Chapter 3: Taxol from endophytic fungus Gliocladium sp.

Production, purification, characterization and bioassays of taxol and its precursor 10 DAB III.

1. Summary
We have isolated endophytic fungi from Indian yew tree, *Taxus baccata* and then screened for taxol production. Out of the forty fungal cultures screened, one fungus *Gliocladium* sp. was found to produce taxol and 10DAB III (10 Deacetyl baccatin III). These compounds were purified by TLC, HPLC and characterized using UV-Spectroscopy, ESI-MS, MS/MS and proton NMR. One liter of *Gliocladium* sp. culture yielded 10 µg of taxol and 65 µg of 10 DAB III. The purified taxol from the fungus showed cytotoxicity towards cancer lines HL-60 (leukemia), A431 (epidermal carcinoma) and MCF-7 (breast cancer).

2. Introduction
Paclitaxel (Taxol®), a tubulin binding diterpenoid was first isolated from the pacific yew tree *Taxus brevifolia* (Wani et al, 1971). Because of its ability to bind specifically to β-tubulin and its cytotoxicity at lower concentrations, it is being used for the treatment of several classical tumors (Amos and Lowe, 1999; Haldar et al, 1995; Haldar et al, 1996; Haldar et al, 1997). In comparison with other antineoplastic agents such as Vinca alkaloids and colchicine, its binding dynamics with tubulin are peculiar, the former enhance microtubule disassembly where as taxol promotes assembly and stabilization (Roberts and Hyams, 1979). Due to the complex structure and limited supply of Taxol, scientists have been posed with the difficulty of finding an alternative potential source of this compound (Gordon et al, 1993). A number of methods for the production of taxol including tissue culture of *Taxus* sp (Jaziri et al, 1996), extraction from endophytic fungi (Strobel et al, 1996) and chemical synthesis (Nicolaou et al, 1994) have been reported. But these procedures are either low yielding or very complex and tedious.

India has a large wealth of medicinal plants with abundance of *Taxus baccata*, hence a screening programme was initiated to isolate endophytic fungi from *Taxus baccata* that
produce taxol. Forty slow growing, few non-sporulating and uncommon endophytic fungi were isolated from *Taxus* bark, stem, leaves (needles) and brought to pure culture state. In this study, the production, purification and characterization of Taxol and its useful precursor 10 DAB III by an endophytic fungus identified as *Gliocladium* sp. is reported.

3. Materials

Taxol immunoassay kit was purchased from Hawaii Biotech, Hawaii, USA. Standard taxol, 10 DAB III and Baccatin III were purchased from M. P. BioMedicals, USA. TLC precoated plates, Silica G for TLC, Silica G for column chromatography and HPLC grade solvents from Merck. NMR grade solvents and tubes from Cambridge Isotope Lab. Inc USA. A431, MCF-7 and HL-60 cell lines from National Center for Cell Sciences (NCCS), Pune, India. Standard 25 cm² tissue culture flasks and 96 well microtiter plates from Nunc. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide from Sigma chemicals. All other analytical grade chemicals and solvents were from Qualigens, Merck, Himedia and Sigma.

4. Methods

A novel endophytic fungus *Gliocladium* sp. having optimum growth at pH 7 and 25°C was isolated from *Taxus baccata* tree growing in West Bengal, India (Sreekanth et al., 2008). This fungus was maintained on Potato Dextrose Agar (PDA) slants. Stock cultures were maintained by subculturing at monthly intervals. After growing the fungus at pH 7.0 and 25°C for 7 days, the slants were preserved at 15°C. From an actively growing stock culture, subcultures were made on fresh slants. After 7 days of incubation at pH 7.0 and 25°C they were used as the starting materials for fermentation experiments.

4.1. Production and isolation of taxol from *Gliocladium* sp.

Production of taxol by the fungus was studied by a two stage fermentation procedure as describe by Stierle et al (1993). In brief, in first stage fungal culture was stabilized in modified mycological medium for 5 days at 25°C at 120 rpm. After the culture stabilization, the biomass was pelleted out by centrifugation (6,000 rpm for 20 min) and washed twice with sterile distilled water. The final biomass obtained was used as
inoculum for the second stage fermentation. Culture was incubated at 25 °C for 21 days, under stationary condition, after which it was harvested and preceded for the further processing.

4.2. Lyopillization and extraction
The culture harvested was freeze dried and powdered using pestle motor. The grounded biomass was weighed and extracted using a mixture of polar and non polar solvents. For maximum compound extraction, chloroform and methanol (9:1) ratio solvent system was used. The organic layer was separated from the aqueous layer using separating funnel. The procedure was repeated thrice and the solvent was dried using anhydrous sodium sulphate and concentrated under vacuum using rotavapor at 40 °C. The crude compound thus obtained was analyzed on TLC for taxol content.

4.3. Thin layer chromatography (TLC)
The crude extract thus obtained was analyzed on TLC on silica gel G (0.25 mm thickness) using chloroform: acetonitrile (7:3) solvent system. The TLC plates were developed with anisaldehyde-sulfuric acid reagent or vanillin sulpheric acid reagent (Wang et al, 2000). Taxanes produced a dark blue spot that later turned gray with use of these reagents. Purity of the isolated compound was checked on TLC in different solvent systems such as (A) chloroform: acetonitrile (7:3); (B) chloroform: methanol (7:1) and (C) ethlyacetate: 2-propanol (95:5).

4.4. Silica Gel Column chromatography and preparative TLC
Purification of the fungal taxol was done by silica gel column chromatography. The crude extract was loaded on silica gel column (60-120 mesh size, 30 cm x 3 cm length/width) pre-equilibrated with chloroform, and eluted with a gradient of chloroform: acetonitrile (chloroform 100, 75:25, 50:50, 25:75, 100% acetonitrile). Fractions containing compounds with Rf value similar to that of the standard taxol were pooled and subjected to preparative TLC on a 0.5mm thick (20 cm x 20 cm) silica plate and developed in solvent system chloroform: acetonitrile (7:3). The putative taxol was scraped and eluted out with chloroform: methanol (9:1).
4.5. HPLC and Spectroscopic analysis

Purity and characteristics of putative taxol were determined by HPLC using C18 Symmetry column (Waters). Sample was taken in 10 μL chloroform, injected in HPLC column and gradient elution was performed using 25 % to 95 % acetonitrile at a flow rate of 0.5 mL / min. A dual wavelength recorder set at 227 and 254 nm was used to detect the compounds eluting from the column (Martin et al, 1998). The absorption maximum of the purified compound was determined by Shimadzu PC 101 Spectrophotometer. Sample was dissolved in HPLC grade methanol and spectral data was collected over 200 to 450 nm range.

4.6. Immunoassay and Quantification of Taxol

Taxol in the crude extracts was qualitatively and quantitatively determined by competitive inhibition enzyme immunoassay (CIEIA) using immunoassay kit (TA01) purchased from Hawaii Biotechnology, Hawaii. The crude extracts were dissolved in methanol and centrifuged to remove the insoluble materials. In brief, this assay was performed in a 96-well microtitre plate coated with Taxol –protein coating antigen. The plate was blocked with 1 % (w/v) BSA in PBS. After washing, the solid phase bound taxol was incubated with samples and taxol standard and a specific antitaxol monoclonal antibody. The taxol in the sample competes with solid phase bound taxol for binding to the monoclonal antibody. The monoclonal antibody bound to the solid phase bound taxol was detected by an alkaline phosphatase conjugated second antibody and alkaline phosphatase substrate, p-nitro phenyl phosphate the inhibition of color development was proportional to the concentration of free taxol present in the samples. The amount of taxol in each sample was calculated from an inhibition curve made using different concentrations of standard taxol supplied with the kit. This technique was used to screen for taxol in each of the fungal extracts. The assay is sensitive to about 1 ng/ mL.

4.7. ESI-MS and MS/MS

Molecular mass of the purified compound was determined by M/S Applied Biosystems API QSTAR pulsar (ESI-MS) mass spectrometer. Samples for the analysis were dissolved in HPLC grade methanol, water, acetic acid in the ratio of 50:50:0.1. Samples were then analyzed by infusion method (injected into MS) at a flow rate of...
5 μL/ min and at a IS voltage of 3800 V in TOF mode. Spectrum from a range of m/z 500 to 900 Daltons was obtained. Fragmentation of the desired molecule was obtained by acquiring the product ion spectrum using MS/MS with similar parameters as used for ESI-MS. Molecular ions of the standard taxol were also obtained for comparison.

4.8. ¹H-NMR (Proton NMR)
¹H-NMR analysis was carried out on a Bruker AV 400 Spectrophotometer at 400 MHz. NMR spectrum was measured with a spectral width of 8223.68 and data was acquired into 32 K data points. An acquisition time of 1.9 s and a relaxation delay of 1.0 s were used. Samples were then dissolved in CDCl₃ (deuterochlorofrom) and scanned overnight. NMR of the standard Taxol was also obtained, however the number of scans acquired for obtaining signals were less in comparison with that of the test.

4.9. Cytotoxicity of fungal taxol on different cancerous cell lines
In general the protocol is as described: Cells were kept frozen (0.5 mL) stock at 2-5 X 10⁸ cells/ mL in 70 % FBS containing 30 % DMSO. Frozen stocks were thawed at 37 ⁰C, then 5 mL fresh animal cell culture medium (all supplements) and 10 % Fetal Calf Serum (FBS) was added into 25 cm² Tissue culture flask. The approximate seeding ratio from frozen stock is one ampoule to two flasks. Cells were grown to 90 % confluency, replacing medium every 48-72 h as required. Subculture cells at 90 % confluence every 48-72 h at a ratio of 1:3 (or 4) for cells. Cells were suspended in medium to a concentration of 1 x 10⁴/mL. 100 μL cell suspension was aliquoted into each well of a 96 well microtiter plate (i.e. 1 x 10⁴ cells/well). Plates were incubated overnight at 37 ⁰C. Complete medium containing test compound in 5 % v/v was serially diluted in 8 wells. All the samples were taken in triplicates.

To each well 10 μL of MTT reagent (5 mg/mL) was added. Then cells were stained for 20 h at 37 ⁰C. At the end of this period, 200 μL acidified isopropanol was added to solubilize the purple formazan crystals produced. Absorbance was measured at 490 nm with a Titertek Multiskan. For each test compound the highest "no effect" concentration was determined, i.e. the highest concentration of test compound to which the cells were exposed and which did not result in a decrease in absorbance, compared to the control situation.
Cytotoxicity of the compound against cell lines HL-60 (leukemia), A431 (epidermal carcinoma) and MCF-7 (breast cancer) was determined by MTT assay (Twentyman and Luscombe, 1987). The cell suspension at a concentration of 1 x 10^4 cells / mL was added in 96 well microtiter plates. Culture media used for HL-60, MCF-7 and A431 were RPMI 1640, MEM and DMEM respectively. Plates containing culture media and test compound were incubated overnight for HL-60, 7-8 days for MCF-7 and 4 days for A431 at 37 °C, 5 % v/v CO₂ and 95 % humidity. All the samples were taken in triplicates. 10 µL of MTT reagent (5 mg/ml) was added to each well and cells were incubated for 1 h at 37 °C. At the end of this period, 200 µL of acidified isopropanol was added and plates were incubated for 4 h to solubilize the purple formazan crystals produced. Absorbance was measured at 490 nm with a Beckman coulter spectrophotometer.

4.10. Antibacterial activity
Eight pathogenic bacteria were tested for the bacterial growth inhibition studies of Taxol, 10DAB III and Baccatin (commercially available). Briefly, bacteria (Staphylococcus aureus, Proteus vulgaris, Streptococci group D, Bacillus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia morganii) grown to mid-logarithmic phase were harvested by centrifugation, washed with 10 mM sodium phosphate buffer (SPB) at pH 7.4, and diluted to 2 x 10^5 colony-forming units (CFU)/mL in SPB containing 0.03 % Luria–Bertani (LB) broth. Taxol, 10DAB III and Baccatin 10 µL of 1 mg/ mL concentration was added in 50 µL of LB medium in 96-well microtitre plates. Each well was inoculated with 50 µL of 5 x 10^4 CFU of bacteria. All the tests were done in triplicates. O.D at 660 nm of the plates was taken at 0, 1, 6, 8, 16 and 21 h time interval. Growth inhibition was calculated accordingly with respect to control.

4.11. Production and Isolation of 10DAB III from Gliocladium sp.
Production of 10 DAB III by the fungus was studied by a two stage of fermentation procedure as described above for taxol production. For 10 DAB III production, the flasks were incubated at 25-27 °C for 14 days as stationary culture (Second stage). Culture filtrate and mycelia obtained were lyophilized, extracted thrice with chloroform: methanol (9:1) and solvent dried at 40 °C in vacuo. The crude extract was then subjected
to silica gel chromatography and eluted with gradient of chloroform: acetonitrile as described earlier. Fractions showing 10 DAB III on TLC were pooled and subjected to further fractionation on preparative TLC using Chloroform: acetonitrile (7:3) as solvent phase. Homogeneity of 10 DAB III was checked using HPLC and molecular mass was determined by ESI-MS as described earlier. 10 DAB III from one liter of culture was estimated by immunoassay kit TA 04 as described for taxol estimation.

5. Results

5.1. Taxol from culture of fungus Gliocladium sp

Extraction of fungal culture yielded dark brown crude compound, which was fractionated on silica gel column with 100 % chloroform and chloroform: acetonitrile (75:25). Partially purified compound with chromatographic properties similar to that of the standard taxol (FIG 1) was obtained. The partially purified taxol on preparative TLC yielded considerably pure compound. Partially purified taxol obtained from preparative TLC showed single dark blue spot that later turned to gray (with Rf value 0.5) when sprayed with the anisaldehyde sulfuric acid reagent / vanillin sulfuric acid reagent on TLC as shown in FIG 2 (Chan et al, 1994). The purity of the putative taxol showed similar chromatographic properties as that of the standard taxol in three different solvent systems A, B and C on TLC (FIG 2).

FIG 1: Thin layer chromatography compounds obtained after Silica Gel column chromatography. Lane A- I and L- P fractions obtained from the gradient of chloroform, acetonitrile. Lane J standard taxol and Lane K standard 10 DAB III. Fractions in lane F and G show putative fungal taxol.
FIG 2: The TLC of fungal taxol purified from culture broth along with standard taxol on silica gel G using chloroform: acetonitrile (7:3) solvent system. Lane A: purified fungal taxol, Lane B: Standard taxol. Detection: Vanillin sulphuric acid.

5.2. HPLC and Spectroscopic analysis
The homogeneity of the purified compound was confirmed by HPLC analysis, which showed a single, symmetrical peak with retention time 25.5 min on C18 symmetry column (FIG 3). Absorbance of the eluting compound showed high intensity at 227 nm and relatively low at 254 nm. The UV absorption analysis showed a peak showing absorption maxima at 227 nm. An absorbance maximum of standard taxol was also obtained for comparison (FIG 4).
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**FIG 3**: HPLC profile of pure fungal taxol with retention time of 25.5 min.

**FIG 4**: UV absorption spectrum of standard taxol and fungal taxol.
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5.3. ESI-MS and MS/MS
Electrospray ionization mass spectrometry yielded a major ion at 854 $m/z$ and at 876 $m/z$. Apart from the major ions, fragment ion at 569 $m/z$ is also seen (FIG 5). In MS/MS fragment ions at $m/z$ 286 and at $m/z$ 570 were seen, but the relative abundance of the same was very low (FIG 6).

FIG 5: ESI-MS spectrum of the $m/z$ 854 (M+H) and $m/z$ 876 (M+Na) ion of fungal taxol.
FIG 6: MS-MS spectrum of fungal taxol showing product ions 570 m/z and 286 m/z attributing to taxane substructure and side chain substructure.

5.4. Proton NMR

$^1$H NMR spectrum showed well resolved signals which were distributed in the region from 1.0 to 8.5 ppm. The three proton signals caused by methyl group and acetate groups were seen in the range of 1.0 to 2.5 ppm which include H16, H17, H18, H19, 10-OAc, 4-OAc, H14α, H14β, 1-OH, 7-OH, H6α and H6β. Multiplets caused by certain methylene groups were also seen in this range. Protons in the taxane skeleton and the side chain such as H10, H13, H3’, H2, H5, H2’, H7, H20α, H20β and H3 including NH
and 2'-OH were observed in the region between 2.5 and 7.0 ppm and the aromatic proton signals caused by the C2 benzoate, C3' phenyl and C3' benzamide groups such as o, m, p-Ph1, o, m, p-Ph2, and o, m, p-Ph3 appeared in the region between 7.0 and 8.5 ppm (FIG 7).

**FIG 7:** ^1^H NMR spectrum of fungal taxol. Proton signals were resolved in the region 1 to 8.5 ppm. The methyl group (H17, H16, H18, and H19) and acetate group (10-OAc, 4-OAc, 1-OH, and 7-OH) protons along with methylene group multiplets were seen in the region 1-2.5 ppm. Other taxane and side chain protons such as H3, H20α, H20β, H7, H2’, H5, H2, H3’, H13, H10 and NH are seen in the region of 2.5 to 7 ppm. Protons from the aromatic groups such as C2 benzoate, C3’ phenyl and C3’ benzamide are seen in the range of 7 to 8.5 ppm. Measured in CDCl₃ (400 MHz).
5.5. Quantification and cytotoxicity

Calculations for quantifying taxol in the sample are as follows:

Mean absorbance of the background wells was calculated and subtracted from all the wells. Mean absorbance of test and standard samples taken in duplicates were also calculated. Absorbance of well #8 is considered as uninhibited reference well. This absorbance is proportional to the concentration of anti-taxol or anti-taxane antibody bound by Taxol–protein coating the well, in the absence of inhibitor Taxol standard (B₀). The absorbance of each of wells #1- #7 is proportional to the concentration of anti Taxol antibody bound by Taxol-protein coating the well, in the presence of that particular concentration of inhibitor Taxol (B). The ratio of the absorbance for each concentration of inhibitor Taxol standard in wells #1- #7 (B) to the absorbance of well #8 (B₀) will provide a B/B₀ value for each standard concentration of Taxol. Using this data a standard curve of B/B₀ (on Y-axis) is constructed against the inhibitor concentrations (on X axis). The ratio of the absorbance for each dilution of all unknown samples to the absorbance of well #8 is B/B₀. Using the B/B₀ values that fall on the linear portion of the standard curve, extrapolate the concentration. Calculate the concentration of the analyte in the sample by multiplying the extrapolated concentration by the dilution factor. Using the above method concentrations of taxol produced by the fungal culture \textit{Gliocladium} sp is 10 µg/lit taxol (FIG 8).

<table>
<thead>
<tr>
<th>Sample/2lit dilution</th>
<th>Sample dilution</th>
<th>Mean absorbance (B)</th>
<th>B/B₀</th>
<th>Extrapolated taxol Conc from Std Curve</th>
<th>Mean Taxol Conc(x dilution) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure fungal Taxol</td>
<td>1/1000</td>
<td>0.075</td>
<td>0.330</td>
<td>10.2 ng/ mL</td>
<td>10.2 µg/ L</td>
</tr>
</tbody>
</table>

Table summarizing the values obtained
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FIG 8: Standard plot obtained using different concentrations of standard taxol and with extrapolated test sample concentration.

Approximately 58% inhibition of cell proliferation was observed with 30 μM fungal taxol against HL-60 leukemia cell line, 46% with 3.65 μM fungal taxol against MCF-7 breast cancer cell line and 53% with 3.65 μM fungal taxol against A431 epidermal carcinoma cell line (FIG 9).

FIG 9: Cytotoxicity of fungal taxol in terms of % cell viability, HL-60 showing 58% cell inhibition with 30 μM of fungal taxol treatment, MCF-7 showing 46% with 3.6 μM and A431 showing 53% cell inhibition with 3.6 μM. Bar shows standard error 3.1%. 
5.6. Antibacterial activity

Out of eight pathogenic bacteria, five of them were susceptible for taxol, 10 DAB III and Baccatin III treatment. Highly susceptible being *E coli* and *Proteus vulgaris* (FIG 10). 10 DAB III showed high activity against all the five susceptible bacteria, the next potent molecule being baccatin III and then taxol. The probable difference in the activity of these molecules may be because of difference in the hydrophobicity. As the hydrophobicity is inversely proportional to antibacterial activity, 10 DAB III being less hydrophobic shows higher activity than taxol and baccatin III.

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5.7. 10 DAB III from culture of Gliocladium sp

Fungal 10 DAB III was isolated from a 14 day old culture. The crude fractions eluted with chloroform: acetonitrile (50:50 and 75:25) showed compounds similar to the standard 10 DAB III. Purified compound showed a single bluish violet spot when sprayed with vanillin sulphuric acid and had R_f value 0.2 (FIG 11). ESI-MS analysis showed molecular ions at m/z 545 and at m/z 567 (FIG 12) (Edward et al, 1994) and the yield was 65 µg / L culture (FIG 13).

FIG 11: TLC showing purified fungal 10 DAB III in comparison with standard on silica gel G using chloroform: acetonitrile (7:3) solvent system A: fungal purified 10 DAB III, B: Standard 10 DAB III. Detection: Vanillin sulphuric reagent.

FIG 12: ESI-MS spectrum showing ions at m/z 545 and m/z 567 of 10 DAB III attributing to M+H and M+Na.
Table summarizing the values obtained

<table>
<thead>
<tr>
<th>Sample dilution</th>
<th>Mean absorbance (B)</th>
<th>B/B0</th>
<th>Extrapolated taxol Conc from Std Curve</th>
<th>Mean calc Taxane Conc(x dilution) (µM/L)</th>
</tr>
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<tbody>
<tr>
<td>1/1000</td>
<td>0.221</td>
<td>0.184</td>
<td>13.0 nM</td>
<td>13.0 µM</td>
</tr>
</tbody>
</table>

**FIG 13**: Standard plot obtained using different concentrations of standard taxane and with extrapolated test sample concentration.

**6. Discussion**

Studies on the isolation of the taxanes produced by *Gliocladium* spp. indicate that the fungus produces taxanes 10 DAB III and taxol in the culture broth *in vitro*. TLC, HPLC and other analyses of the extracts from culture broth confirm this conclusion. In addition to 10 DAB III and taxol, this fungus also produces few unidentified taxanes, although in trace amounts. To the best of our knowledge, this is the first report of isolation of 10 DAB III and taxol from *Gliocladium* sp. isolated from Indian Yew tree *Taxus baccata*.

However, Wang et al, 2000 and others (Strobel et al, 1996) have isolated taxanes from different endophytic fungal species (*Taxomyces andreanae, Pestalotiopsis, Pestalotia,*...
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*Fusarium, Alternaria* and others) obtained from *Taxus* sp. that are common to Europe, Asia and North America. The necessary precursor molecules such as sodium benzoate, sodium acetate, biotin, pyridoxal required for the formation of the taxane ring and L-phenylalanine required for the formation of side chain were provided in the growth medium, as they are essential for taxol production as reported earlier (Stierle et al, 1993). Endophytic fungi producing taxol and baccatin III in 3 weeks have been reported earlier (Noh et al, 1999), but the fungus *Gliocladium* sp. produces taxol in 21 days. However, it does produce 10 DAB III after 14 days.

The ability of *Gliocladium* sp. to make taxol was confirmed by isolation of a compound having chromatographic properties similar to those of standard taxol in three solvent systems A, B and C which showed a single dark bluish violet spot on TLC when sprayed with anisaldehyde reagent or vanillin sulfuric acid reagent. Putative taxol on HPLC C18 symmetry column showed a single symmetrical peak at retention time of 25.5 min that confirmed its homogeneity. Absorption maximum of the purified fungal taxol was found to be at 227 nm as reported earlier (Chan et al, 1994). In ESI-MS, molecular ions at \( m/z \) 854 attributing to the \((\text{M}+\text{H})^+\) and at \( m/z \) 876 attributable to \((\text{M}+\text{Na})^+\) confirmed its molecular weight to be 853 (Edward et al, 1994). MS/MS showed fragment ions at \( m/z \) 570 attributable to taxane ring substructure and at \( m/z \) 286 for the side chain substructure which are usually seen as taxol fragment ions (Thomas, 1992). \(^1\text{H} \)NMR spectrum was identical with that of the standard taxol spectrum (Chmurny et al, 1992). Taxol obtained per liter of culture was estimated to be approximately 10 μg. The concentration of taxol produced is neither low nor high when compared with the other reports (Guo et al, 2006; Strobel et al, 1996). However, this is the first report on taxol from an endophyte of yew tree growing in India. Anti-tumor activity of taxol was checked against HL-60 leukemia cell line, A431 epidermal carcinoma cell line, and MCF-7 breast cancer cell line, showing 58 %, 53 % & 46 % inhibition with 30 μM, 3.65 μM & 3.65 μM, fungal taxol respectively. Taxol showed less cytotoxicity on HL-60 cell line. However, in case of MCF7 and A431, the cell-growth inhibition was significantly high even at low taxol concentration. A number of *Taxus* sp. has been reported to produce 10 DAB III (Xueli et al, 1998) and we have also isolated this compound from a 14-day old fungal culture. ESI-MS showed a molecular
ion at $m/z$ 545 and at $m/z$ 567 attributable to (M+H)$^+$ and (M+Na)$^+$, inferring its mass to be 544 (Edward et al, 1994). 10 DAB III produced was 65 $\mu$g / L culture.

7. References

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