Ravindra H. Patil
Vijay L. Maheshwari *Editors*

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Potential Source of Compounds of Commercial and Therapeutic Applications

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Preface

Endophytes are the microorganisms that reside in the living internal tissues of the plant without showing any apparent symptom of their presence. During their life cycle, they establish a symbiotic or parasitic relationship with the host plant. The horizontal transfer of genes from the host plant to endophytes apparently enables them to mimic the host chemistry. Their potential was first recognized when taxol-producing endophytic fungi was isolated from yew plant. Recently, these microorganisms have attracted a lot of scientific attention worldwide because of their huge potential for novel phytochemicals, pharmaceuticals and lead compounds. Hundreds of new novel endophytic fungi have been isolated, identified and systematically studied in the last decade. Moreover, more than 200 different bioactive compounds are reported from these endophytic microorganisms. Among the endophytic microorganisms, endophytic fungi are the most thoroughly explored group for biotechnological applications. Many groups across the globe are working on the different aspects of endophyte biology and exploring the therapeutic potential of endophyte-derived compounds. However, a systematically compiled information/documentation is not available as far as biotechnological potential of these microorganisms is concerned. This book is a compilation of existing information based on current research and knowledge in the field of endophyte natural products, endophyte ecology and diversity. It contains many interesting articles highlighting the biodiversity of endophyte-derived natural products and their medicinal and pharmaceutical potentials. The chapters also represent a detailed outlook of biotechnological potential of endophytic microorganisms for the compounds of commercial, agricultural and therapeutic applications.

Alternaria is a genus belonging to ascomycete and generally comprises of members which cause spoilage, decay and decomposition of agricultural produce, and some exist as opportunistic human pathogens. In Chap. 1, Sanjay Saxena, presented an update on specialized members of *Alternaria* genus existing as endophyte and the new chemical entities produced by them possessing agrobiological and pharmacological properties. There are several instances which show that endophytic microbes isolated from such plants introduced as bio-inoculants in their close cultivars suppress plant pathogens, reduce biotic stress, help crop to grow successfully in nutrient-deficit soil and provide resistance against aphids. In Chap. 2, Mili et al. highlighted endophytic fungal diversity of wild and domesticated crop plants

and their applications as plant growth promoter and biological control agents. There has been an exponential rise in different types of cancers among the global population due to a variety of factors. In Chap. 3, Singamaneni et al. discussed novel anticancer metabolites from endophytic microorganisms. The Indo-Pak region has deep historical roots of traditional medicine. In both Pakistan and India, the local population has relied upon the Unani-Tibb (Graeco-Arabic) and Ayurveda medicinal systems for centuries. Ilyas et al. in Chap. 4 highlighted the traditional plants of the Indo-Pak region, their endophytes particularly the genus Actinomycetes and the recent studies done on their diverse metabolites. Midhun and Mathews in Chap. 5 discussed pharmacological uses of endophytes as a novel source of drugs against several diseases and other promising therapeutic use.

The north-eastern region (NER) has been in spotlight for its high biodiversity and traditional knowledge and has been a priority for leading conservation agencies of the world. Among the north-eastern states, Assam is very rich in medicinal herbs and plants, epiphytic orchids and wild edible leafy vegetables. In Chap. 6, Talukdar et al. highlighted the endophytic fungi harbouring in some ethno-medicinal plants of Assam and their potential for applications as antimicrobial agents. Samanta et al. provided a detailed diversity of antimicrobial, antioxidant and anticancer compounds from endophytic microorganisms in Chap. 7. In Chap. 8 by Alwis et al., the importance of the endophyte diversity of tropical rainforests and mangroves of Sri Lanka is discussed. This chapter focused on the novel metabolites isolated from endophytic fungi of Sri Lanka and their bioactivities in terms of therapeutic and agricultural aspects.

The process of harnessing natural products of endophyte is not only time-consuming but also tedious. The endophytic microorganisms are first isolated from the selected plant. Usually, a large number of isolates are obtained and tested by bioassay-based screening for desired activity. Moreover, the distribution of bioactive metabolites is varying in different plant tissues. The desired metabolites are isolated through a lengthy extraction and purification process. Patil et al. in Chap. 9 reviewed the dynamics of endophytes and their natural products within the cell, imaging mass spectrometry (IMS) for in situ probing of metabolites of endophytic organisms, process workflow of DESI-MS and provides details of the identified secondary metabolites of endophytic microorganisms using DESI-MS platform in the last 10 years.

Drought, high salinity, metal toxicity and temperature extremities are abiotic stresses hampering plant's growth and development. Defensive strategies adapted by plants induce cascade of signals ranging from phytohormones to secretion of osmolytes. Endophytic microbes cause phytohormone-based signalling modulation in host plants. Chapter 10 by Cosoveanu et al. presents the dynamics of phytohormones-producing endophytes in plant microbial interaction under stress. Lack of a holistic and comprehensive approach towards extraction and characterization of secondary metabolites has been a stumbling block in the realization of full potential of endophytes. In Chap. 11, Kannakazhi Kantari et al. have reviewed the novel and recent exogenous methods available for activating biosynthetic gene clusters for screening large number of isolated endophytic fungi for various

bioactivities; various genome mining strategies employed for detecting and analysing the gene clusters and biosynthetic pathways and metabolomics approaches useful for speedy isolation of bioactive metabolites. Chapter 12 of the book by Preethi et al. takes a comprehensive review of the endophytes as a source of compounds with commercial and therapeutic applications.

We appreciate the patience and cooperation extended by all the contributors of this book. Thanks are also due to the Publisher Springer Nature Singapore Pvt. Ltd., Singapore, for giving us this opportunity. We hope that the contents of the book will serve as a useful resource for all those working in the area and will be received with enthusiasm and interest.

Shirpur, Maharashtra, India
Jalgaon, Maharashtra, India
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Ravindra H. Patil
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About the Editors

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Biologically Active Secondary Metabolites from Endophytic *Alternaria* Species

1

Sanjai Saxena

Abstract

Microbial secondary metabolites have contributed immensely in the development of a variety of medicines, namely antibiotics, metabolic inhibitors, immunomodulatory agents, antioxidants and anticancer agents. Endophytism in the past decade has further opened avenues of exploration and exploitation of new chemical entities produced during the plant–microbe interaction for pharmaceutical as well as agricultural applications. *Alternaria* is a genus belonging to ascomycete and generally comprise of members which cause agricultural spoilage, involved in decay and decomposition and some exist as opportunistic human pathogens. The genus is a prolific producer of secondary metabolites, which are finding applications in the agrochemical as well as pharmaceutical industry. The present review is an update on specialized members of *Alternaria* genus existing as an endophyte and the new chemical entities produced by them possessing agrobiological and pharmacological properties.

Keywords

Bioactive compounds · Ascomycete · Plant · Microbe interactions · Endophytism · Fungi

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1.1 Introduction

Endophytes are microorganisms which reside within the plant tissues without any symptomatic signatures of their existence, and their relationship with the host fluctuates from a saprobe to a latent pathogen or commensalistic/mutualistic symbiont (Stone et al. 2000). Endophytes play an imperative role in combating with the abiotic and biotic stresses encountered by the host plant apart from improving the growth and yield (Rodriguez et al. 2009). During the course of their interactions within the plant system, there is a lot of chemical communication happening which is mediated by a variety of signal molecules which have largely remained underexplored (Saikkonen et al. 2010; Paramanantham et al. 2019).

The concept of endophytism evolved with the discovery of taxol-producing endophytic fungus *Taxomyces andreanae* from the taxol-producing plant *Taxus brevifolia* (Stierle et al. 1995). This discovery raised questions on the ability of endophytic fungus as a producer of the host phytochemicals due to horizontal gene transfer from the host to the endophyte during the course of evolution or vice versa? (Suryanarayanan 2013). However, in due course many other endophytic isolates were found to produce taxol which were isolated from unrelated plants suggesting the biogenetic capabilities of the endophytic fungus being far more than their hosts (Porras-Alfaro and Bayman 2011). Subsequently a spectrum of endophytic fungi was explored from the medicinal plants to assess the possibility of fermentative production of phytomedicines. Today, endophytic fungi producing phytomedicines such as camptothecin, podophyllotoxin, huperzine and vincristine have been well studied (Aly et al. 2011b; Nisa et al. 2015). Apart from producing the phytomedicines, the endophytic fungi exist under a variety of biotic and abiotic stress which render them metabolically active thereby producing novel chemical entities which help in sustaining themselves in the torrid internal environment of the plants (Braga et al. 2016; Rana et al. 2020). Over the last two decades, endophytic fungi have been recognized as a lucrative and prolific source of novel chemistries which could be developed into newer medicines as well as environment and user-friendly agrochemical (Zhang et al. 2006; Gouda et al. 2016; Martinez-Klimova et al. 2017; Al-Ani 2019). However, this is just the tip of the iceberg since every plant on the earth, under different ecological niches, harbours a novel set of endophytic microorganisms (fungi as well as bacteria). Thus, exploration and exploitation of endophytes are alive and well in the present millennium.

The genus *Alternaria* was established in 1817 with *Alternaria alternata* as a type isolate. *Alternaria* belongs to the family Dematiaceae and commonly exists as a saprophyte deriving energy through cellulolytic activity. Generally, *Alternaria* species are inhabitants of soil or decaying (plant based) organic matter. However, some members of this genus are opportunistic pathogens and cause diseases in economically important plants such as ornamentals, vegetables like broccoli, cauliflower, tomatoes citrus, apples, and oil crops (Thomma 2003). They have also been identified as post-harvest pathogens. Secondary metabolites produced by the members of the genus *Alternaria* existing as a saprophyte or a pathogen have been largely explored and studied; however, bioactive compounds produced by

endophytic species of *Alternaria* are relatively a newer aspect (Lou et al. 2013). Existence of *Alternaria* species as an endophyte has been recorded in tropical forests of South India wherein it was isolated from trees belonging to different families. Some compelling examples of *Alternaria* species existing as an endophyte in different plant families and reported to exhibit potential bioactivities include Lamiaceae, Polygonaceae (Aly et al. 2008), Rhodomelaceae (Gao et al. 2009), Lythraceae (Kjer et al. 2009), Rubiaceae, Sapindaceae, Vitaceae, Euphorbiaceae, Cupressaceae (Soltani and Hosseyni Moghaddam 2014), Fabaceae, Calophyllaceae, Cactaceae and from plants in the 'restingas' area of Brazil (Das et al. 2017). Approximately 268 secondary metabolites have been reported from different *Alternaria* species till 2013. In this chapter we shall discuss the bioactive metabolites produced by the members of the genus *Alternaria* existing as an endophyte for their presumptive use as pharmaceuticals and agrochemicals.

1.2 Pharmaceutically Active Metabolites

The discovery of drugs from microbes began with the serendipitous discovery of Penicillin from the blue mould *Penicillium notatum* by Sir Alexander Fleming which marked the beginning of the golden era of antibiotics in the pharmaceutical research and development (Aly et al. 2011a). Ever since the discovery of Penicillin, fungi have been extensively explored for bioactives which have been developed into different drug classes such as antibiotics, antifungal, immunomodulatory, anti-hypercholesterolemic, anticancer, anti-obesity agents and in treating neurodegenerative diseases by producing inhibitors which could hinder in the formation of toxic protein aggregates (Saxena et al. 2019). Fungal endophytes are also a prolific source of novel bioactive compounds which could propel the development into newer drugs (Aly et al. 2010). The bioactive compounds which have been produced by endophytic *Alternaria* species can be classified as antimicrobial agents, cytotoxic agents (anticancer agents), anti-diabetic agents, anti-parasitic agents and enzyme inhibitors. In the upcoming paragraphs, an attempt has been made to discuss these promising bioactive compounds on their pharmacological actions.

1.2.1 Antimicrobial Agents

With the burgeoning incidences of human pathogenic microorganisms encountering resistance to the current armamentarium of antimicrobials, clinicians are finding it difficult to treat drug-resistant chronic infections caused by these superbugs. Hence there is a demand for novel chemical entities with either a unique mode of action or a novel chemical scaffold against which these superbugs have not developed a resistance mechanism. *Alternaria* sp. Samif01 was isolated as an endophyte existing in the roots of *Salvia miltiorrhiza* Bunge, produced **alternariol-9-methyl ether (1)** which exhibited a potential in vitro antibacterial activity against the test panel of microorganisms with a Minimal Inhibitory Concentration (MIC) range between

25 and 75 μg/mL. Other compounds with antibacterial activities produced by *Alternaria* sp. Samif01 are **altenuisol (2)**, **4-hydroxy alternariol-9-methyl ether (3)** and **alternariol (4)**. Altenuisol exhibited a potent activity against *Staphylococcus aureus*, while alternariol is active against both *Bacillus subtilis* and *S. aureus*, i.e. exhibits a broad-spectrum antibacterial activity (Jin et al. 2017). Another compound reported from *Alternaria alternata* Tche-153 was **altenusin (5)** which was isolated from ethyl acetate extract of the liquid culture. *Alternaria alternata* Tche-153 was isolated from the Thai medicinal plant *Terminalia chebula*. Altenusin exhibited a potent azole synergistic activity against *Candida albicans* of which ketoconazole exhibited the highest synergism. As *Candida* species are encountering resistance to azole class of antifungals, Altenusin holds a promise to be used as an antifungal combination with ketoconazole and other members of the azole family for combating refractory candidiasis (Phaopongthai et al. 2013). *Paracoccidioides brasiliensis* is a human pathogenic fungus which causes systemic mycosis known as Paracoccidioiodomycosis. This was being treated by drugs like sulphonamides, amphotericin B and itraconazole. However, in the recent past these drugs have been found to become ineffective which is getting evident by the extension in the duration of the antifungal course or relapses. This has created a demand for new antifungals for this disease. Altenusin isolated from *Alternaria* sp. UFMGCB55 exhibited a potent antifungal activity against 11 clinical isolates of *Paracoccidioides brasiliensis.* All the test isolates were highly susceptible to altenusin with MIC values between 1.9 and 3.9 μg/mL. Thus, altenusin hold a promise as a lead for developing a new antifungal agent. Yet another promising antifungal compound reported from *Alternaria brassisicola* MP-408 which is existing as an endophyte in *Malus halliana* is **Herbarin A (6)**. Herbarin exhibits a pronounced antifungal activity against *Trichophyton rubrum* and *Candida albicans* with a MIC of 15.6 μg/mL. *Alternaria brassisicola* MP-408 also produced **Alterchromone A (7)** which exhibited antibacterial as well as anti-candidal activity. The MIC for antibacterial activity was 3.9 μg/mL for *E. coli* and *Bacillus subtilis*, while it was 1.8 μg/mL for *Pseudomonas florescence*. Similarly, for anti-candidal activity the MIC was 3.9 μg/mL (Gu 2009). Recently an endophyte of *Ocimum tenuiflorum* L., *Alternaria tenuissima* OE7 has exhibited a potential antifungal activity against human pathogenic fungi including *Microsporum gypseum*, *Trichophyton rubrum*, *Candida albicans* and *C. tropicalis*. Thus, *Alternaria tenuissima* OE7 appears to be a promising candidate for exploration of antifungal compounds in the drug development programme (Chatterjee et al. 2020). **Solanopyrone P (8)**, **solanopyrone A (9)** and **solanopyrone C (10)** were isolated from the fermentation broth of *Alternaria tenuissima* SP07 which existed as an endophyte in the roots of Chinese medicinal plant *Salvia przewalskii*. These compounds exhibited a potent antibacterial activity against Gram-positive human pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA). Another known metabolite **alternariol methyl ether (11)** was also produced by *A. tenuissima* SP07 and exhibited a similar antibacterial activity against the Gram-positive bacteria (Wang et al. 2014b). Another promising antifungal secondary metabolite is **(3R,6R)-3-benzyl-6-isopropyl-4-methylmorpholine-2,5-dione (12)**, which exhibits a potent activity against

Alternaria solani, *Colletotrichum gloeosporioides* and *Phyricularia grisea.* It was isolated from *Alternaria atrans* MP-7 which is existing as an endophyte in *Psidium guajava. Alternaria atrans* MP-7 also produces a derivative of fusaric acid, **Atransfusarin (13)** which exhibited a remarkable antifungal activity against *Alternaria solani*, *P. grisea* and *C. gloeosporioides* with an MIC of 6.25 μM as compared to the positive controls, carbendazium and fusaric acid (Yang et al. 2019c). Another compound possessing a strong antibacterial activity against Gram-positive pathogenic microbes is **altersetin (14)** isolated from *Alternaria* sp. P0506, existing as an endophyte in *Vinca minor.* This compound has exhibited an appreciable activity in the murine sepsis model wherein it was intraperitonially injected within a concentration range of 10/25 mg/kg body weight. *Staphylococcus aureus* was used as the test organism to develop the murine sepsis model (Hellwig et al. 2002). Fermentation broth of *Alternaria* sp. MHE 68 isolated from *Pelargonium sidoides* yielded **cyclodecasiloxane (15)** which exhibited a potent broad-spectrum antibacterial activity including drug-resistant enterococcal strains, viz. *Enterococcus faecium* and *Enterococcus gullinarum* (Manganyi et al. 2019). Another endophytic isolate of *Alternaria* isolated from *Schinus terebinthifolius*, *Alternaria alternata* LGMF626 exhibited a potent anti-MRSA activity with an MIC of 18.52 μg/mL of the cell-free extract. Further fractionation of the extract yielded **E-2-hexyl-cinnamaldehyde (16)** and **3-benzylhexahydropyrrolo-[1,2-α] pyrazine-1,4-dione (17)** and **3-isobutylhexahydropyrrolo-[1,2-α]pyrazine-1,4-dione (18)** (Tonial et al. 2016). E-2-hexyl-cinnamaldehyde exhibited an MIC of 18.52 μg/mL against *Staphylococcus aureus* while the isolated pyrrolopryazine alkaloids showed a combined MIC of 55.55 μg/mL.

1.2.2 Anti-Viral Agents

Viruses are the most enigmatic microbes as they are living inside a host and non-living outside the host. Essentially, viruses require a host to replicate or reproduce and, in this process, they affect the host. The human body has mechanisms to combat the viral intruders; however, it depends on the extent and nature of the viruses and the immune status of the host. Over past two decades, there has been a spurt of viral infections across the globe either as major or minor outbreaks, epidemics and pandemics, the most recent being SARS-CoV-2 apart from influenza, herpes, dengue, etc. Getting vaccinated is one of the most probable methods to develop resistance to viral infections; however, it really takes a long time to develop a suitable vaccine. Chemotherapeutic interventions also exist in treating viral diseases which largely act on their replication system inside the host. However, owing to their evolutionary mechanism, they evolve into the drug-resistant versions, thereby making it more difficult for the clinicians to deal with these diseases. With this premise, it is pertinent as well as a necessity to explore novel chemical structures which are effective as well as potent to combat viruses and could be possibly converted into a therapeutic entity.

Fungi have already been recognized as fountainheads of novel chemical entities possessing diverse pharmacological activities including anti-viral activity which primarily is assessed using different in vitro assays. However, fungal endophyte producing anti-viral agents is fairly a newer resource. The premise of fungal endophytes producing anti-viral agents could be hypothesized as a mechanism to help in overcoming the biotic stress encountered by the host plant when attacked by a plant virus. Apart from developing them into therapeutic entities, they can be used in developing topical anti-viral disinfectants. *Alternaria tenuissima* QUE1se exists as an endophyte in the stem of *Quercus emoryi* and found to produce Altertoxins, viz. **altertoxin V (19)**, **altertoxin I (20)**, **altertoxin II (21)** and **altertoxin III (22)** which inhibit HIV-1 virus at a concentration of 0.5, 2.20, 0.3 and 1.5 μM, respectively. All the compounds appear to be promising for being developed into anti-HIV therapeutics after carrying out Quantitative Structural Activity Relationships (QSAR) studies (Bashyal et al. 2014). *Alternaria solani* residing in the roots of *Aconitum transsectum* Diels. has furnished a metabolite, **7-dehydroxyl-zinniol (23)**, which has a moderate anti-hepatitis B virus activity (Ai et al. 2012). Similarly, another endophytic isolate *Alternaria alternata* PGL-3 was isolated from the peel of *Puncia granatum*. The ethyl acetate extract of the culture broth of this fungus exhibited highly potent inhibition of HCV NS3/4a protease with an IC_{50} value of 17 μg/mL and yielded two previously known compounds **alternariol (4)** and **alternariol-9-methyl ether (11)**. Thus, it could be concluded that the anti-HBV of *Alternaria alternata* PGL-3 is attributed to these compounds (El-Kassem et al. 2019).

1.2.3 Anti-Parasitic Agents

Tropical diseases largely comprise of pathogenic parasites which are vector or non-vector-borne and predominate in populations where there is poor hygiene and sanitation; the living conditions are poor and have close proximity with animals. It is estimated that more than 1 billion people across the globe are suffering from these tropical parasitic diseases. The pathogenic parasites in tropical regions generally comprise of protozoans, helminths, bacteria and viruses. However, we shall more emphasize on the parasitic diseases caused by the protozoans and helminths which have limited options for the treatment unlike bacteria and viruses for which vaccines can be designed and developed. As these parasitic organisms are difficult to cultivate inside the laboratory, have multicellular complex organization and complete their life cycle in multiple hosts, hence developing vaccines is very difficult. Therefore, small molecule drugs appear to be the best option in treating these diseases. Endophytic fungi existing in tropical plants are highly diverse and can probably be the best source in screening and isolation of compounds which possess anti-parasitic activity. *Alternaria* sp. UFMGCB 55 existing as an endophyte in *Trixis vauthieri* (*Asteraceae family*) collected from Brazil exhibited anti-trypanocidal activities. Further isolation of bioactive extract of this endophytic isolate yielded a biphenyl, **altenusin (5)**. Further it was found that the trypanocidal activity of altenusin was

mediated through inhibition of the enzyme trypanothione reductase (TR). The IC_{50} value of altenusin for TR was found to be 4.3 ± 0.3 μM. Thus, altenusin is a promising lead for developing a novel lead/therapeutic drug for the treatment of trypanosomiasis and leishmaniasis (Cota et al. 2008). Another isolate, *Alternaria alternata* P1210 existing as an endophyte in roots of halophyte *Salicornia* sp. in Spain furnished two compounds **(±)-alternarlactones A (24)** and **alternarlactones B (25)**. These compounds exhibited anti-parasitic activity against *Leishmania donovani* and *Plasmodium falciparum* (Shi et al. 2019). Two perylenequinone class of compounds, namely **3,6a,9,10-tetrahydroxy-7,8-epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene (26)** and **3,6a,7,10-tetrahydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydroperylene (altertoxin I) (20)**, have been isolated from *Alternaria* sp. DC401 existing as an endophyte in *Pinus ponderosa.* Both these perylenequinone compounds have exhibited anti-leishmanial activity with an IC_{50} and IC_{90} values 3.14 and 14.69 mg/mL, respectively, against *Leishmania donovani*. However, *L. donovani* exhibited a higher susceptibility to Altertoxin I with an IC_{50} of 1.4 mg/mL and IC_{90} of 5.754 mg/mL. This also showed antimalarial activity against chloroquine-resistant and -sensitive clones of *Plasmodium falciparum* with IC_{50} of 2.459 and 3.164 mg/mL (Idris et al. 2015). Thus, possibilities exist where bioactive agents of endophytic *Alternaria* species could be exploited as anti-parasitic agents directly or indirectly.

1.2.4 Cytotoxic Agents

Cancer is a generic term given to a set of diseases which can affect any part of the human body. They generally result due to formation of abnormal cells which exhibit uncontrolled growth and proliferation. As these abnormal cells have higher demand for energy due to their continuous proliferative tendency, they invade in other parts of the body thereby affecting the organs and organ systems. This eventually leads to failure of the organ systems and proves fatal. Today, cancer is the second leading cause of global deaths with about 9.6 million deaths registered in 2018.

One of the major strategies to prevent the spread of these abnormally proliferating cells is chemotherapy. Chemotherapy entails the use of a chemical compound of natural or synthetic origin which eventually kills the abnormal cell to prevent further proliferation. This action of the therapeutic chemical is referred to as cytotoxicity. However, challenges do exist in the treatment of cancer. The chemotherapeutic agents used do not have a mechanism of differentiating between healthy and abnormal cells which results in deleterious side effects. Apart from the deleterious side effects due to chemotherapy, the other major challenge is the cost of chemotherapy as well as refractory behaviour of the cancerous cells. Hence, natural product chemists, medicinal chemists and cancer biologists are in a continuous foray to explore novel direct acting new chemical entities from biological matrices which have a directed action on the target cell with minimal side effects. Endophytic fungi have been in centre stage for production of plant-based anticancer agents ever since the discovery of *Taxomyces andreanae* from *Taxus brevifolia* which produced taxol

in the free fermenting conditions (Stierle et al. 1995). Since then a number of endophytic fungi have been explored, documented which have the inherent potential to produce anticancer phytomedicines such as camptothecin, podophyllotoxin, vincristine and vinblastine via fermentative route (Aly et al. 2010; Kharwar et al. 2011; Rana et al. 2020). As endophytic fungi have been recognized as a treasure trove of new chemical entities with a spectrum of bioactivities, it becomes relevant to explore their cytotoxic effects using in vitro assays. *Alternaria alternata* existing as an endophyte in *Paeonia lactiflora* was found to produce **alternate C (27)** which exhibited cytotoxic activities against MDA-MB-231 and MCF-7 cell lines with an IC_{50} of 20.1 and 32.2 μM, respectively (Wang et al. 2019). Bianthraquinone derivatives, i.e. alterporriol family of compounds were first reported from *Alternaria porri*. An isolate, *Alternaria* sp. Z69-6B existing as an endophyte in fruit of a mangrove plant *Aegiceras corniculatum*, from China, has been found to produce three new bianthraquinone derivatives named as alterporriol K, alterporriol L and alterporriol M. **Alterporril K (28)** and **alterporriol L (29)** exhibited a moderate cytotoxic activity towards MDA-MB-435 and MCF-7 cells with an IC_{50} values in range of 13.1–29.1 μM (Huang et al. 2011). **Xanalteric acid I (30)** and **Xanalteric acid II (31)** were isolated from the solid rice culture of *Alternaria* sp. existing as a leaf endophyte in *Sonneratia alba* and exhibit a moderate activity against L5178Y cells at a concentration of 10 μg/mL (Kjer et al. 2009). Crude ethyl acetate extract of *Alternaria phargmospora*, an endophyte isolated from leaves of *Vinca rosea*, yielded **5-butyl-6-(hydroxymethyl)-4-methoxy-2H-pyran-2-one (32)** and **4-methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one (33)** which exhibited appreciable anti-leukaemic activity against HL60 cells with an IC_{50} of 2.2 and 0.9 μM, respectively. The activity of these compounds was also found against the immortalized myelogenous leukaemia cell lines K562 with an IC_{50} of 4.5 and 1.5 μM, respectively (Metwaly et al. 2014). **Alternariol (4)**, **altenusin (5)** and **altertoxin II (21)** have also been isolated from the culture broth of endophytic *Alternaria* sp. EK10.4.5W existing in the seeds of *Ziziphusjujuba.* Alternariol, Altenusin and Altertoxin II have exhibited a potential cytotoxic activity against mouse lymphoma cell lines L5178Y with an IC_{50} of 1.7, 6.8 and 6.2 μM which was comparable to the positive control kahalalide F having an IC_{50} of 4.3 μM (Orfali et al. 2017). *Alternaria* sp. R6, a mangrove plant endophytic fungus has been found to produce derivatives of resveratrol—**resveratroaldehyde A (34)**, **resveratroaldehyde B (35)** and **resveratroaldehyde C (36)**. Resveratroaldehyde A and C have exhibited an in vitro broad-spectrum inhibitory activity against three human cancer cell lines, MDA-MB-435, human liver Hep G2 and human colon HCT-116 by MTT assay with IC_{50} <50 μM. However, resveratroaldehye A and resveratroaldehyde B exhibited potential activity against the cell lines MDA-MB-435 and HCT-116 with an IC_{50} <10 μM (Wang et al. 2014a). **Tenuazonic acid (37)** and **Vivotoxin II (38)** were for the first time reported from the endophytic fungus *Alternaria* sp. 28, from the bark of medicinal plant *Ginkgo biloba*. Both these compounds exhibited significant cytotoxic effect in the brine shrimp assay with mortality rate of 73.6% and 68.9%, respectively, at a concentration of 10 μg/mL (Quin et al. 2009).

1.2.5 Enzyme Inhibitors

One of the most sought out targets for drug development are enzymes. All the metabolic processes occurring in humans are driven by the catalytic power of the enzymes. They animate life. Despite the fact that enzymes are indispensable for existence of life, sometimes alterations in their expression levels or activity may lead to diseases.

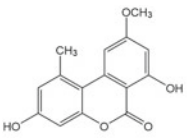
A significant percentage of available oral therapeutic agents used clinically today inhibit enzymes. Thus, enzyme inhibitors form an important drug class (Copeland et al. 2007). Enzyme inhibitors from endophytic fungi are also being explored for developing new drugs. Xanthine oxidase (XO) is an important enzyme involved in metabolism of purines and converting them into uric acid which is eventually excreted in urine. However, hyperuricemia results when the uric acid concentration in blood serum goes beyond 6–7 mg/dL. More recently XO has also been implicated in oxidative stress during a stroke or a heart attack. Hence XO inhibitors are playing a critical role in combating oxidative stress and hyperuricemia (Kapoor and Saxena 2014). **Alternariol (4)** from *Alternaria brassisicola* ML-P08 exhibits a potential XO inhibitory activity with an IC_{50} of 15.5 μM (Gu 2009). Diabetes is a chronic metabolic disorder when the pancreas does not produce enough insulin, or the insulin is not effectively used in controlling the blood sugar level. There are several enzymes which convert the complex carbohydrates into glucose in our body during the process of digestion. Thus, these enzymes play a critical role in the input of glucose in blood. However, if these enzymes are inhibited, the breakdown of complex carbohydrates would drastically reduce and help in the management of blood glucose levels. Two α-glucosidase inhibiting compounds, **prenylcephalochromin A (39)** and **prenylcephalochromin B (40)**, were isolated from *Alternaria* sp. ZG22, symbiotically residing in *Dasymaschalon rostratum*. Prenylcephalochromin A exhibited an IC_{50} of 2.9 mM while for prenylcephalochromin B the value was 2.8 mM for α-glucosidase inhibition when compared to the positive control acarbose (a clinically approved drug) which exhibited an IC_{50} value of 2.3 mM (Song et al. 2019a). Another anti-diabetic agent reported was **2,4,6-triphenylaniline (41)** which was isolated from *Alternaria longipes* strain VITN14G existing as a mutualistic symbiont in *Avicenna officinalis*, a mangrove plant. Both α-amylase and β-glucosidase inhibitory activities were exhibited by 2,4,6-triphenylaniline with an IC_{50} value of 27.05 and 39.02 μg/mL, respectively (Ranganathan and Mahalingam 2019). Hence these compounds hold a promise to be developed into anti-diabetic agents for blood-glucose-level management.

Similarly, for the treatment of Alzheimer's dementia (AD), acetylcholine esterase is a suitable enzyme target. Due to presence/overproduction of this enzyme, the acetylcholine levels are drastically reduced in AD. Thus, inhibiting this enzyme would help in restoring the acetylcholine levels in brain, hence acetylcholine esterase is a promising drug target (Guizior et al. 2015). Aromatic polyketide dimer **bialternacin E (42)** has been isolated from *Alternaria* sp. NF2128 which exists an endophyte in Chinese medicinal plant *Maianthemum bifolium* and found to inhibit

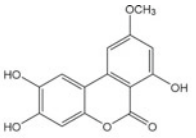
acetylcholine esterase with an IC_{50} of 15.5 μM (Yang et al. 2019a). *Alternaria alternata* existing as an endophyte in *Catharanthus roseus* has been found to produce **altenuene (43)** (Fig. 1.1) which exhibited a significant butyrylcholine esterase inhibitory activity and holds promise for therapeutic development for AD (Bhagat et al. 2016).

1.2.6 Miscellaneous Pharmacological Activities

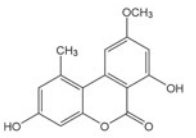
Endophytic *Alternaria* species have also been reported for other pharmacological actions such as anti-platelet/anti-thrombin activity and radical scavenging activity. Some endophytic isolates of *Alternaria* have been reported to produce high titres of known polyfunctional compounds such as resveratrol, swainsonine and taxol. *Alternaria alternata* MT-47, an endophyte of *Huperzia serrata* (Chinese club moss), produces four secondary metabolites, viz. **alternatin D (44)**, **alternariol (4)**, **(−)-alternarlactam (45)** and **stemphyperylenol (46)**, which exhibit the potential to be developed into anti-platelet and anti-coagulants by inhibiting the release of ATP from thrombin-activated platelets (Yang et al. 2019b). *Alternaria* sp. R6 was isolated as an endophyte from the root of *Myoporum bontioides* and produced two new compounds, viz. **(±)-(4R*,5S*,6S*)-3-amino-4,5,6-trihydroxy-2-methoxy-5-methyl-2-cyclohexen-1-one (47)** and **(±)-(4S*,5S*)-2,4,5-trihydroxy-3-methoxy-4-methoxycarbonyl-5-methyl-2-cyclopenten1-one (48)**. Both of these compounds exhibited a very potent ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] radical scavenging activity (Wang et al. 2014b). More recently, altenusin has been identified as an agonist of farnesoid X receptor (FXR). FXRs are responsible for maintaining homeostasis of bile acids, glucose and lipids. Hence FXR agonists play an important role in treatment of non-alcoholic fatty liver disease (NAFLD). NAFLD is a chronic liver disease and is responsible for type 2 diabetes and obesity globally. The EC_{50} values of altenusin to act as a novel agonist for treatment of NAFLD is 3.2 ± 0.2 μM. Hence, altenusin represents the first is class of non-steroidal FXR agonist with a promise to treat NAFLD and associated metabolic syndrome (Zheng et al. 2017). *Alternaria oxytropis* exists as an endophyte in Locoweed (*Oxytropis lambertii*) and produces **Swainsonine (49)** which already possesses multiple biological activities such as anti-viral, antibacterial and anti-tumour (Song et al. 2019b). Similarly, *Alternaria tenuissima* TER995 existing as an endophyte in the bark of *Terminalia arjuna* was recognized as one of the highest **Taxol (Paclitaxel) (50)** (Fig. 1.1) producing strains, i.e. 124.32 μg/mL, reported till date (Ismaiel et al. 2017). *Alternaria* sp. MG1, an endophyte of *Vitis vinifera* L. cv. *Merlot*, was found to produce **resveratrol[3,5,4-trihydroxy stilbene] (51)** (Shi et al. 2012). Resveratrol is a novel molecule with multiple bioactivities involved in using human diseases with particular reference to cancer, cardiovascular diseases and ischaemic injuries (Saxena and Srivastava 2014).



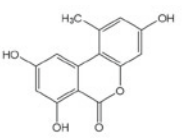
(1)
Alternariol-9-methyl ether



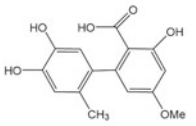
(2)
Altenuisol



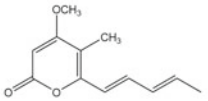
(3)
4-hydroxy alternariol-9-methyl ether



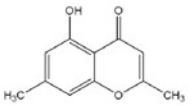
(4)
Alternariol



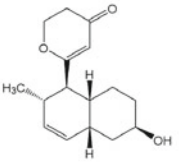
(5)
Altenusin



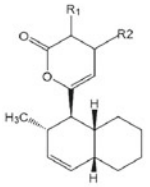
(6)
Herbarin A



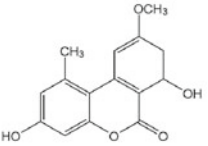
(7)
Alterchromone A



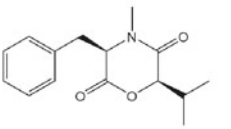
(8)
Solanopyrone P



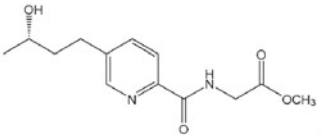
(9) Solanopyrone A [R_1= CHO, R_2= OCH_3]
(10) Solanopyrone C [R_1= CHO, R_2=NH-CH_2-CH_2-OH]



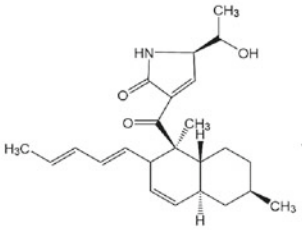
(11)
Alternariol methyl ether



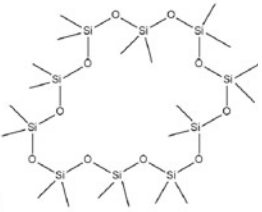
(12)
(3R, 6R)-3-benzyl-6-isopropyl-4-methylmorpholine-2,5-dione



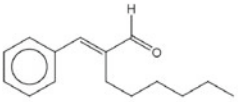
(13)
Atransfusarin



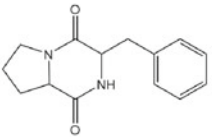
(14)
Altersetin



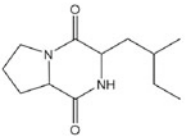
(15)
Cyclodecasiloxane



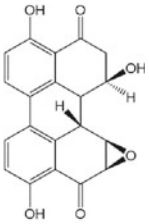
(16)
Cyclodecasiloxane



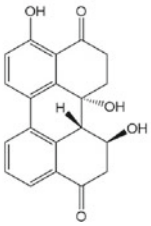
(17)
3-benzylhexahydropyrrolo-[1,2-α] pyrazine-1,4-dione



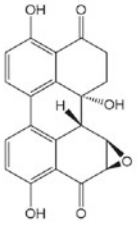
(18)
3-isobutylhexahydropyrrolo-[1,2-α] pyrazine-1,4-dione



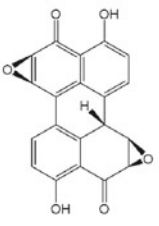
(19)
Altertoxin V



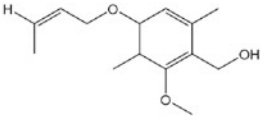
(20)
Altertoxin I



(21)
Altertoxin II

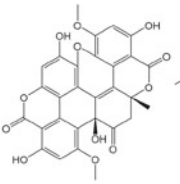


(22)
Altertoxin III

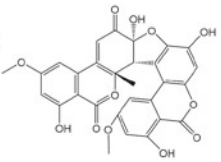


(23)
7-dehydroxyl-zinniol

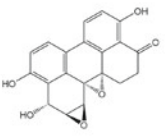
Fig. 1.1 Bioactive secondary metabolites produced by endophytic *Alternaria* species



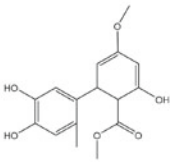
(24)
(±)-Alternarlactone A



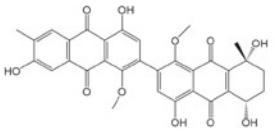
(25)
(±)-Alternarlactone B



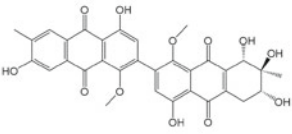
(26)
3,6a,9,10-tetrahydroxy-7, 8- epoxy-4-oxo-4,5,6,6a,6b,7,8,9-octahydroperylene



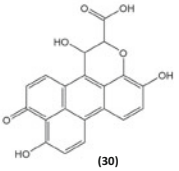
(27)
Alternate C



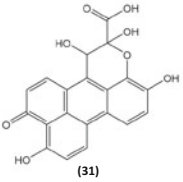
(28)
Alterporriol K



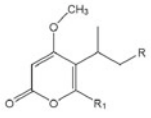
(29)
Alterporriol L



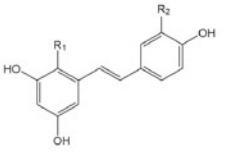
(30)
Xanalteric Acid I



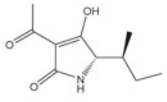
(31)
Xanalteric Acid II



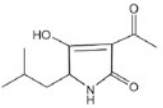
(32) 5-butyl-6-(hydroxymethyl)-4-methoxy-2H-pyran-2-one [R=CH_2CH_3, R_1=CH_2OH]
(33) 4-methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one [R=$COCH_3$, R_1=CH_3]



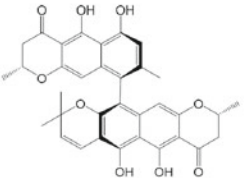
(34) Resveratroaldehyde A [R_1=H, R_2=CHO]
(35) Resveratroaldehyde B [R_1=CHO, R_2=CHO]
(36) Resveratroaldehyde C[R_1=CHO, R_2=H]



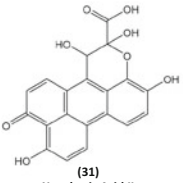
(37)
Tenuazonic acid



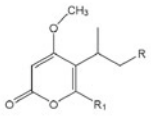
(38)
Vivotoxin II



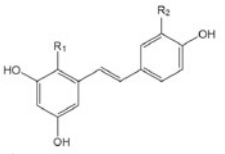
(39)
Prenylcephalochromin A



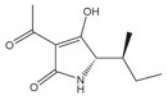
(31)
Xanalteric Acid II



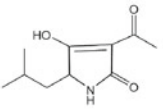
(32) 5-butyl-6-(hydroxymethyl)-4-methoxy-2H-pyran-2-one [R=CH_2CH_3, R_1=CH_2OH]
(33) 4-methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one [R=$COCH_3$, R_1=CH_3]



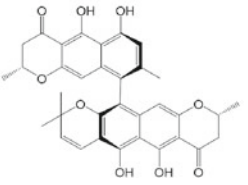
(34) Resveratroaldehyde A [R_1=H, R_2=CHO]
(35) Resveratroaldehyde B [R_1=CHO, R_2=CHO]
(36) Resveratroaldehyde C[R_1=CHO, R_2=H]



(37)
Tenuazonic acid



(38)
Vivotoxin II



(39)
Prenylcephalochromin A

Fig. 1.1 (continued)

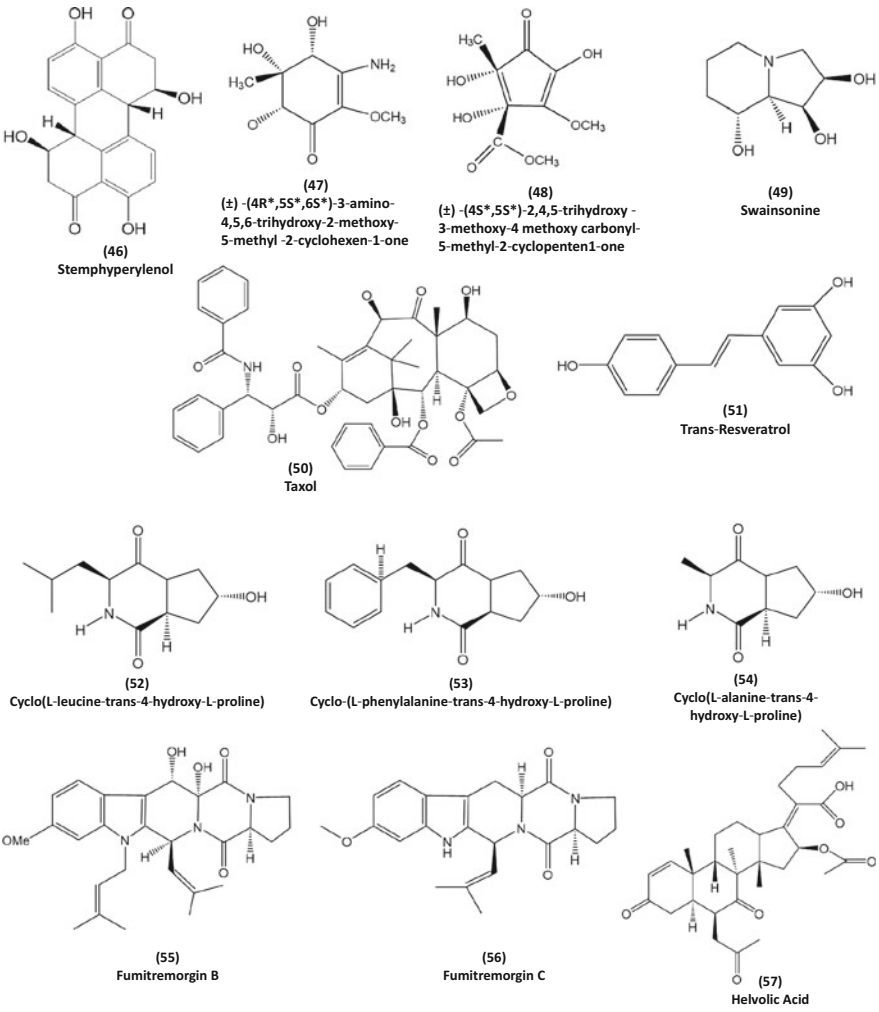


Fig. 1.1 (continued)

1.3 Agrochemically Active Metabolites

Global agriculture is undergoing a renaissance with adoption of environmentally benign and sustainable technologies, directed towards drastic reduction in input of xenobiotics of pure synthetic origin. One of the major aspects of making the world food secure is through reduction in the pre- and post-harvest losses drastically apart from carrying out crop improvement programmes. Agrochemicals thus have played a significant role as pesticides comprising of herbicides, fungicides, insecticides, nematicides and rodenticides. However, their indiscriminate use has led to severe

ecological and health implications. This demands for alternatives which could be less toxic or benign to the chemical alternatives being used currently.

Plant–microbe interactions could possibly provide safer alternatives safer/benign chemicals as compared to synthetic chemical compounds. As endophyte co-evolve with their host and also help in combating with biotic and abiotic stressors, they could possibly be the sources of biorational compounds produced as a result of this interaction and possibly be developed into next generation of safer agrochemicals (Segaran and Sathiavelu 2019). The members of the genus *Alternaria* have been responsible for pathogenesis of major economically important crops with their relationship ranging from a saprobe to an endophyte (Woudenberg et al. 2015). *Alternaria* species produce secondary metabolites which can be classified as phytotoxins and mycotoxins while they are interacting with the host in the capacity of a pathogen. The phytotoxins produced by *Alternaria* sp. can be classified as host-specific and non-host-specific (Meena and Samal 2019). Many of these phyto/mycotoxins have been developed into biorational agrochemicals (Saxena and Pandey 2001;Copping and Duke 2007; Saxena 2014). However, exploiting the relationship of this genus, as an endophyte, is relatively a newer aspect but gaining threshold with the exploration of bioactivities of the signal molecules involved during the endophytic relationship.

Endophytic *Alternaria* J46 was isolated from the stem of medicinal plant *Platycladus orientalis*. The culture filtrate of *Alternaria* J46 exhibited a significant inhibition in seed germination of large crabgrass, bromegrass and barnyard grass. Further purification of the extract led to the isolation of **tenuazonic acid (37), vivotoxin II (38)** and **cyclo(L-leucine-trans-4-hydroxy-L-proline) (52)** (Fig. 1.1). Tenuazonic acid holds a promise towards the development of a biobased herbicide for controlling weeds in cotton crop. In a pot trial tenuazonic acid exhibited an EC_{50} between 119 and 795 μg/mL against 14 weeds but did not have any toxicity or damage to cotton (*Gossypium hirsutum*), Tobacco (*Nicotianatabacum*) and Asian Copperleaf (*Acalyphaaustralis*), even at a concentration of 1000 μg/mL. The field trial of tenuazonic acid controlled the two weeds, *Amaranthusretroflexus* and *Digitariasanguinalis* without affecting the Cotton (Hao et al. 2015). **Alternariol-9-methyl ether (1)** which was isolated from endophytic *Alternaria* sp. Samif01 was also found to exhibit anti-nematode activity with IC_{50} values of 98.17 and 74.62 μg/mL, against *Bursaphelenchus xylophilus* and *Caenorhabditis elegans* respectively (Jin et al. 2017). *Plasmopara viticola* Berl. et De Toni is one of the most destructive pathogens which causes grapevine downy mildew disease in the tropics. Antifungal compounds produced by endophytic fungi can be used as biorational pesticides replacing synthetic chemical-based fungicides (Musetti et al. 2007). Diketopiperazines are dipeptides which have been previously implicated with antifungal activity. Recently three diketopiperazines have been reported from an endophytic *Alternaria alternata* (Fr.) Keissel which reportedly have been found to control this fungal pathogen in green house trial. These promising compounds are **cyclo-(L-phenylalanine-trans-4-hydroxy-L-proline) (53)**, **cyclo(L-leucine-trans-4-hydroxy-L-proline) (53)** and **cyclo(L-alanine-trans-4-hydroxy-L-proline) (54)** (Musetti et al. 2007). These further warrants field trials for their development into

commercial biorational fungicide to prevent grapevine from the infection of *Plasmopara viticola*.

Another isolate of *Alternaria alternata* existing as an endophyte in *Azadirchta indica* A. Juss. was found to have insecticidal activity when its ethyl acetate extract was tested against *Sodoptera litura*, a phytophagous insect causing economic losses to crops globally (Kaur et al. 2013). Similarly chloroform extract of *Alternaria* sp. Cas1, an endophytic isolate of *Ricinus communis*, also exhibits lethal effects on *S. litura* (Singh et al. 2012). Endophytic *Alternaria* species are being explored for their anti-phyto pathogenic activity for possible use as plant antibiotics or biofungicide. *Alternaria* sp. FL25 exists as an endophyte in the leaves of *Ficuscarica* and produced f**umitremorgin B (55)**, **fumitremorgin C (56)** and **helvolicacid (57)** which was isolated from the ethyl acetate extract of the culture filtrate. All the three compounds exhibited potential antifungal activity against the test panel of phytopathogenic fungi by in vitro microbroth dilution assay. Fumitremorgin B and C exhibited a potent fungicidal activity with an MIC in the range of 3.13–25 μg/mL against the test panel fungi, while helvolic acid exhibited a fungistatic activity with an MIC range of 1.56–12.5 μg/mL (Chengliang and Yangmin 2010). Thus, now a trend has sent in where endophytic *Alternaria* species are being explored for bioactive compounds with applications in agriculture.

1.4 Future Perspectives

With an upsurge of global disease burden, comprising of new and emerging infections as well as metabolic disorders, there is an increasing demand for newer chemical scaffolds in the drug development pipeline. Studies of fungal endophytes in the last two decades have clearly proven that endophytic fungi serve as excellent biomatrices for novel and untapped chemical entities with potential for new drug development. The variable ecological relationship of these endophytic fungi also makes them amenable sources of a variety of agroactive agents which could replace the currently used harmful synthetic agrochemicals. *Alternaria* species has a wide range of occurrence in nature, i.e. from a saprobe, a facultative pathogen to an endophyte. Members of *Alternaria* genus existing as saprobes and as pathogens have been widely explored for their secondary metabolite which is recognized as mycotoxins and phytotoxins. These secondary metabolites could be possibly exploited directly as crop protection agents or could provide leads for developing safer agrochemicals. With increasing number of studies on endophytic fungal diversity, it is expected that newer isolates of *Alternaria* sp. having endophytic existence shall be identified which shall open up immense opportunities for their exploitation as sources of plant-based medicinals/phytochemicals as well as of novel chemical entities which would possess potential for pharmaceutical and agrochemical development.

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