INTRODUCTION

The most promising source of ‘first-in-class’ drugs has been derived from the natural source like plants and microorganisms which are the most productive and consistent sources (Newman and Cragg, 2007). The most productive and consistent sources (Newman and Cragg, 2007). The endophytic fungi based discovery of pharmacological agents has drawn the attention of scientific community remarkably (Strobel and Daisy, 2003). The untapped potential of these endophytic fungi has not been used adequately due to less exploration. Schulz (1993) observed that these organisms have made their presence in almost all plant tissues studied so far without showing any symptoms. They play a role in plant growth, physiological activities and enable them to cope with biotic stresses (Carroll, 1988; Hallmann and Sikora, 1996; Azevedo et al., 2000; Azevedo and Araujo, 2007). They also promote the growth of plants by enhancing their nitrogen fixing capabilities (Verma et al., 2001; Rahman and Saiga, 2005). These fungi are a reservoir of pharmacologically important bioactive secondary metabolites (Deshmukh and Verekar, 2009; Kharwar et al., 2011; Deshmukh et al., 2015; Deshmukh et al., 2017) and would be helpful in developing new drugs of clinical importance and management of plant disease (Murray et al., 1992; Kusari et al., 2013; Kumar et al., 2013; Chowdhary and Kaushik, 2016).

Smith et al. (2008) reported that endophytes are present in one or more number in nearly 300,000 species of terrestrial plants. Strobel and Daisy (2003) rightly remarked that endophytes are a goldmine for a diverse class of bioactive metabolites. Pestalotiopsis sp. can be considered as the “E. coli of the rain forests”. Considering the range of metabolites obtained from P. microspora, it was called as a “microbial factory”. A range of compounds with diverse structures has been described from these fungi that include ambuic acid, cryptocandin, taxol, tolraemic acid, subglutinol A and B and several others.

Newer strains and novel metabolites derived from endophytes have great potential to inhibit or eradicate variety of infections caused by pathogens like fungi, bacteria, viruses and protozoa that infect animals and humans (Tan and Zau, 2001). Endophytes in suspension culture produce secondary metabolites during growth. Parameters like temperature, media composition and aeration rate are known to affect the quantity as well as the variety of compounds produced (Strobel et al., 2004), like steroids, xanthones, phenols, isocoumarins, perylene derivatives, quinines, furandiones, terpenoids, depsipeptides and cytochalasins (Zhang et al., 2006; Gunatilaka, 2006; Kharwar et al., 2011; Deshmukh et al., 2015; Bedi et al., 2018). Dreyfuss and Chapela (1994) estimated that approximately one million endophytes exist in nature. The discovery of such a large number of novel bioactive metabolites is possible because fungal endophytes display genetic diversity that can be exploited for the production of bioactive compounds (Gunatilaka, 2006). Some of the metabolites isolated from endophytic fungi at Basic Research Centre of Hoechst Marion Roussel Limited and Research Centre of Piramal Enterprises, Mumbai are listed in Table 1 and Figure 1 and 2.

Metabolites with Anticancer activity:

PM181110 (1) (Fig. 1) a novel depsipeptide, was isolated from Phomopsis glabrae (PM0509732), an endophytic fungus harboring within the leaves of Pongamia pinnata (L.)
Pierre, collected from Karnala Bird Sanctuary of Maharashtra. The compound displayed the mean IC$_{50}$ value of 0.089 μM towards a set of 40 human cancer cell lines and ex vivo efficacy (mean IC$_{50}$= 0.245 μM) towards 24 human tumor xenografts (Vereket et al., 2014).

Ophiobolin A (2) (Fig.1) was derived from the endophytic fungus Bipolaris setariae of the Parthenium hysteronphorus collected from Mumbai, India. It showed IC$_{50}$ of 0.4-4.3 μM and restricted cell growth of haematological cancer. In contrast, IC$_{50}$ of 20.9 μM was recorded for normal cells. Ophiobolin A was also observed to check the phosphorylation of S6, ERK and RB, the effector proteins of PI3K/mTOR, Ras/Raf/ERK and CDK/RB pathways, respectively. It checked the progress of cell cycle and induced cell death in MDAMB-231 cancer cell line with simultaneous inhibition of pS6, pAKT, pERK, pRB and cyclin D1 proteins. The anti-cancerous property was as a result of simultaneous inhibition of pS6, pERK and RB, the effector proteins of different cancer regulatory pathways like PI3K/mTOR, Ras/Raf/ERK and CDK/RB pathways (Bhatia et al., 2016).

Altersolanol A (3) (Fig. 1), a derivative of anthraquinone was obtained from Phomopsis sp. isolated from Nycanthes arbor-tristis which was collected from Mumbai. Altersolanol A showed activity against 34 human cancer line in vitro with mean IC$_{50}$ (IC$_{50}$) values of 0.005 μg /mL (0.024 μg /ml), respectively (Mishra et al., 2015). The cellular activity of Altersolanol A has been studied in detail, which inhibits kinase that follows caspase-dependent pathways for inducing apoptosis. Altersolanol A inhibited a variety of kinases which suggested that the kinase inhibition might be the mode behind cytotoxic activity (Debbab et al., 2009). Altersolanol A blocks NF-B transcriptional activity (Teiten et al., 2013).

Heptelic acid (4) (Fig. 1) is a sesquiterpene lactone, also known as Koningic acid (KA) was derived from Trichoderma sp. of Azadirachta indica. The compound exhibited moderate activities towards T47D, SKOV-3, KM-12, NAMALWA, MDAMB-231, NCI-H460, HOP-62, Colo-205, TK10, Ovcar-3, BXPC3, HL-60, WM-266-4, DU145, HCT-116, A549 cancer cell lines with IC$_{50}$ < 1 μg/mL (Rahier et al., 2015). Early on, KA was found as a glycolytic pathway inhibitor that inhibited ATP synthesis, thus inhibiting glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which is involved in the conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate (Endo et al., 1985). KA permanently blocks GAPDH via blocking the active site of the enzyme with a cysteine residue (Sakai et al., 1988, 1990, 1991; Kato et al., 1992; Cane and Sohng, 1994).

Sclerotiorin (5) (Fig. 1) was isolated from Cephalotheca faveolata, occurring within the petiole of Eugenia jambolana. It showed cytotoxicity against HCT-116, H460, ACHN, Panc-1 and Calu-1 cell lines with the IC$_{50}$ value of 0.63, 1.6 1.2, 1.6 and 2.1μM, respectively. While in MCF10A, it showed an IC$_{50}$ >10μM. Sclerotiorin induced apoptosis in HCT116 cells via the triggering of BAX and downregulation of BCL-2 that results in stimulation of cleaved caspase-3 thereby causing the death of cancerous cells (Giridharan et al., 2012).

An unidentified fungus (PM0651480) isolated from the leaves of Mimosops elengi yielded Ergoflavin (6) (Fig. 1). Ergoflavin showed IC$_{50}$ values of 1.9 and 1.2 μM for TNF-α and IL-6, respectively. The compound showed IC$_{50}$ value of 1.2, 4.0, 2.4, 8.0, 1.5 μM against ACHN, H460, Panc1, HCT16, Calu1 cancer cell lines, respectively (Deshmukh et al., 2009).

Secalonic acid D (SAD) (7) (Fig. 1) was produced from Aspergillus aculeatus, harboring within a marine sponge Cinachyra cavernosa collected from Mandapam, Tamilnadu. It was cytotoxic against Panc-1, H-460, ACHN, Calu-1, HCT-116 and WI-38 cell lines with IC$_{50}$ value of 0.2,0.2, 0.19, 0.2, 0.2 and 4.9 μg/mL (Deshmukh, unpublished data). Previously it was isolated from Paeclomyces sp. (tree 17), a mangrove endophytic fungus that showed cytotoxicity against KB cells with an IC$_{50}$ <1 μg/mL and checked activity of topo isomerase I with an IC$_{50}$ of 0.16 μM (Guo et al., 2007). SAD was earlier isolated from mangrove-derived endophytic fungus No. ZSU44 and exhibited cytotoxicity against HL60 and K562 cells, with IC$_{50}$ values of 0.38 and 0.43 μM, respectively. PI assay/Annexin V-FITC and western blot also confirmed its apoptosis inducing activity. It also checked G1 phase progress resulting in cell cycle arrest due to downregulation of c-Myc. The reduction in the activity of c-Myc: cell-cycle arrest occurred by the activation of GSK-3β followed by β-catenin degradation (Zhang et al., 2009). Penicillium oxalicum, isolated from the roots of Catharanthus roseus was observed to produce SAD that possesses potent antitumor activity. SAD was observed to inhibit tumor formation by targeting

Fig. 1. Structures of anticancer metabolites obtained from Endophytic fungi.
<table>
<thead>
<tr>
<th>No.</th>
<th>Endophytic fungal strain</th>
<th>Host plant(s)</th>
<th>Plant part or tissue</th>
<th>Locality of host plants</th>
<th>Isolated metabolite(s)</th>
<th>Biological activity</th>
<th>Tested systems</th>
<th>Activity response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phomopsis glabrae</td>
<td>Pongamia pinnata</td>
<td>Leaf, Karnala Bird Sanctuary, Raigad (MS), India</td>
<td>TM 181110 (1)</td>
<td>Anti-cancer</td>
<td>40 human cancer cell lines</td>
<td>Mean IC&lt;sub&gt;50&lt;/sub&gt; value 0.005 µM</td>
<td>Mean IC&lt;sub&gt;50&lt;/sub&gt; value 0.245 µM</td>
<td>Verkar et al., 2014</td>
</tr>
<tr>
<td>2.</td>
<td>Bipolaris turcica</td>
<td>Parthenium hysterophorus</td>
<td>Leaf, Mumbai, India</td>
<td>Ophiobolism A (2)</td>
<td>Anti-cancer</td>
<td>A2780, PC3, MDA-MB-231, MCF-7, MM1M, RPMI8226, U266H 68 and Jurkat cell lines</td>
<td>Mean IC&lt;sub&gt;50&lt;/sub&gt; value 0.63-3 µM</td>
<td>Bhuta et al., 2016</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Phomopsis sp.</td>
<td>Acanthus indica</td>
<td>Leaves, Mumbai, India</td>
<td>Anticancer</td>
<td>-</td>
<td>Anticancer</td>
<td>-</td>
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<tr>
<td>4.</td>
<td>Trichoderma sp.</td>
<td>Acanthus indica</td>
<td>Leaf, Mumbai, India</td>
<td>Heptelic acid (4)</td>
<td>Anti-cancer</td>
<td>T971, SKOV-3, KM-12, SAMA SAMALWA, MDAMB-231, NCI-H460, HEP-2, Colo-205, TK10, Ovcar-3, B sprinkle, HL-60, WM-266-4, DU145, HCT-116, A-549 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.21 µM, 0.41 µM, 0.48 µM, 0.48 µM, 0.49 µM, 0.51 µM, 0.63 µM, 0.66 µM, 0.69 µM, 0.76 µM, 0.84 µM, 0.89 µM, 0.93 µM, 0.99 µM</td>
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<tr>
<td>5.</td>
<td>Coprinus cinereus</td>
<td>Eugenia jambolana</td>
<td>Leaf, Mumbai, India</td>
<td>Sclerotinin (5)</td>
<td>Anticancer</td>
<td>ACHN, Panc1, Calu-1, HCT-116, and H460 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 1.2 µM, 1.6 µM, 2.1 µM, 2.3 µM, 3 µM, 6.3 µM, 6.6 µM, 7 µM, 10 µM</td>
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<tr>
<td>6.</td>
<td>Unidentified fungus</td>
<td>Acmisopogon elongii</td>
<td>Leaf, Mumbai, India</td>
<td>Ergosterol (6)</td>
<td>Anti- inflammatory activity</td>
<td>Identified TNE-1 and IL-6</td>
<td>-</td>
<td>-</td>
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<td>7.</td>
<td>Aspergillus Aculeata</td>
<td>Marine sponge Cinachyra sp.</td>
<td>Mandapam, Tamilnadu, India</td>
<td>Seclalomic acid D (7)</td>
<td>Anti-cancer</td>
<td>Panc1, H460, ACHN, Calu-1, HCT-116, and WI-38 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.2-0.2 µM, 0.19 µM, 0.2-0.2 µM, 0.2-0.2 µM and 0.9 µg/mL</td>
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<td>8.</td>
<td>Dichomosycys cepitae</td>
<td>Anomum squamosum</td>
<td>Bark, Thane, India</td>
<td>Gliotoxin (8)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT-116, Calu cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.1-0.4 µM</td>
<td>-</td>
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<tr>
<td>9.</td>
<td>Dichomosycys cepitae</td>
<td>Anomum squamosum</td>
<td>Bark, Thane, India</td>
<td>Acetyl derivative of Gliotoxin (9)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT-116, Calu cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.1-0.4 µM</td>
<td>-</td>
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<tr>
<td>10.</td>
<td>Peronospora sp.</td>
<td>Unidentified plant</td>
<td>Tegur, Assam, India</td>
<td>Penicilamin A (10)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu cancer cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.8-1.5 µg/mL</td>
<td>-</td>
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</tr>
<tr>
<td>11.</td>
<td>Peronospora sp.</td>
<td>Unidentified plant</td>
<td>Tegur, Assam, India</td>
<td>Penicilamin B (11)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu cancer cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.8-1.5 µg/mL</td>
<td>-</td>
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<tr>
<td>12.</td>
<td>Humicola fasciculata</td>
<td>Mangifera indica</td>
<td>Mumbai, India</td>
<td>Radicicol (12)</td>
<td>Anticancer</td>
<td>ACHN, Panc1, Calu-1, H460, HCT 116, MCF 10A cell line</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 0.29 µM, 0.45 µM, 0.41 µM, 0.27 µM and 2.7 µM respectively</td>
<td>-</td>
<td></td>
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<tr>
<td>13.</td>
<td>Unidentified fungus</td>
<td>Acacia marina</td>
<td>Thane creek, Mumbai, India</td>
<td>Hostycin (13)</td>
<td>Anticancer</td>
<td>ACHN, Panc1, Calu-1, H460, HCT 116, MCF 10A cell line</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 1-3 µg/mL, in different cancer cell lines</td>
<td>-</td>
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<tr>
<td>14.</td>
<td>Unidentified fungus</td>
<td>Acacia marina</td>
<td>Thane creek, Mumbai, India</td>
<td>Deoxyhostycin (14)</td>
<td>Anticancer</td>
<td>ACHN, Panc1, Calu-1, H460, HCT 116, MCF 10A cell line</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 2-2.5 µg/mL</td>
<td>-</td>
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<tr>
<td>15.</td>
<td>Unidentified fungus</td>
<td>Physalospora angula</td>
<td>Mumbai, India</td>
<td>Triquinone (15)</td>
<td>Anticancer</td>
<td>pERK, pS6</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; value 1.9 µM, 1.9 µM, IC&lt;sub&gt;50&lt;/sub&gt; = 0.14 µM</td>
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<td>16.</td>
<td>Curvularia sp.</td>
<td>Cissus sinensis</td>
<td>Mumbai, India</td>
<td>5-Hydroxycurvulactin (16)</td>
<td>Anticancer</td>
<td>HDERK, pS6 Proteosome pRB</td>
<td>-</td>
<td>-</td>
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</table>
Translating Endophytic Fungal Research Towards Pharmaceutical Applications

Cont. ......

<table>
<thead>
<tr>
<th>Sr. No.</th>
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<th>Activity response</th>
<th>References</th>
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<tr>
<td>17.</td>
<td>Unidentified fungus</td>
<td>Rauvolfia monosperma</td>
<td>India</td>
<td>Radixin (17)</td>
<td>Anticancer</td>
<td>pERK, p56, proteasome, PRb</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 5.5, 8.9, 23.1; &gt;100µM; IC&lt;sub&gt;50&lt;/sub&gt; in the range of 1-3 µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<td>18.</td>
<td>Chaetomium sp.</td>
<td>Phyllanthus sp.</td>
<td>Stem, Thane, India</td>
<td>Chaetoglobosin A (18)</td>
<td>Anticancer</td>
<td>pERK, p56, proteasome, PRb</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; &lt;3 µg/mL, &gt;3µg/mL, &gt;3µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<td>19.</td>
<td>Xylaria juruensis</td>
<td>Nychanthus arborvitosis</td>
<td>Leaf, Mumbai, India</td>
<td>Cytochalasin D (19)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu1 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; in the range of 0.3-1 µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<td>20.</td>
<td>Pencillium sp.</td>
<td>Unidentified plant</td>
<td>Mumbai, India</td>
<td>Mycophenolic Acid (20)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu1 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; between 0.8 to 1.5 µM in different cancer cell lines</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<tr>
<td>21.</td>
<td>Unidentified fungus</td>
<td>Embelia ribes</td>
<td>Mumbai, India</td>
<td>Dichlorodiaportin (21)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu1 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; &lt;1 µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<tr>
<td>22.</td>
<td>Unidentified fungus</td>
<td>Thevetia sp.</td>
<td>Mumbai, India</td>
<td>PR-Toxin (22)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu1 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 0.3-3 µg/mL in different cancer cell lines</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<tr>
<td>23.</td>
<td>Trichoderma sp.</td>
<td>Azadirachta indica</td>
<td>Mumbai, India</td>
<td>Viridiol (23)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu1 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; &lt;1 µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<td>24.</td>
<td>Mycoleptodiscus terrestris</td>
<td>Vallisneria sp.</td>
<td>Mumbai, India</td>
<td>A52068 A (24)</td>
<td>Anticancer</td>
<td>ACHN, H460, Panc1, HCT16, Calu1 cell lines</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 1-3 µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<td>25.</td>
<td>Lepidosaphera nicotiae</td>
<td>Unidentified plant</td>
<td>Rajkot, India</td>
<td>Mutolide (25)</td>
<td>Anti-inflammation</td>
<td>TNF-α and IL-6</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 2.77 and 1.07 µM</td>
<td>Shah et al., 2015</td>
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<tr>
<td>26.</td>
<td>Humicola fuscaatra</td>
<td>Ficus glomerata</td>
<td>Thane, India</td>
<td>Brefeldin A (26)</td>
<td>Antiinflammation</td>
<td>TNF-α and IL-6</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 0.3 and 0.02 µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
</tr>
<tr>
<td>27.</td>
<td>Trichuras sp.</td>
<td>India</td>
<td>Trichurasin A (27)</td>
<td>Antiinflammation</td>
<td>TNF-α and IL-6</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; and 0.3µg/mL, IC&lt;sub&gt;50&lt;/sub&gt; and 0.8µg/mL</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<tr>
<td>28.</td>
<td>Dendryphion nanum</td>
<td>Ficus religiosa</td>
<td>Leaf, India</td>
<td>Herbarin A(29)</td>
<td>Anti-inflammation and Anti-diabetic</td>
<td>TNF-α and IL-6</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 0.60 and 0.60 µM, IC&lt;sub&gt;50&lt;/sub&gt; 0.3 µg/mL, Toxicity &gt; 10 µg/mL</td>
<td>Mishra et al., 2013</td>
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<td>29.</td>
<td>Alternaria longissima</td>
<td>Sphaeranthus sp.</td>
<td>Not reported, India</td>
<td>Ustilagininodin (30)</td>
<td>Antidiabetic</td>
<td>GU A</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; 0.1 µM, Toxicity &gt; 1 µM</td>
<td>(Deshmukh et al. unpublished Data).</td>
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<tr>
<td>30.</td>
<td>Alternaria phaeospermum</td>
<td>Unidentified grass</td>
<td>Not reported, India</td>
<td>Anthrichitin (31)</td>
<td>Anti-fungal</td>
<td>Fungicidal efficiency against Pyricularia oryzae infection of rice and B. cinerea infectionof cucumber at 5000 ppm</td>
<td>75 and 85%</td>
<td>Vijayakumar et al., 1996</td>
</tr>
</tbody>
</table>

angiogenesis in human MCF-7 breast tumor xenografts and human umbilical vascular endothelial cells (HUVEC) by impeding HIF-1α/VEGF interaction. Studies suggested that SAD inhibited cell viability through the Akt/mTOR/p70S6K pathway. The addition of neutralizing VEGF antibodies or by Akt inhibitors perifosine and GSK69069, reversed the antiangiogenic effects of SAD. It exhibited both extrinsic and intrinsic apoptotic properties. It was observed to alter the
amount of G1/S transition phase proteins, thereby leading to cell cycle inhibition. For ascertaining SAD as a potent cancer-specific therapeutic agent, deciphering its mode of action and preclinical evaluation has been recommended (Guru et al., 2015).

Gliotoxin (8) and its acetyl derivative have been (9) (Fig. 1) isolated from endophytic fungus Dichotomomyces epeit residing in Annona squamosa. Gliotoxin displayed IC_{50} values between 0.1-0.4 µM against different cancer cell lines, whereas acetyl derivative of gliotoxin exhibited IC_{50} values in the range of 0.7-1.6 µg/mL in different cancer cell lines (Deshmukh, unpublished data). Gliotoxin was originally isolated from Gliocladium fimbritatum (Johnson, et al., 1943), later on isolated from Aspergillus fumigatus (Glister and Williams, 1944) and Penicillium terikozkowsii (Johnson, et al., 1953). Gliotoxin is a proven cytotoxic fungal-derived metabolite which belongs to epipolythiodioxopiperazine (ETP) class of compounds (Vigushin et al., 2004). The histone methyltransferase activity was inhibited by dimeric ETPs (Iwasa et al., 2010). It was also reported from Penicillium sp. It showed inhibitory activity against HMT G9a with IC_{50} value 2.6 µM and cytotoxicity on P388 Cell lines with the IC_{50} value of 0.056 µM (Sun et al., 2012).

Cytotoxic compounds Periconiasin A (10) and B (11) (Fig. 1) were identified from Periconia sp. isolated from an unidentified plant collected from Tejpur, Assam. Periconiasins A exhibited activity against ACHN, H460, Panc1, HCT16, Calu1 cancer cell lines with IC_{50} in the range of 0.8 to 1.5 µg/mL. Similarly, periconiasin B exhibited cytotoxicity against ACHN, H460, Panc1, HCT16, Calu1 cancer cell lines with IC_{50} in the range of IC_{50} 1-3 µg/mL in different cancer cell lines (Deshmukh, unpublished data). Both the compounds were previously isolated from Periconia sp. F-31 obtained from Annona muricata (Deshmukh, unpublished data). Periconiasin A selectively inhibited HCT-8 and BGC-823 cell lines with IC_{50} values of 0.9 and 2.1 µM, respectively. Periconiasin B displayed selective inhibition of growth in BGC-823, Bel-7402 and HCT-8 with IC_{50} values of 9.4, 5.1 and 0.8µM, respectively (Zhang et al., 2013).

Radicicol (12) (Fig. 1) was isolated from Humicola fuscaotra of Mangifera indica collected from Mulund, Mumbai. Radicicol exhibited cytotoxicity against ACHN, Panc1, Calu1, H460, HCT116, MCF 10A cell lines with IC_{50} values of 0.29, 0.45, 0.41, 0.27, 0.29 and 2.7 µM, respectively. Based on the efficacy of pancreatic cancer cells, radicicol was further profiled for molecular signature in Panc-1 cells using high content screening tools. The results revealed that, radicicol up-regulates p21 and p53 significantly and also showed notable downregulation of NFKB and STAT3 protein levels at 6 hrs in Panc-1 cells. In addition, the levels of pAKT^{S473}, pRB^{S780} were significantly downregulated in Panc-1 cells. Radicicol also showed upregulation of caspase3 when compared to untreated control cells (Deshmukh, unpublished data). Radicicol is an antifungal macrolactone antibiotic that inhibits protein tyrosine kinase. Radicicol induces the differentiation of HL-60 cells into macrophages, blocking cell cycle at G1 and G2. It suppresses NIH 3T3 cell transformation by diverse oncogenes such as src, ras, and mos and also suppresses the expression of mitogen-inducible cyclooxygenase-2. As a cell differentiation modulator, radicicol has anti-angiogenic activity in vivo, inhibiting the proliferation of and plasmogen activator production by vascular endothelial cells (Kwon et al., 1992; Oikawa et al., 1993; Zhao et al., 1995; Shimada et al., 1995; Channugam et al., 1995; Pillay et al., 1996; Schulte et al., 1999; Wu et al., 2013).

An unidentified mangrove endophytic fungus of Avicennia marina collected from Thane creek yielded Bostrycin (13) (Fig. 1) and Deoxybostrycin (14) (Fig. 1). Bostrycin exhibited cytotoxicity against ACHN, Panc1, Calu1, H460, HCT116, MCF 10A cell lines with IC_{50} in the range of 1.2-3.5µg/mL in different cancer cell lines. Similarly deoxybostrycin exhibited cytotoxicity against ACHN, Panc1, Calu1, H460, HCT116, MCF 10A cell lines with IC_{50} in the range of 2.2-5.7µg/mL in different cancer cell lines (Deshmukh, unpublished data). Mangrove fungus No. 1403 also yielded bostrycin. Saccharomyces cerevisiae was used as a model wherein at G1 phase cell cycle was ceased, finally leading to time- and dose-dependent cell death by inhibiting cell proliferation using bostrycin. Bostrycin also leads to mitochondrial destruction by decreasing mitochondrial membrane electric potential, during apoptosis. The cell death was induced in YCA1 null yeast strain but was partially rescued in A1F1 null mutant both in respiratory media and fermentative. This strongly suggests that cell death is induced by mitochondria facilitated but caspase-independent pathway (Xu et al., 2010). Both bostrycin and deoxybostrycin were also obtained from marine fungus Nigrospora sp. (No. 1403) occurring in Kandelia candel wood. Bostrycin showed cytotoxicity with IC_{50} values of 2.64, 5.39, 5.90, 4.19, 6.13, and 6.68 µM against A549, Hep-2, Hep G2, KB, MCF-7, and Adr cell lines respectively (Xia et al., 2011).

Some other compound viz. Triticone (15) (Fig. 1) was extracted from unidentified fungus of Physalia angula. Triticone displayed IC_{50} for pERK = 1.9 µM, pS6 = 1.9 µM and cytotoxicity in different cancer cell lines having IC_{50} of ~0.1µg/mL (Deshmukh et al., unpublished data). Previously triticone with phytotoxic properties was isolated from plant pathogenic fungus Drechslera tritici which also inhibits enzymes with the functional group carrying SH as a component of the active site, e.g. the protease-ficin (Kenfield et al., 1988; Sugawara et al., 1988).

3ß-Dehydrocurvularin (16) (Fig. 1) was isolated from Curvularia sp. residing in Citrus sinensis. The compound exhibited IC_{50} for pERK = 30; pS6 = 7.4; Proteasome = 29.3; pRb > 30 µg/mL; and cytotoxicity in different cancer cell lines having IC_{50} of ~0.1µg/mL (Deshmukh et al., unpublished data). Previously it was reported from Alternaria macrospora as a phytotoxic compound (Robeson et al., 1985).

Radicin (17) (Fig. 1) was isolated from an unidentified fungus obtained from Butea monosperma. This compound displayed in vitro potency IC_{50} pERK = 5.5; pS6 = 8.9; proteasome=25.1; pRb > 100 M and cytotoxicity in range of 1-3 µg/mL against different cancer cell lines (Deshmukh,
unpublished data). It was isolated earlier from Alternaria radicina and Bipolaris coicis as a phytotoxic compound (Nakajima et al., 1997; Solfrizzo et al., 2004).

Chaetoglobosin A (18) (Fig. 1) was obtained from endophytic fungus Chaetomium sp. harboring stem of plant Phyllanthus sp. The compound exhibited IC_{50} of <3 μg/mL for pERK and >3 μg/mL for pS6 and proteasome (Deshmukh, unpublished data). It was previously reported as cytotoxic compound and was isolated from the same fungus (Sekita et al., 1973; Umeda, 1975).

Other cytotoxic compound Cytochalasin D (19) (Fig. 1) was isolated from Xylaria juruensis, an endophyte residing in Nyctanthes arbor-tristis. The compound displayed IC_{50} values between 0.3-1 μg/mL in different cancer cell lines (Deshmukh, unpublished data). It was previously reported from Xylaria arbuscular an endophytic fungus from healthy tissues of Cupressus lusitanica (Amarala et al., 2014). Cell-permeable cytochalasin D, causes cell arrest at G1-S transition by activation of p53 dependent pathways and checks actin polymerization. It prevents polymerization of actin monomers by binding to the F-actin polymer (Heptinstall et al., 1998).

Investigation of the endophytic fungus Penicillium sp. obtained from an unidentified plant led to the isolation of an anticancer compound. Mycophenolic Acid (20) (Fig. 1). The compound exhibited cytotoxic activity with IC_{50} values between 0.8 to 1.5 μM against different cancer cell lines (Deshmukh, unpublished data). Mycophenolic acid was discovered for the first time from Penicillium glaucum (now called P. brevicompactum) from spoiled corn and possess broad-spectrum antiviral, antifungal, antibacterial, anticancer, and anti-psoriasis properties (Silverman et al., 1997). Mycophenolate mofetil is a prodrug for mycophenolic acid, an immunosuppressive agent that is in use for transplant recipients and used to treat several inflammatory conditions (Moder, 2003). It is also used for curing autoimmune disorders like lupus nephritis (Appel, 2012).

Dichlorodiaportin (21) (Fig. 1) was obtained from an unidentified fungus from Embelia ribes. It exhibited cytotoxicity against different cell lines and showed IC_{50} values of < 1 μg/mL. It was previously reported from Penicillium naigovense (Larsen and Breinholt, 1999). It was also reported from Trichoderma sp. 09 and endophytic fungus of Myoporum bontioides with anti fungal activity against Colletotrichum musae and Rhizoctonia solani (Li et al., 2016).

PR-Toxin (22) (Fig. 2) was isolated from an unidentified fungus from Thevetia sp. The compound showed IC_{50} values between 0.3-3 μg/mL in different cancer cell lines (Deshmukh et al., unpublished data).

Viridiol, a steroidal antibiotic (23) (Fig. 2) was isolated from Trichoderma sp. harboring in Azadirachta indica. Viridiol exhibited cytotoxicity with IC_{50} <1 μg/mL. It was earlier reported from Trichoderma viride. Viridiol exhibit phytotoxic (Shows necrotic activity on plants) and antifungal properties. It is also a Phosphatidylinositol 3-kinase (PI3K) inhibitor (Moffatt et al., 1969; Hanson et al., 1988; Jones and Hancock, 1987; Jones et al.; 1988; Andersson et al., 2010; Caou et al., 2010).

A52688 (24) (Fig. 2) was obtained from Mycoleptodiscus terrestris, from the leaf of Vallisneria sp. A52688 exhibited cytotoxic activity towards different cell line with IC_{50} values between 1-3 μg/mL. It was earlier reported from the same fungus with antibacterial and anti-neoplastic activity (Anderson et al., 1985).

Metabolites with Anti-inflammatory activity:

An anti-inflammatory compound Mutolide (25) (Fig. 2) was obtained from Lepidosphaeria nicotiae, of an unidentified plant and also from a coprophilous fungus Lepidosphaeria sp. In LPS-induced inflammation, the compound checked cytokines TNF-α and IL-6 secretion from THP-1 and mononuclear cells of the human peripheral blood (IC_{50} 1.27 and 1.07 μM, respectively). In anti-hCD3/anti-hCD28 stimulated hPBMCs, the compound was found active to inhibit release of pro-inflammatory cytokine IL-17. NF-κB has prominent role involved in the release of pro-inflammatory cytokines including IL-17. Mutolide was found effective in inhibiting NF-κB activation and translocation. However, the compound was not much effective in checking the activity of p38 MAPK enzyme, a serine/threonine kinase responsible for cytokine secretion. In the LPS-induced acute model of inflammation in Balb/c mice, a dose of 100 mg/kg of mutolide was found to inhibit secretion of TNF-α (Shah et al., 2015).

Brefeldin A (26) (Fig. 2), a lactone was isolated from Humicola fuscoatra, an endophyte residing in Ficus glomerata. The compound inhibited TNF-α and IL-6 with IC_{50} of 0.3 and 0.02 μg/mL, respectively (Deshmukh unpublished data). It was previously reported from Eupenicillium brefeldianum as an antiviral agent (Tamura et al., 1991; the mechanism of action of Brefeldin A is to inhibit GTP exchange factor (GEF) activity, which is responsible for the activation and localization of small GTPases in eukaryotic cells.

**Fig. 2.** Structures of anticancer, anti-inflammatory, antidiabetic and antifungal metabolites
Metabolites with anti-diabetic activity:

Herbarin (29) (Fig. 2) was a naphtoquinone with anti-inflammatory and anti-diabetic activity was detected from Dendryphion nanum harboring leaf of Ficus religiosa. Herbarin displayed anti-inflammatory activity by inhibiting IL-6 (IC50 0.60 μM) and TNF-α (IC50 0.60 μM) and antidiabetic activity in GUA assay with IC50 value of 0.3 μg/mL and Toxicity at >10 μg/mL (Mishra et al., 2013).

Another antidiabetic metabolite, Ustilaginoidin A (30) (Fig. 2) was detected from Alternaria longissima from Sphaeranthus sp. Ustilaginoidin A exhibited IC50, GUA 0.1 μM; Toxicity > 1 μM (Deshmukh Unpublished data). It was reported from Claviceps virens with weak antitumor cytotoxicity to human epidermoid carcinoma (Koyama et al., 1988; Koyama and Natori, 1988).

Metabolites with Antifungal activity:

A cyclic depsipeptide Arthrichitin (31) (Fig.2) was obtained from Arthrinium phaeospermum derived from unidentified grass, and also from (as LL156256g) the marine fungus Hypoxylon oceanicum (Vijayakumar et al., 1996; Schlingmann et al., 1998). This compound showed antifungal activity against Candida sp., Trichophyton sp. and several phytopathogens. It has low in vitro potency to be used in the clinic, but analogs with improved activity could be developed (Vijayakumar et al., 1996). Arthrichitin (31) also displayed fungicidal efficiency of 75 and 85% against Pyricularia oryzae infecting rice and Botrytis cinerea infecting cucumber at 5000 ppm (Vijayakumar et al., 1996).

Some of the strategies of cultivation of these fungi:

The cultivation of fungi for production of secondary metabolites needs optimizing the parameters that involve many strategies. Different approaches like changes in pH, aeration, temperature, the design of culture flask, modulation of medium to flask volume ratio, media composition, or by biotic elicitation through co-culture, abiotic stimulation using physical and chemical stress or by epigenetic modulation (Bode et al. 2002; Cichewicz, 2010; Pettit, 2011; Marmann et al. 2014; Bertrand et al. 2014). Some of the approaches used for getting chemical diversity are described briefly here.

One factor at a time (OFAT):

One factor at a time approach is a classical way for finding optimum condition for the processes. Response Surface Methodology (RSM) and Plackett-Burman design are a most accepted way for the optimizing processes. The Plackett-Burman design (Plackett and Burman, 1946) constitutes two level factorial design that reduces the number of trials and finds out the most prominent factor that affects productivity. Response surface methodology (RSM) (Box and Behnken, 1960) is another approach based on polynomial regression fitting, significance analysis and stationary point location that determines the best concentration of chosen factor in order to get desired response. In different organism and systems, it has been implemented to optimize most critical factors that affect the level of secondary metabolite produced (Garyali et al., 2014; Luo and He, 2004; Srivastava and Srivastava, 2012; Wang et al., 2013; Xu et al., 2006).

Garyali et al. (2014) reported the increased production of taxol using OFAT approach in fermentation by using Fusarium redolens. Due to the use of RSM, fungal taxol production rate increased by 3 folds compared to un-optimized medium.

Chemical epigenetic modifiers:

In fungi, certain genes remain silent and are not expressed during the entire life in normal condition, but under stress condition their expression takes place. These silent genes or cryptic genes can be induced to express using epigenetic modulators. Epigenetic modifiers act without altering DNA sequence but alter expression levels. Chemical inhibitors like HDAC or DNMT are effective in regulating gene cluster such that there is a significant increase in the production of metabolites. DNA methyltransferase (DNMT) inhibitors like procaine, hydralazine, 5-azacytidine, 5-aza-2’-deoxycytidine, and procainamide and/or histone deacetylase (HDAC) inhibitors like suberoyl anilidehydroxamic acid (SAHA), valproic acid and sodium butyrate are commonly used epigenetic modifiers (Williams et al., 2008; Takahashi et al., 2016).

Simultaneous feeding of the HDAC inhibitor, SAHA, and the DNMT inhibitor, 5-azacytidine, to the growth media of Pestalotiopsis acacae resulted in significant changes in the production of secondary metabolites, and led to production of three novel aromatic compounds, 2’-hydroxymethyl-4’-methylphenyl-2, 6-dihydroxy-3-(2-isopentenyl) benzoate,4,6-dihydroxy-7-hydroxymethyl-3-methyl coumarin and 4,6-dihydroxy-3,7-dimethyl coumarin, along with five known polyketides endocorcin, pestalotiollide B, pestalotiopyrone G , scirpyrone A and 7-hydroxy-2-(2-hydroxypropyl)-5-methylchromone (Yang et al., 2013).

Brominated resorcylic acid lactones, along with other four already known, aigialomycin B,11 zeanolin,12 LL-Z1640-1,12,13 and LL-LZ1640-2 were isolated from the marine-derived fungal strain Cochliobolus lunatus (TA26-46) culture was treated with sodium butyrate a HDAC inhibitor (Zhang et al., 2014). There was 10 fold increase in the production of fumiquinazoline C when Aspergillus fumigatus (GA-L7) culture was treated with valproic acid, an epigenetic modifier,
Co-culture of different strains

One of the promising strategies to obtain chemically different compounds is the co-culture strategy. This is because the microorganisms live in an extremely biodiverse community in their natural habitats. Sharing the same niche, interferes with their morphology, growth, adaptation, and development patterns, that may also include changes in secondary metabolite production as a result of chemical interaction among the organisms (Kusari et al., 2014; Pamphile et al., 2017). A promising and complex environment is created by co-culturing for new secondary metabolites production due to intercommunication between different organisms. The compound australidixanthone and sesquiterpene (+)-austrosene along with other five deciphered compounds were demonstrated by Ebrahim et al. (2016) obtained from the EtOAc extract of axenically cultured Aspergillus austroafricanus, a fungus that harbors the leaves of aquatic plant Eichhornia crassipes. There was an increase up to 29 times for several diphenyl ethers, including the new austramide, in mixed cultures where the same strain was grown with Streptomyces lividans or with Bacillus subtilis.

FUTURE PERSPECTIVES

Endophytic fungi from the Indian subcontinent have immense potential to produce diverse metabolites. The true potential of these fungi has not been fully explored. In the Indian subcontinent, one third of the plants are endemic, and there are nine phytogeographic zones, two hotspots of biodiversity, six wetlands and 8000 km of coastal areas with endemic mangroves. India is a country which has different geographical regions and climate zones ranging from tropical to alpine (Himalayas) and has cold and hot deserts. Exploration of the diversity of endophytic fungi will give a boost to the natural product drug discovery.

We need to study the whole genome sequence of microbes and generate bioinformatics data based information. It will help to predict the presence of genes/gene clusters responsible for the synthesis of novel classes of chemical scaffolds.

The genome editing system (CRISPR/Cas9) is a powerful tool to manipulate genomes of different organisms. Only a few studies exist that employ genome editing approach in filamentous fungi (Shi et al., 2017). In Trichoderma reesei CRISPR/Cas9 generated site specific alteration in target genes (Liu et al., 2015). The system also offered an opportunity for simultaneous manipulation of multiple genes. The observations recorded from CRISPR/Cas9 mediated genome editing in T. reesei are promising and extend the opportunity for other filamentous fungi.

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