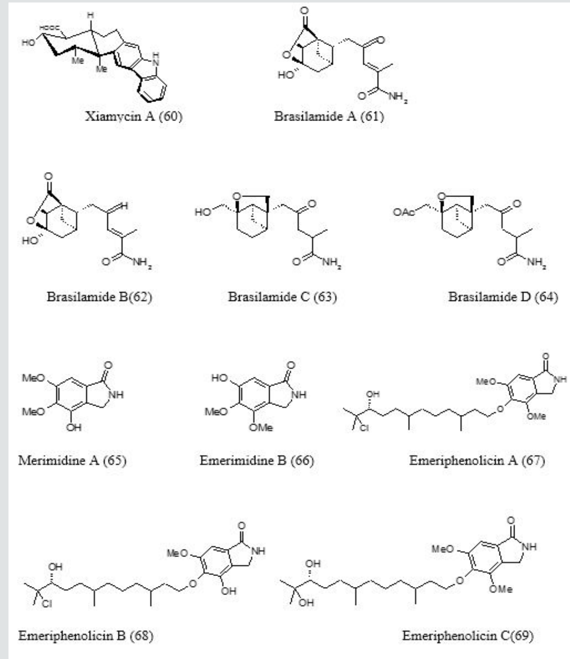


Figure 4a: Nitrogen containing antiviral metabolites from plant endophytes.

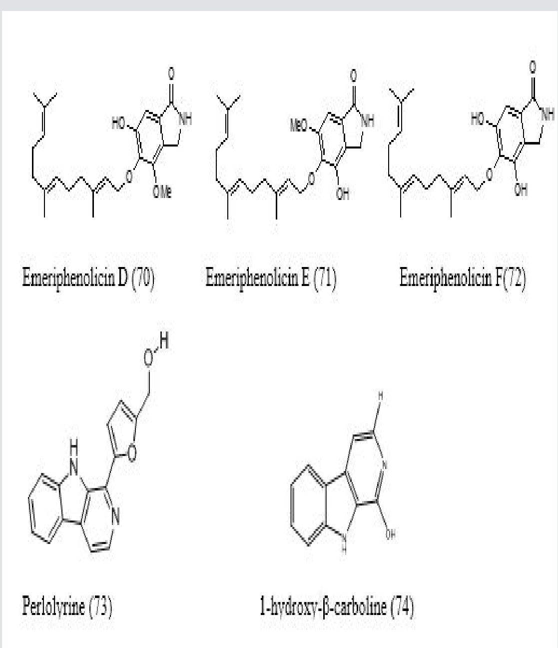


Figure 4b: Nitrogen containing antiviral metabolites from plant endophytes.

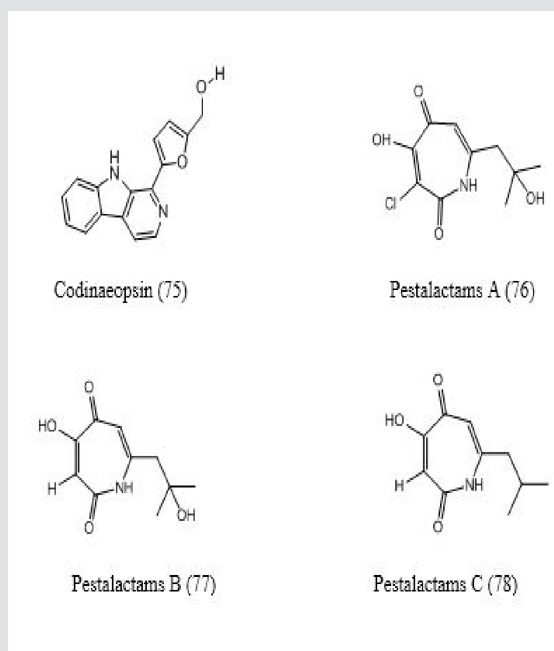
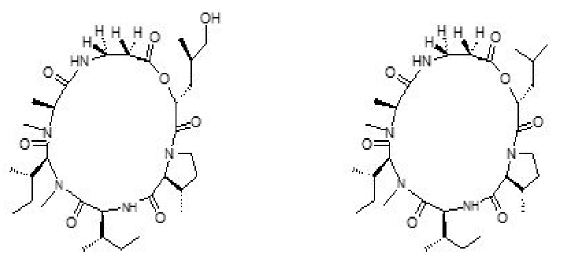


Figure 5: Nitrogen containing antimalarial metabolites from plant endophytes.

Table 1: Medicinal plants producing nitrogenous secondary metabolites from endophytes and their pharmacological activity.

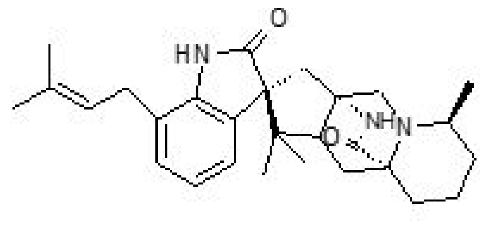
Sl no	Name of the Medicinal Plants	Endophytic organisms/species	Nitrogen containing secondary metabolite	Reported activity	Ref
1	*Catharanthus roseus* (Apocynaceae)	*Mycelia sterilia*	Vincristine (1)	Anticancer	13
2	*Glochidion ferdinandi* (Phyllanthaceae)	*Eupenicillium* sp	Trichodermamides A- C (2-4)	Anticancer	14
3	*Aegiceras corniculatum* (Primulaceae)	*Penicillium* sp. GQ-7	Penicillenol A1- A2 (5-6) Penicillenol B1- B2 (7-8) Penicillenol C1- C2 (9-10)	Anticancer	15
4	*Prumnopitys andina* (Podocarpaceae)	*Penicillium janczewskii K. M. Zalessky,*	Cycloaspeptide A (11)	Anticancer	16
5	*Quercus variabilis* (Fagaceae)	*Penicillium* sp	Penicidones A-C (12-14)	Anticancer	17
6	*Cynodon dactylon* (Poaceae)	*Aspergillus gumigatus*	9 deacetoxy fumigaclavine (15)	Anticancer	18
7	*Ficus carica* (Moraceae)	*Alternaria* sp. FL25	Fumitremorgins B (16) Fumitremorgins C (17)	Anticancer	19
8	*Salvia officinalis* (Lamiaceae)	*Chaetomium species*	Cochliodinol (18) Isocochliodinol (19)	Anticancer	20
9	*Nothapodytes foetida* (Icacinaceae) *Campotheca acuminate* (cornaceae)	*Entrophosphora* *Neurospora crusa* *Fusarium solane*	Campothecin (20) 9 methoxy campothecin (21) 10 hydroxy campothecin (22)	Anticancer	21,22
10	*Taxus yunnanensis* (Taxaceae) Himalayan yew	*A. alternata Fusarium redolens,*	Taxol (23)	Anticancer	23
11	*Rhizophora stylosa* (Rhizophoraceae)	*Aspergillus nidulans* MA-143	Aniquinazolines A–D (24-27)	Anticancer	27
12	*Cynodon dactylon* (Poaceae)	*Aspergillus fumigatus* CY018	Asperfumoid (28)	Antibacterial	29
13	*Ginkgo biloba* L (Ginkgoaceae)	*Xylaria* sp	7-amino-4-methyl coumarin (29)	Antibacterial	30
14	*Garcinia dulcis* (Roxb.) Kurz. (Clusiaceae)	*Phomopsis* sp. PSU-D15	Phomoenamide (30) Phomonitroester (31)	Antibacterial	31
15	*Acrostichum aureurm*	*Penicillium* sp.	Cyclo(Pro-Thr) (32) Cyclo (Pro-Tyr) (33)	Antibacterial	32
16	*Zea maize*	*Acremonium zeae*	Pyrrocidines A-B (34-35)	Antibacterial	33
17	*Kandelia candel* (Rhizophoraceae)	*Streptomyces* sp. HKI0595	Xiamycin B (36) Indosespene (37) Sespenine (38)	Antibacterial	34
18	*Indigofera enneaphylla* L. (Poaceae)	Alternaria alternata SVJM015	Tenuazonic acid (39)	Antitubercular	35
19	*Porteresia coarctata* (Roxb.) (Poaceae)	*Penicillium chrysogenum*, (MTCC 5108),	3,1”-didehydro-3[2”(3”,3”-dimethyl-prop-2-enyl)-3”-indolylmethylene]-6-methyl pipera-zine-2,5-dione (40)	Antibacterial	36
20	*Bruguiera gymnorrhizain* (Rhizophoraceae)	Streptomyces sp. HKI0576	Divergolides A–D (41-44)	Antibacterial	37
21	*Catunaregam tomentosa* (Rubiaceae)	*Curvularia geniculata*	Curvularides A–E (45-49)	Antifungal	40
22	*Alpinia galanga* a (L.) (Zingiberaceae)	*Streptomyces* sp. LJK109	3-methylcarbazole (50) 1-methoxy-3-methylcarbazole (51)	Antifungal	41
23	Xinglong Chinese tree	*Pestalotiopsis adusta*	Pestalachloride A (52)	Antifungal	42
24	*Gloriosa superba* (Colchicaceae)	*Aspergillus* sp.	KL-4 (53)	Antifungal	43
25	*Ginkgo biloba* (Ginkgoaceae)	*Chaetomium globosum* No.04	Chaetoglobosin A (54) Chaetoglobosin D (55) Chaetoglobosin G (56) Chaetoglobosin R (57)	Antifungal	44
26	*Melia azedarach* (Meliaceae)	*Aspergillus fumigatus* LN-4	12β-hydroxy-13α-methoxyverruculogen TR-2(58) 3-hydroxyfumiquinazoline A (59)	Antifungal	45
27	*Bruguiera gymnorrhiza* (Rhizophoraceae)	*Streptomyces* sp. GT2002/1503	Xiamycin A (60)	Antiviral	46

28	*Acer truncatum Bunge* (Sapindaceae)	*Para coniothyrium brasiliense.* Verkley (M3–3341)	Brasilamides A-D (61-64)	Antiviral	47
29	*Aegiceras corniculatum* (Primulaceae)	*Emericella* sp. (HK-ZJ)	Merimidine A (65) Emerimidine B (66) Emeriphenolicin A-F (67-72)	Antiviral	48
30	*Xylocarpus granatum* (Meliaceae)	*Jishengella endophytica* 16111	Perlolyrine (73) 1-hydroxy-β-carboline (74)	Antiviral	49
31	*Vochysia guatemalensis* (Vochysiaceae)	*Codinaeopsis gonytrichoides*	Codinaeopsin (75)	Antimalarial	53
32	----	*Pestalotiopsis* sp. (BRIP 39872	Pestalactams A-C (76-78)	Antimalarial	54
33	---	*Trichothecium roseum*	Trichomides A-B (79-80)	Immunosuppressive	55
34	*Cistanche deserticola* (Orobanchaceae)	*Penicillium chrysogenum* No. 005	Chrysogenamide A (81)	Neurocyte protection	56



Trichomide A (79)   Trichomide B (80)

Figure 6: Nitrogen containing immunosuppressive metabolites from plant endophytes.



Chrysogenamide A (81)

Figure 7: Nitrogen containing miscellaneous metabolites from plant endophytes

rivatives, Merimidine A (65), Emerimidine B (66), Emeriphenolicin A-F (67-72) were isolated from endophytic fungus *Emericella* sp. (HK-ZJ) associated with the inner bark of the mangrove plant A*egiceras corniculatum* (Primulaceae). Compound 66 exhibited anti-influenza and antiviral (H_1N_1) activities using the cytopathic effect (CPE) inhibition assay.[48] Perlolyrine (73) and 1-hydroxy-β-carboline (74) were isolated from the fermentation broth of an endophytic actinomycetes *Jishengella endophytica* 16111, present in the root of the mangrove plant, *Xylocarpus granatum* (Meliaceae). Compound 74 exhibited moderate anti-H_1N_1 virus activity .they showed anti-influenza A (H_1N_1) virus activity with the half maximal inhibitory concentration (IC_{50}) and selectivity index (SI) value of 38.3μg/ml.[49] Figure 4 shows important antiviral metabolites from endophytes.

NITROGEN CONTAINING ANTIMALARIAL METABOLITES

Malaria has a major health threat in developing countries and yearly more than 200 million new malaria cases are reported,[50] emphasising the urgent need for new drugs against malaria. In India, its management is challenging due to the huge population and large geographical area by rapid growth[51] and the resistance developed by the malaria parasite.[52] Antimalarial metabolites obtained from endophytes may come up with the need. A new tryptophan polyketide hybrid, Codinaeopsin (75) was obtained from endophytic fungi *Codinaeopsis gonytrichoides* present in *Vochysia guatemalensis*, (Vochysiaceae) a white yemeri tree collected in Costa Rica. Compound 75 is active against 3D7 strain of Plasmodium falciparum, with an IC_{50} value of 2.3 μg/ml.[53] Fermentation culture from the endophytic fungus *Pestalotiopsis* sp. (BRIP 39872) from *Melaleuca quinquenervia* (Myrtaceae) yielded three novel caprolactams, Pestalactams A-C (76-78). Compounds 76 and 78 were tested against two different strains of the malaria parasite *Plasmodium falciparum* (3D7 and Dd2) and both the compounds displayed antimalarial activity, with 16-41% parasite growth inhibition achieved at 25 μM.[54] The structures of antiviral metabolites from endophytes are depicted in Figure 5.

NITROGEN CONTAINING IMMUNOSUPPRESSIVE METABOLITES

The immunomodulatory compounds are divided into immunosuppressive and immunoregulatory drugs. Many of them have been obtained from endophytes from plants. Emerging of new diseases as autoimmune disorders, use of immunosuppressive drugs in the market has been increased. Immunosupressive drugs are used to prevent allograft rejection in transplant patients and to treat auto immune diseases such as rhematoid arthritis and insulin dependent diabetes. As a result, researchers are focusing on the production of such drugs from the alternative source as endophytes.

Trichomides A (79) and B (80), two new cyclodepsipeptides were isolated from the endophytic fungus *Trichothecium roseum*. Trichomide A has immunosuppressive effect more selectively than cyclosporine A. It was found that trichomide A decreases the expression of Bcl-2, increases the expression of Bax, and has a small or negligible effect on the expressions of p-Akt, CD25, and CD69 as depicted in Table 1.[55] The structures are listed in Figure 7.

NITROGEN CONTAINING METABOLITES WITH MISCELLANEOUS ACTIVITIES

Compounds bearing antioxidant activity are effective against damage caused by reactive oxygen species and oxygen-derived free radicals, contributing to numerous pathological effects, such as DNA damages, carcinogenesis, and cellular degeneration. Chrysogenamide A (81), a new member of the macfortine group of alkaloids, was identified from *Penicillium chrysogenum* No. 005, an endophytic fungus associated with *Cistanche deserticola* (Orobanchaceae). It exhibited a neurocyte protection effect against oxidative stress-induced cell death in SH-SY5Y cells.[56] Other activities such as molluscicidal activity of endophyte JJ18 from *Pseudolarix kaempferi* Gord was also reported.[57]

CONCLUSION

The objective of this paper is to review the diversity of nitrogenous secondary metabolites from endophytes with various biological/pharmacological activities such as anticancer, antimicrobial, antifungal, antiviral, antimalarial, etc in the plant tissues. This review contains 81 nitrogenous compounds with various activities against plant and human pathogens, produced by endophytes inhabiting in various medicinal plants. Endophytes have the evidence of producing same or novel secondary metabolites with a wide-range of pharmacological activities which shows potential use in agricultural, industrial and medical areas. Thus, nitrogenous secondary metabolites produced by endophytes originate from various biosynthetic pathways belonging to different structural groups such as alkaloids, amines, peptides, etc. Endophytes producing bioactive natural compounds have shown promising potential and usefulness concerns for human health and safety. Modern biotechnology, as genetic engineering, metabolic technology and microbial fermentation process, showed advantages to understand and manipulate the importance of microorganism resources making beneficial for the mankind. However, there is need to understand and discover about the host/entophyte relationship before utilizing endophytes in the discovery of medicinally important compounds. In conclusion, a more comprehensive understanding of the biochemistry, genetics and biology of endophyte and host, may lead to new opportunities for developing medicinally important products to alleviate various human ailments.

ACKNOWLEDGMENT

The authors are thankful to the Principal, Soniya Education Trust's College of Pharmacy, Dharwad, for encouragement and support. Authors are also thankful to the librarians of SET's College of Pharmacy, Dharwad and Karnatak University, Dharwad for providing the necessary digital library facilities for review of literature.

CONFLICT OF INTEREST

None

REFERENCES

1. Reinhold HB, Hurek T. Living inside plants: bacterial endophytes. Curr. Opin. Plant Biol. 2011;14(4):435-43.
2. Source: Boundless "Nitrogen Compounds." Boundless Chemistry. Boundless; 2015.
3. Dompeipen EJ, Srikandace Y, Suharso WP, Cahyana H, Simanjuntak P. Potential endophytic microbes selection for antidiabetic bioactive compounds production. Asian J. Biochem. 2011;6(6):465-471.
4. Owen NL, Hundley N. Endophytes-the chemical synthesizer inside plants. Sci Prog. 2004;87(2):79–99.
5. Kogel KH, Franken P, Huckelhoven R. Endophyte or parasite—what decides. Cur Opin in Plant Bio. 2006;9(4):358-63.
6. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. J Antibiot. 2009;62:5-16.
7. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Nat. Prod. Rep. 2001;18(4):448-59.
8. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbial Mol Biol Rev. 2003;67(4):491-502.
9. Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J Nat Prod. 2004;67(2):257-68.
10. Pimentel MR, Molina G, Dionısio AP, Marostica MR, Pastore GM. The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotech Res Inter. 2011;1-11.
11. Gangadevi V, Muthumary J. Taxol, an anticancer drug produced by an endophytic fungus Bartalinia robillardoides Tassi, isolated froma medicinal plant, *Aegle marmelos* Correaex Roxb. World J Micro Biotech. 2011;24(5):717-24.
12. Pasut G. Veronese FM. PEG conjugates in clinical development or use as anticancer agents: an overview. Adv Drug Del Rev. 2009;61(13):1177–88.
13. Moore A, Pinkerton R. Vincristine: can its therapeutic index be enhanced? Pediatr. Blood Cancer. 2009;53(7):1180-7.
14. Davis RA, Longden J, Avery VM, Healy PC. The isolation, structure determination and cytotoxicity of the new fungal metabolite, Trichodermamide C. Bioorganic & Medicinal Chemistry Letters. 2008;18(9):2836-9.
15. Lin ZJ, Lu Z, Zhu TJ, Fang YC, Gu QQ, Zhu WM. Penicillenols from *Penicillium* sp. GQ-7, an endophytic fungus associated with *Aegiceras corniculatum*. Chem. Pharm. Bull. 2008;56(2):217-21.
16. Schmeda HG, Hormazabal E, Rodriguez JA, Theoduloz C. Cycloaspeptide A and pseurotin A from the endophytic fungus *Penicillium janczewskii*. Z Naturforsch C. 2008;63(5-6):383-8.
17. Ge HM, Shen Y, Zhu CH, Tan SH, Ding H, Song YC, Tan RX. Penicidones A-C, three cytotoxic alkaloidal metabolites of an endophytic Penicillium sp. Phytochemistry. 2008;69(2):571-6.
18. Ge HM, Yu ZG, Zhang J, Wu JH, Tan RX. Bioactive alkaloids from endophytic *Aspergillus fumigates*. J. Nat Prod. 2009;72(4):753-75.
19. Ma Y, Feng C, Zhang H, Zhou X. Two indole alkaloids produced by endophytic fungus FL25 from *Ficus carica*. Chem. Res. Appl. 2009;21:1173-5.
20. Debbab AH, Aly RA, Edrada-Ebel, Muller WEG, Mosaddak M, Hakiki A, *et al*. Bioactive secondary metabolites from the endophytic fungus *Chaetomium* sp. isolated from *Salvia officinalis* growing in Morocco. Biotechnol. Agron. Soc. Environ. 2009;13(2):229-34.
21. Kusari S, Zuhlke S, Spiteller M. An endophytic fungus from *Camptothec acuminata* that produces camptothecin and analogues. J. Nat. Prod. 2009;72(1):2–7
22. Ravindra N, Kharwar AM, Surendra KG, Andrea S, Donald S. Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat. Prod. Rep. 2011;28(7):1208.
23. Garyali S, Kumar A, Reddy MS. Taxol production by an endophytic fungus, *Fusarium redolens*, isolated from Himalayan yew. J Microbiol Biotechnol. 2013;23(10):1372-80.
24. Sun D, Ran X, Wang J. Isolation and identification of a taxol producing endophytic fungus from *Podocarpus*. Acta Microbiol. Sin. 2008;48(5):589-95.
25. Zhao K, Ping W, Li Q, Hao S, Zhao L, Gao T, *et al. Aspergillus niger* var. taxi, a new species variant of taxol producing fungus isolated from Taxus cuspidata in China. J. Appl. Microbiol. 2009;107(4):1202-7.
26. Venkatachalam R, Subban K, Paul MJ. Taxol from *Botryodiplodia theobromae* (BT115) an endophytic fungus of *Taxus baccata*. J. Biotechnol. 2008;136:S189-90.
27. Chun Y, Xiao ML, Chun Shun L, Wang MH, Xu GM, Wang BG. Aniquinazolines A–D, Four New quinazolinone alkaloids from marine derived endophytic fungus *Aspergillus nidulans*. Mar Drugs. 2013;11(7):2682-94.
28. Marcellano JP, Collanto AS, Fuentes RG. Antibacterial Activity of Endophytic

Fungi Isolated from the Bark of *Cinnamomum mercadoi*. Pharmacog J. 2017;9(3):405-9.

29. Wang FW, Hou ZM, Wang CR, Li P, Shi DH. Bioactive metabolites from Penicillium sp., An endophytic fungus residing in *Hopea hainanensis*. World J Microbio Biotech. 2008;24(10):2143-7.
30. Liu X, Dong M, Chen X, Jiang M, Lv X, Zhou J. Antimicrobial activity of an endophytic *Xylaria* sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. Appl Microbiol Biotechnol. 2008;78(2):241-7.
31. Rukachaisirikul V, Sommart U, Phongpaichit S, Sakayaroj J, Kirtikara K. Metabolites from the endophytic fungus *Phomopsis* sp.PSU-D15. Phytochem Lett. 2008;69(3):783-7.
32. Cui HB, Mei WL, Miao CD, Lin HP, Kui H, Dai HF. Antibacterial constituents from the endophytic fungus *Penicillium* sp. 0935030 of a mangrove plant *Acrostichum aureurm*. Chin J Antibiot. 2008;7:4.
33. Wicklow DT, Poling SM. Antimicrobial activity of pyrrocidines from *Acremonium zeae* against endophytes and pathogens of maize. Phytopathology. 2009;99(1):109-15.
34. Ling D, Armin M, Heinz H, Wen Han L, Christian H. A family of multicyclic indol sesquiterpenes from a bacterial endophyte. Org. Biomol. Chem. 2011;9(11):4029-31.
35. Sonaimuthu V, Parihar S, Thakur JP, Luqman S, Saikia D, Chanotiya CS, *et al*. Tenuazonic acid: a promising antitubercular principle from *Alternaria alternate*. Microbiol Res. 2011;2(2):63-5.
36. Prabha D, Cheryl R, Naik CG, D'Souza L. Isolation and characterization of antibacterial compound from a Mangrove-Endophytic Fungus, *Penicillium chrysogenum* MTCC 5108. Ind J Microbiol. 2012;52(4):617-23.
37. Ding L, Maier HH, Fiebig H, Gorls WH, Lin G, Peschel C, *et al*. Divergolides A–D from a Mangrove endophyte reveals an unparalleled plasticity in ansa-Macrolide biosynthesis. Chem. Int. 2011;50(7):1630-4.
38. Kavitha KS, Satish S. Bioprospecting of some medicinal plants explored for antifungal activity. Pharmacog J. 2016;8(1):59-65.
39. Akanksha B, Pavan A. Review fungal endophytes: as a store house of bioactive compound. Wor J pharm Pharmaceut sci. 2014;3(9):228-37.
40. Chomcheon P, Wiyakrutta S, Aree T, Sriubolmas N, Ngamrojanavanich N, Mahidol CS,*et al*. *Curvularides* A–E: antifungal hybrid peptide–polyketides from the endophytic fungus *Curvularia geniculata* Chem. Eur. J. 2010;16(36):11178-85.
41. Thongchai T, Srisakul C, Wanwikar R, Waya SP. Antifungal activity of 3- methylcarbazoles from *Streptomyces* sp. LJK109; an endophyte in *Alpinia galanga*. J Appl pharmaceut Sci. 2012;02(03):124-8.
42. Li E, Jiang L, Guo L, Zhang H, Che Y. Pestalachlorides A-C, antifungal metabolites from the plant endophytic fungus *Pestalotiopsis adusta*. Bioorg. Med. Chem. 2008;16(17):7894-9.
43. Budhiraja A, Nepali K, Sapra S, Gupta S, Kumar S, Dhar KL. Bioactive metabolites from an endophytic fungus of *Aspergillus* species isolated from seeds of Gloriosa superba Linn. Med chem res. 2013;22(1):323-9.
44. Zhang G, Zhang Y, Qin J, Qu X, Liu J, Li X, *et al*. Antifungal metabolites produced by *Chaetomium globosum* No. 04, an endophytic fungus isolated from *Ginkgo biloba*. Ind J micro. 2013;53(2):175-80.
45. Xiao JL, Qiang Z, Zhang AL, Gao JM. Metabolites from *Aspergillus fumigatus*, an endophytic fungus associated with *Melia azedarach*, and their antifungal, antifeedant, and toxic activities. J. Agric. Food Chem. 2012;60(13):3424-31.
46. Ding L, Maier A, Fiebig HH, Lin WH, Hertweck CA. Family of multicyclic indolosesquiterpenes from a bacterial endophyte. Org. Biomol. Chem. 2011;9(11):4029-31.
47. Liu L, Gao H, Chen X, Cai X, Yang L, Guo L, *et al*. Brasilamides A–D: sesquiterpenoids from the plant endophytic fungus *Paraconiothyrium brasiliense*. Europ J organic Chem 2010;(17):3302-6.
48. Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D, *et al*. Antiviral isoindolone derivatives from an endophytic fungus *Emericella* sp. associated with *Aegiceras corniculatum*. Phytochem. 2011;72(11):1436-42.
49. Wang P, Kong F, Wei J, Wang Y, Wang W, Hong K, *et al*. Alkaloids from the mangrove-derived actinomycete *Jishengella endophytica* 161111. Marine drug. 2014;12(1):477-90.
50. WHO World Malaria Report. 2011; http://www.who. int/mediacentre/factsheets/fs 094.
51. Shah NK, Dhillon GPS, Dash AP, Arora U, Meshnick SR, Valecha N. Antimalarial drug resistance of *Plasmodium falciparum* in India: changes over time and space. The Lancet infec disease. 2011;11(1):57–64.
52. Anderson T. Mapping the spread of malaria drug resistance. PLoS Medicine. 2009;6:e1000054.
53. Renee K, Jon C. Codinaeopsin, an antimalarial fungal Polyketide. Org. Lett. 2008:10(18):4149–4151.
54. Davis RA, Carroll AR, Andrews KT, Boyle GM, Tran TL, Healy PC, *et al*. Pestalactams A–C: novel caprolactams from the endophytic fungus *Pestalotiopsis* sp. Org biomolec chem. 2010;8(8):1785-90.
55. Zhang AH, Wang XQ, Han WB, Sun Y, Guo Y, Wu Q *et al*. Discovery of a new class of immunosuppressants from *Trichothecium roseum* co-inspired by cross kingdom similarity in innate immunity and pharmacophore Motif. Chem. Asian J. 2013;8(12):3101-7.
56. Lin ZJ, Wen JN, Zhu TJ, Fang YC, Gu QQ, *et al*. Chrysogenamide A from an endophytic fungus associated with *Cistanche deserticola* and its neuroprotective effect on SH-SY5Y cells. J. Antiobiot. 2008;61(2):81-5.
57. Chen J, Han BX, Guo SB, Wang Y, He J, Zhou XK, *et al*. Molluscicidal activity against *Oncomelania hupensis* of endophyte JJ18 from *Pseudolarix kaempferi* Gord. Pharmacog Res. 2009;1(6):421.