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Detection of taxol, an anticancer drug, from selected coelomycetous fungi

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Abstract: The fungal extraction of taxol, an anticancer drug, was studied by screening fifteen different coelomycetous cultures. Of these fungi, *Botryodiplodia theobromae*, *Pestalotiopsis menezesiana* and *Pestalotiopsis uvicola* showed positive results for the production of taxol. The taxol production by them was confirmed by TLC, HPLC, UV and Mass spectroscopic analyses and compared with authentic taxol. The fungal taxol extracted was subjected to its efficacy test on different cancer cell lines by *in vitro* solid tumor selective assay. All the three fungal extracts showed cytotoxic activity. *P. menezesiana* produced 274.9 µg/L of taxol in laboratory condition which gives scope for larger production by means of industrial fermentation. The discovery of such microbiological production of drug can revolutionize the search for effective pharmaceutical agents to control tumour.

Keywords: *Botryodiplodia theobromae*, *Pestalotiopsis menezesiana, P. uvicola*, fungal taxol, cytotoxicity test, cancer cell lines.

Introduction

The Taxanes are a group of drugs that includes paclitaxel (Taxol) and docetaxel (Taxotere), which are used in the treatment of cancer. Taxanes have a unique way of preventing the growth of cancer cells as they affect subcellular structures called microtubules, which play an important role in cell functions. Taxanes stop the microtubules from breaking down; cancer cells become so clogged with microtubules that they cannot grow and divide (Georg & Chen, 1994).

Paclitaxel is a complex diterpenoid alkaloid, and the structure of this novel compound was reported first by Wani *et al*. (1971). The molecular formula is $C_{47}H_{51}NO_{14}$, and it contains 11 stereo centers. The other chemical characteristics are: the molecular weight 853.93, UV spectrum (0.002% methanol) maximum absorption at 227(±2) nm, melting point 213-217, impurities (7-epi-10-deacetyl taxol) (HPLC):< 0.5solubility; practically insoluble in water, easily soluble in methanol, chloroform and ethyl ether.

In 1989, National Cancer Institute supported researchers at the Johns Hopkins Oncology Centre, U.S.A. reported that tumors shrank or disappeared in 30 percent of patients who received pacitaxel for the treatment of advanced ovarian cancer. Paclitaxel was later approved as initial treatment for ovarian cancer in combination with cisplatin. Paclitaxel is a compound that was originally isolated from the bark of pacific Yew tree (*Taxus brevifolia*). Early research using paclitaxel was limited due to difficulties in obtaining the drug. The amount of paclitaxel in Yew bark is small, and extracting it is a complicated and expensive process. As the demand for paclitaxel grew, NCI, in collaboration with other government agencies and the pharmaceutical company Bristol-Myers Squib, worked to increase the availability and find other sources of paclitaxel besides the bark of the pacific Yew tree. This work led to the production of a semi-synthetic form of paclitaxel derived from the needles and twigs of the Himalayan Yew tree (*Taxus baccata*), which is a renewable resource. This form of paclitaxel has now replaced the drug derived from the bark of the pacific Yew tree.

Strobel *et al.* isolated *Pestalotiopsis microspora* as endophyte from the inner bark of *Taxus wallichiana* which produced taxol in mycelial culture. The [^{14}C] Acetate and [^{14}C] phenylalanine served as precursors (Strobel *et al.,* 1996a). The occurrence of taxol in other endophytes such as *Taxomyces andreanae, Pestalotia sp., Pestalotiopsis sp., Fusarium sp., Alternaria sp., Pithomyces sp.,* and *Monochaetia sp.* was also recorded (Strobel *et al.,* 1996b). The culture amendments *viz*. lowering of phosphate and addition of sodium benzoate increased the taxol production. The taxol was assayed by competitive enzyme immunoassay (Li *et al.,* 1998).

In the present study, fifteen different coelomycetous cultures were initially screened for their ability to produce taxol by using chromatographic methods. Of the fifteen fungi, only three showed positive results for taxol production. The extracts of fungal taxol were subjected to cytotoxicity test on different cancer cell lines by *in vitro* solid tumor related assay. Three fungi were shown to possess higher toxicity against cancer cells compared

to the rest of the fungi. The results of the study are presented here.

Material and Methods

Screening of the fungi for taxol production:

The fifteen coelomycetous fungi screened for the production of taxol includes *Botryodiplodia theobromae* Pat. (Strain 1&2), *Colletotrichum* sp., *Cytospora chrysosperma* Pers.ex Fr., *Cytosporella sycina* (Sacc.) Sacc., *Gloeosporium* sp. (Strain 1,2&3), *Pestalotiopsis menezesiana* (Bres. & Torr.) Bissett, *P. uvicola* (Speg.) Bissett, *P.funerea* (Desm.) Stey., *Phoma* sp., *Phomopsis* sp. (Strain 1&2), and *Phyllostica* sp.

Out of fifteen, only three were observed to produce taxol which was initially detected by TLC. The colour and Rf value of the sample was same as that of the authentic taxol. Analysis of the crude extracts by HPLC revealed the presence of taxol in the extracts. The retention time of the samples coincided with that of the authentic taxol. Further, the production of taxol was confirmed by MS. Thus, *Botryodiplodia theobromae* (Strain 1), *Pestalotiopsis menezesiana* and *P. uvicola* were shown to produce taxol.

Sample preparation:

The above three endophytic fungi were grown in 2 liter Erlenmeyer flasks containing 500 ml M1D medium supplemented with 1g soytone per liter. After appropriate incubation time of 21 days, the entire culture was passed through 4 layers of cheesecloth. To the culture filtrate, 0.25 g Na_2CO_3 was added while shaking to reduce the contamination of fatty acids that might contaminate taxol. The culture filtrate was extracted with two equal volumes of methylene chloride and the organic phase was taken to dryness at reduced pressure at 35ºC. The residue was then dissolved in 1ml methylene chloride. This sample was further purified through column chromatography.

Column chromatography:

A 1.5 X30 cm column of silica gel was loaded with sample dissolved in methylene chloride. Sample elution was done in a stepwise manner with the following solvent systems in 70 ml:

Methylene chloride (100%)
Methylene chloride:ethylacetate(20:1 v/v)
Methylene chloride:ethylacetate(10:1 v/v)
Methylene chloride:ethylacetate (6:1 v/v)
Methylene chloride:ethylacetate (3:1 v/v)
Methylene chloride:ethylacetate (1:1 v/v).

Fractions having same mobility as the authentic taxol were combined and evaporated to dryness. The residue was subjected to TLC.

Preparative thin layer chromatography (TLC):

Preparative TLC was done on 1mm and 0.25 mm Merck precoated silica gel plates which were developed successively in each of the following solvent systems:

A) Chloroform: methnol 7:1 v/v,
B) Chloroform: acetonitrile 7:3 v/v,
C) Ethylacetate:2-propanol 95:5 v/v,
D) Methylene chloride:tetrahydrofuran 6:2 v/v,
E) Methylene chloride/methanol: dimethyl formaldehyde 90:9:1 v/v/v.

The taxol was detected with 1% w/v vanillin / sulphuric acid reagent after gentle heating; it appeared as a bluish spot fading to dark gray after 24 hours (Cardellina, 1991).

Spectroscopic analysis:

After chromatography, the area of plate containing putative taxol was carefully removed by scrapping off the silica at the appropriate R_f and exhaustively eluted with methanol. The purified sample of taxol was analysed by UV absorption, dissolved in 100% methanol at 273 nm (Wani *et al*., 1971) in a Beckman DU-40 Spectrophotometer and compared with authentic taxol (Paclitaxel-SIGMA Grade).

High performance liquid chromatography (HPLC):

To further confirm the presence of taxol, the fungal samples were subjected to HPLC (Shimatzu 9A model) using a reverse phase C_{18} column with a UV detector. Twenty µl of the sample was injected each time and detected at 232 nm. The mobile phase was methanol / acetonitrile / water (25:35:40, by vol.) at 1.0 ml min^{-1}. The sample and the mobile phase were filtered through 0.2 µm PVDF filter before entering the column. The concentration of taxol was calculated as follows:

$$\text{Concn. of taxol} = \frac{\text{Total sample area X Dilution of standard X Purity of standard}}{\text{Total standard area X Dilution of sample}} \times 100$$

Mass spectroscopy:

Electro spray mass spectroscopic method was done for three samples namely *Botryodiplodia theobromae*, *Pestalotiopsis menezesiana* and *P. uvicola* to confirm the presence of taxol. The samples were dissolved in methanol: water: acetic acid (50: 50: 1 by vol). It was injected with a spray of 2 µl / min

and a spray voltage 50 by the loop injection method.

Cytotoxicity for fungal taxol: In vitro solid tumor assay (DMSO method):

Crude samples of the three fungi were tested for their cytotoxicity against cancer cell lines by disk diffusion assay. (Courtesy Henry Ford Health System, Drug Discovery and Development Program, Detroit, USA). The disk diffusion assay defines the differential killing among the 7 cell types examined.

1. L1210-murine Leukemia
2. CCRF-CEM-human Leukemia
3. murine Colon 38 – Solid tumor
4. Human colon H116 - Solid tumor
5. Human Lung H125 - Solid tumor
6. Haematopoietic progenitor cell –murine
7. CFU-GM-Human Normal Cell

Both antiproliferative response and differential activity was observed. The samples were dissolved in DMSO. A volume of 15µl of each sample was dropped onto a 6.5mm disks (Baxter filter disks). The disks were allowed to dry overnight and then placed on the edge of the Petri dish. The plates were incubated for 7-10 days and examined by an inverted stereomicroscope (10X) for the measurement of inhibition which was taken from the edge of the filter disk to the beginning of the normal-sized colony formation.

Results and discussion:

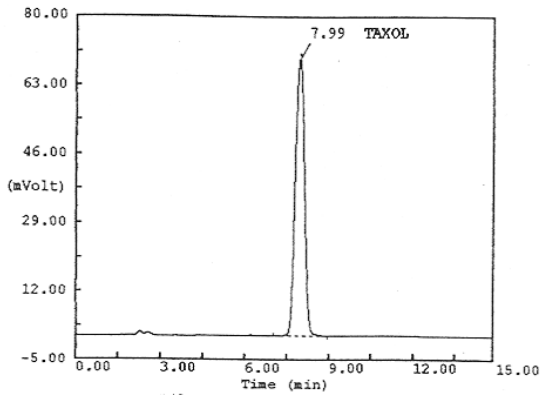
Fifteen coelomycetous cultures were screened for the production of Taxol. Of these, the results of only three fungi viz., *B. theobromae*, *P. menezesiana* and *P. uvicola* are presented in this investigation. They were grown on MID medium and incubated for 21 days at 21º C. After growth, the culture filtrates were analyzed for the production of taxol using chromatographic methods. They were purified through Column chromatography and analyzed using HPLC and MS.

Table 1. Concentration of taxol in the samples analyzed

S. No.	Sample	Conc. of taxol (µg/L)
1	*Botryodiplodia theobromae*	118.7
2	*Pestalotiopsis uvicola*	118.4
3	*P. menezesiana*	274.9

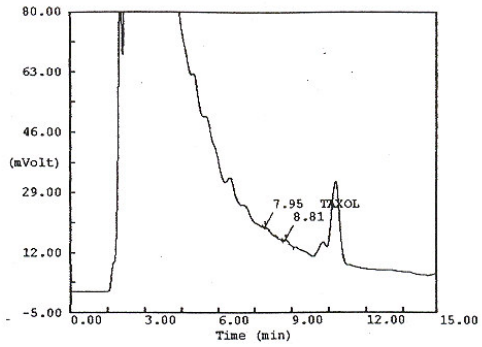
Fractionation was done by using preparative HPLC, in 90:10 Acetonitrile: 2mM Ammonium acetate and 25:45:30 methanol: Acetonitrile: 2mM acetic acid to isolate the fractions at 254 nm and 273 nm. The HPLC result of each fungus is given separately (Table 1 & Fig.1a-d). Samples selected for fractionation were from *Botryodiplodia theobromae*, *Pestalotiopsis menezesiana,* and *P.uvicola*. The fractions were subjected to UV spectroscopy and MS for presence of taxol.

Fig. 1a. HPLC of authentic taxol



For Electrospray method, the sample was dissolved in methanol: water: acetic acid (50:50:1 by vol.). It was injected with a spray of 2µl/min and a spray voltage of 50 by the loop injection method. Authentic taxol yielded an $(M+H)^+$ peak at 854.5 and 876.5. The result of each fungus is given separately (Fig. 3a-d).

Fig. 1b. HPLC of fungal extract of Botryodiplodia theobromae (1)

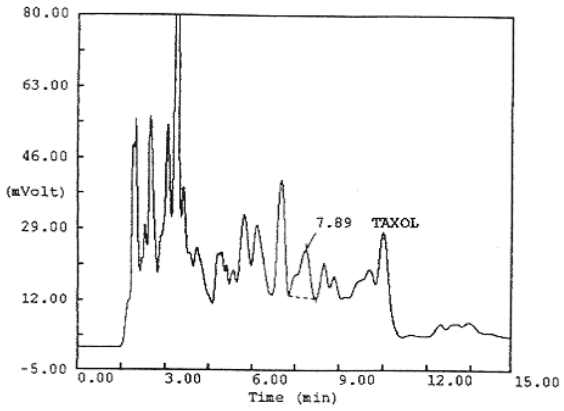


The presence of taxol in the three fungal extracts analyzed and compared with the mass spectrum of the authentic taxol at 854 and 876.5 m/z (Fig. 3a-d & Table 2).

The toxicity of *Taxus* plant has been studied thoroughly and attributed to Taxine, a complex mixture that was first isolated from the leaves of *Taxus* in 1856. However Taxines are relatively abundant in plants and they serve as alternative starting material for semi-synthetic production of paclitaxel. The taxoids of interest can be divided into paclitaxel, cephalomannine, 10-DAB III, taxines and

taxinines. In the present study the mass to charge values of the standard taxane mixture is used to compare and check the presence of other taxoid derivatives in the fungal extracts (Georgios *et al*., 1999). The results obtained are shown in Table 3.

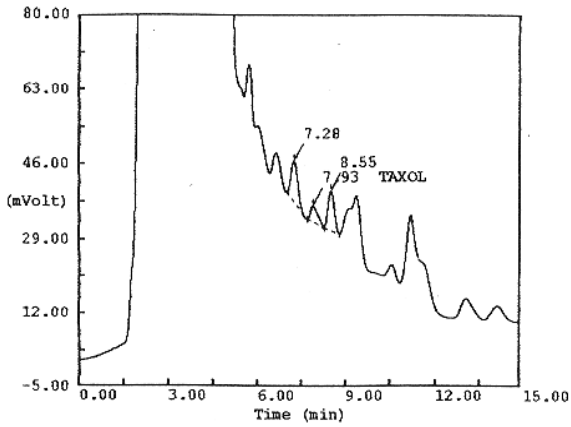
Fig. 1c. HPLC of fungal extract of Pestalotiopsis menezesiana



Cytotoxicity tests for fungal taxol:

The extracts were tested for their cytotoxicity on various cancer cell lines by *in vitro* solid tumor selective assay. The disk diffusion assay defines the differential killing among the seven cell types examined. Two Leukemias cell types (murine L1210 and human CCRF-CEM), three solid tumor cell types (murine Colon 38, human Colon H116 and human Lung H125), a murine and a human normal cell (hematopoietic progenitor cell, CFU-GM) were used. The antiproliferative and differential activity were the end points. There were four positive outcomes. Murine solid tumor selectivity relative to normal cell, human solid tumor selectivity relative to normal cell, human solid tumor selectivity relative to their respective leukemia cell and murine solid tumor selectivity relative to their respective leukemia cell. Samples demonstrating all 4 selective outcomes were first priority for subsequent bioassay-directed fractionation. The magnitude of the zonal difference as well as the potency was used to prioritize the samples. The greater the difference and potency, higher is the priority. For *Pestalotiopsis menezesiana* in the first run a zone of 200 units for L1210 and 700units for Colon 38 is selective since the zone difference is greater than 250 units. In the second run the normal cell (CFU-GM) was found to be 100 units and so there is selectivity between the Colon 38 and CFU-GM > 1000 units means that the plate was wiped out and the zone was greater than this value, even after diluting 1:4 and re-run it showed a greater difference. A similar pattern is observed for *P.funerae*, *P.uvicola,* and *Botryodiplodia theobromae* (Table 3).

Fig. 1d. HPLC of fungal extract of Pestalotiopsis uvicola

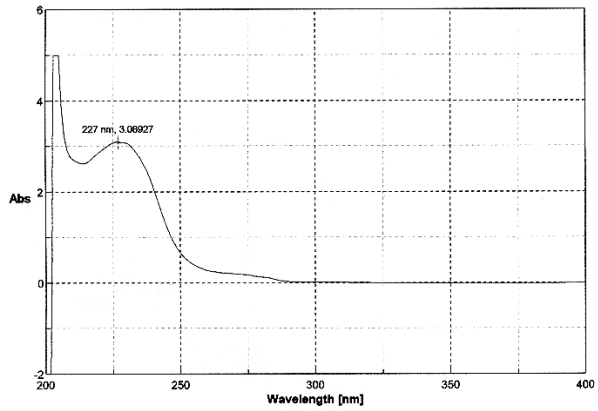


B. theobromae could be potential taxol producers. The above mentioned fungi showed higher toxicity against cancer cell types when compared to the rest of the fungi tested.

Wall and Wani from North Carolina discovered that an extract of the Yew tree bark has antitumor activity and the compound was named “Paclitaxel” or “Taxol” (Wall & Wani, 1995). The interest in developing the drug increased after the mechanism of action of tubulin polymerization was studied by Schiff *et al* (1979). It was discovered that paclitaxel prevents cell division by promoting disassembly of microtubules-skeletal structures that assemble and divide throughout the life of a cell. A large number of microtubules are formed at the start of cell division and as cell division comes to an end, these microtubules are normally broken down. However, paclitaxel prevents microtubules from breaking down. In the presence of this drug, cancer cells, which attempt to divide frequently, become so clogged with microtubules that they cease to grow and divide. In 1983, NCI conducted clinical trials for the safety of the drug and its effectiveness against various types of cancers especially ovarian and breast cancers and also the drug was used in treatment of AIDS-related Kaposi’s sarcoma. The supply of paclitaxel was limited due to several difficulties in obtaining the drug. The concentration of the

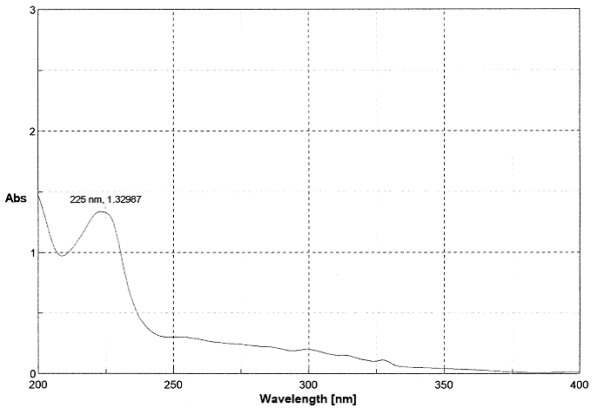
compound in Yew bark is low and the extraction procedure is complex and expensive.

Fig. 2a. UV spectrum of authentic taxol



The Yew tree is a limited resource and it grows very slow. The ecological point of cutting down the tree for the extraction of taxol should also be considered. As demand for paclitaxel increased, researchers have been exploring new ways to increase the availability of the drug. The American Company Bristol-Myers Squib produces taxol through a semi-synthesis process from *Taxus baccata*. Many European and Indian companies are using semi synthetic methods for the production of Taxol. Total synthesis of taxol was not successful for commercial production. In China large Yew tree plantations are raised to increase supply of paclitaxel. Similar to *Taxus baccata, T. yunnanensis* and *T. chinensis* are also found to be new sources of taxol.

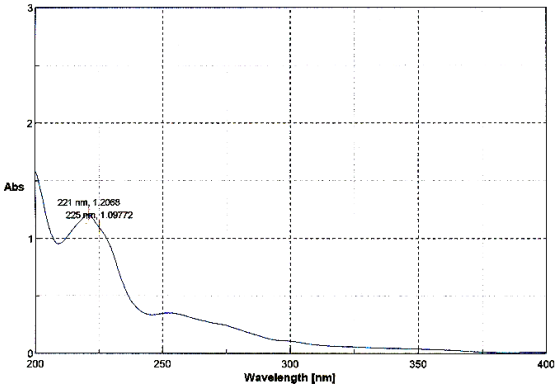
Fig. 2b. UV spectrum of Botryodiplodia theobromae



Pestalotiopsis microspora isolated as an endophyte from *Taxus baccata* was found to produce taxol. *Taxomyces andreanae*, an endophytic fungus isolated from *T. brevifolia* was found to produce taxol. Stierle *et al*. suggested that improved cultural techniques, addition of activators and application of genetic engineering for strain improvement may help commercial production of taxol in fermenters (Stierle *et al*., 1995).

B. theobromae produced 118.7 µg/L of taxol in culture. The production of taxol by this fungus is confirmed by HPLC analysis with retention time of 7.95 min (Fig. 1b) while the authentic taxol showed at 7.99 min (Fig. 1a).

Fig. 2c. UV spectrum of Pestalotiopsis menezesiana



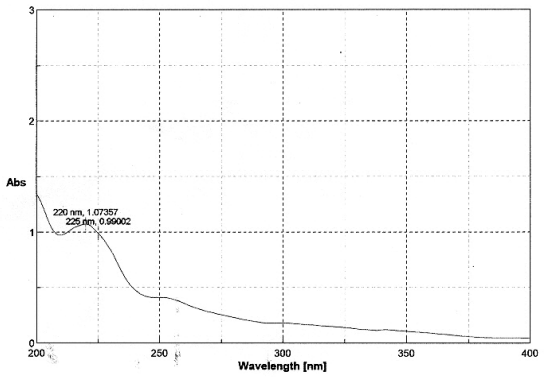
Electrospray mass spectroscopy yielded a characteristic (M+H)+ peak for authentic taxol at 854.5 and 876.5 m/z (Fig. 3a). On comparison the fungal taxol also yielded a (M+H)+ at 877.6 (Fig. 3b). There were other prominent peaks at 563, 924, 813 and 986 m/z, which could be classified as taxoid derivatives according to Georgios *et al*. (1999). The sample was subjected to UV spectroscopy (Fig. 2b) and the absorbance was at 225 nm similar to authentic taxol (Fig. 2a). The HPLC and Mass spectroscopic analyses of the sample confirmed the production of taxol by *B. theobromae* (1). Results of the HPLC analysis of the three samples showed the amount of taxol produced by each fungus (Fig. 1a-d). The maximum amount of taxol was produced by *P. menezesiana* i.e. 274.9 µg/L, followed by *B. theobromae* which produced 118.7 µg/L. *P. uvicola* produced 118.4 µg/L (Table 1).

Table 2: LC/MS analysis of the samples

Mixture of Taxane Standard Compunds	Standards *m/z	PM m/z	PU m/z	BT m/z
10 DAB III	562	562.2	563.8	563.7
Baccatin III	604	-	-	-
7-xylosyl-10-deacetyl taxol B	922	-	-	924
Taxinine M	704	-	-	-
7-xylosyl-10-deacetyl taxol	944	-	-	-
7-xylosyl-10-deacetyl taxol C	938	-	-	-
10-deacetyl taxol	812	814.4	-	813.8
7-xylosyl taxol	986	-	-	986.6
Cephalomanine	832	-	-	-
7-Epi-10 deacetyl taxol	812	814.4	-	813.8
Paclitaxel	854	855.7	854	-
Taxol C	848	-	-	-
7-Epi taxol	854	855.7	854	-
Taxol	876.5	-	-	877.6

m/z = mass to charge, PM= *Pestalotiopsis menezesiana*; PU= *P. uvicola*; BT= *Botryodiplodia theobromae* (* Ref: HPLC analysis of taxoids, Georgios *et al.*, 1999).

Fig. 2d. UV spectrum of Pestalotiopsis uvicola



The presence of taxol in the samples of the above fungi were confirmed by using UV spectroscopy and Mass spectroscopy; Liquid Chromatography / Mass Spectroscopy analyses (Table 3) and authentic taxol was used for reference in the analyses. The authentic taxol yielded a characteristic (M+H)+ at 854.5 and 876.5 m/z (Fig. 3a) while the fungal taxol i.e. *Pestalotiopsis uvicola* yielded a (M+H)+ peak at 854 and 563.2 m/z (Fig. 3d) which was observed to be a taxoid derivative (Georgios *et al.,* 1999). It was calculated that in culture *P. uvicola* produced 118.4 µg/L and the amount could be increased if the medium is amended with activators. Improved culture techniques may also be applied to enhance the production of taxol. HPLC analysis of the samples showed the retention time at 7.93 min and the authentic taxol showed retention time at 7.99 min (Fig. 1a, d). The UV spectrum showed absorbance at 225 nm similar to that of authentic taxol (Fig. 2a, d). All the above mentioned results confirmed the production of taxol by *P. uvicola* though the amount is 118.4 µg/L.

P. menezesiana produced the highest amount of taxol i.e. 274.9 µg/L in culture which is adjudged as the best for the production in large quantities through fermentation. In HPLC the fungal taxol showed retention time at 7.89 min (Fig 1c) and the authentic taxol showed retention time at 7.99 min (Fig. 1a). Identity of the fungal taxol was also confirmed by electrospray mass spectroscopy. Authentic taxol yielded a characteristic (M+H)+ peak at 854.5 and 876.5 m/z (Fig. 3a) while the fungal taxol yielded a (M+H)+ peaks at 855.7. It also peaked at 562 and 814.4 m/z which is classified as taxoid derivative (Fig. 3c) (Georgios *et al.*, 1999). When the samples of

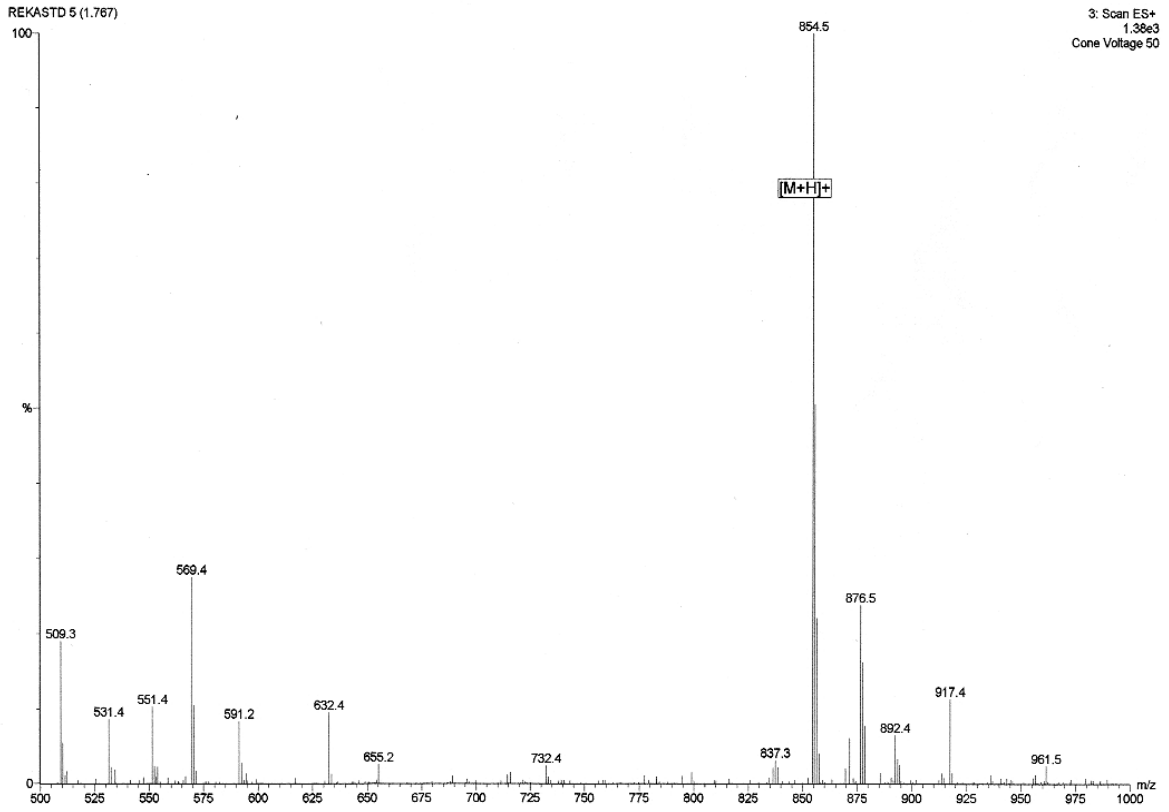
Table 3: Cytotoxicity tests of fungal taxol on cancer cell lines

Sample/extract	Dilution	L1210	Colon 38	H-116	H-125M	CEM	CFU-GM
DMSO		0	0	0	0	0	0
Pestalotiopsis menezesiana		**200**	**700**	50	50		**200**
"	1/4	**250**	**700**				**100**
Pestalotiopsis uvicola		**450**	**>1000**	400	400	500	**350**
"	1/4	**150**	**>1000**				**100**
"	1/16	**50**	**600**				**350**
Botryodiplodia theobromae (1)		300	**500**	200	150		**200**
"	1/4	**200**	**450**				**200**

the fungus were checked by using UV spectroscopy (Fig. 2c), the sample showed absorbance at 225 nm similar to that of authentic taxol (Fig. 2a). The above mentioned UV, HPLC and LC/MS analyses recorded and confirmed the production of taxol by *P. menezesiana* in culture. Since it produces 274.9 µg/L of taxol, the fungus could be selected for the production of large amount of taxol using fermenters. It is suggested that better culture conditions, addition of activators and selection of good strains will definitely improve the amount of taxol production in this fungus.

The fungal samples were tested for cytotoxicity on different cancer cell lines by *in vitro* solid tumor selective assay. Among the 7 cell types examined, two Leukemias, three solid tumors and a murine & a human normal cell types were selected. The disk diffusion assays defines differential killing among the 7 cell types examined (Table 3) using the extracts of *P. menezesiana, P. uvicola* and *B. theobromae*. These three fungi were shown to possess higher toxicity against cancer cells. Therefore, it is suggested that these fungi

Fig. 3a. Mass spectral analysis of authentic taxol



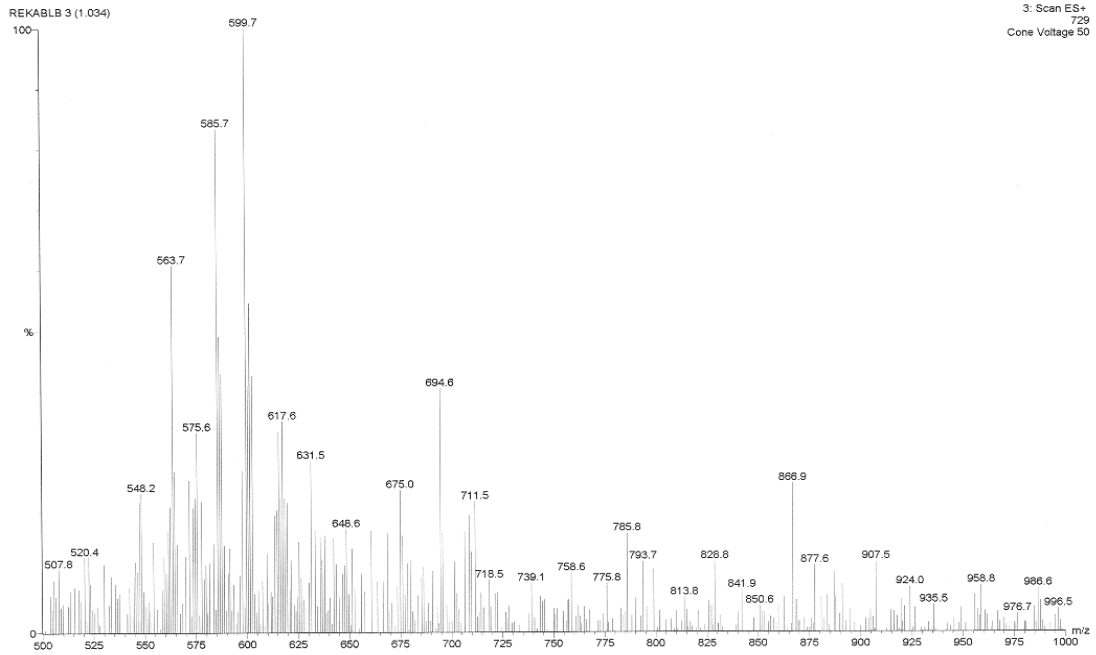
should be given priority for further research on their toxicity on cancer cells.

Studies were conducted on *P. microspora*, a taxol producing endophyte, from *Taxus wallichiana*, to induce the perfect stage (teleomorph) of the fungi in the laboratories as a critical first step to study the inheritance of taxol biosynthetic genes. It was discovered that the perithecial stimulating factor (PSF) stimulates the formation of the perfect stage (Metz *et al*., 2000). The data suggested that plant products may play a role in regulating the biology of endophytic microbes. Taxol could be one such product in the taxol producing fungi. This should be proved by further studies with taxol producing fungi. In the present investigation several coelomycetes were isolated from various sources and further screening of them showed that three of the fungi positively produced taxol in culture. Different techniques such as UV spectroscopy, HPLC, TLC and Mass spectroscopy were employed to confirm the presence of taxol in the culture filtrates. The fungal filtrates may be evaluated not only for evidence of taxol but also for other bioactive compounds. This effort will involve tremendous time and expenditure. In the present study particular attention was paid to compounds with anti cancer activity. The significance of finding a fungus capable of producing taxol should not be understated, since such a discovery will revolutionize the search for effective pharmaceutical agents.

The use of microbial fermentation in the production of bioactive substances, especially taxol, has several advantages. Industrial production of bioactive compounds like taxol requires reproducible, dependable productivity. If a fungus is the source organism, it can be grown in tank fermenters to produce an inexhaustible supply of taxol. The added advantage is that the fungi usually respond favorably to routine cultural techniques. Normally tissue cultures of Yew tree bark for taxol extraction will take a longer time when compared with the fungi. Also, tissue culture needs specialized technique and conditions have to be maintained whereas in fungi one can easily alter culture conditions for the production of different bioactive compounds (Demain, 1981).

Mass spectroscopy and chromatographic methods confirmed that the culture filtrates of the fungi examined in the present study contained a compound identical to authentic taxol. Stierle *et al*. suggested that it should be proved by radiolabelling experiments that the fungal taxol is truly produced *de novo* by the specific fungus (Stierle *et al*., 1995). It should be made sure that taxol is not an accidental contamination of any other fungal culture. Chlorocholine chloride (CCC), a known blocking agent of gibberellin was found to suppress taxol production (Lavell *et al.,* 1995). They suggested that a fungus or a bacterium

Fig. 3b. Mass spectral analysis of Botryodiplodia theobromae (1)

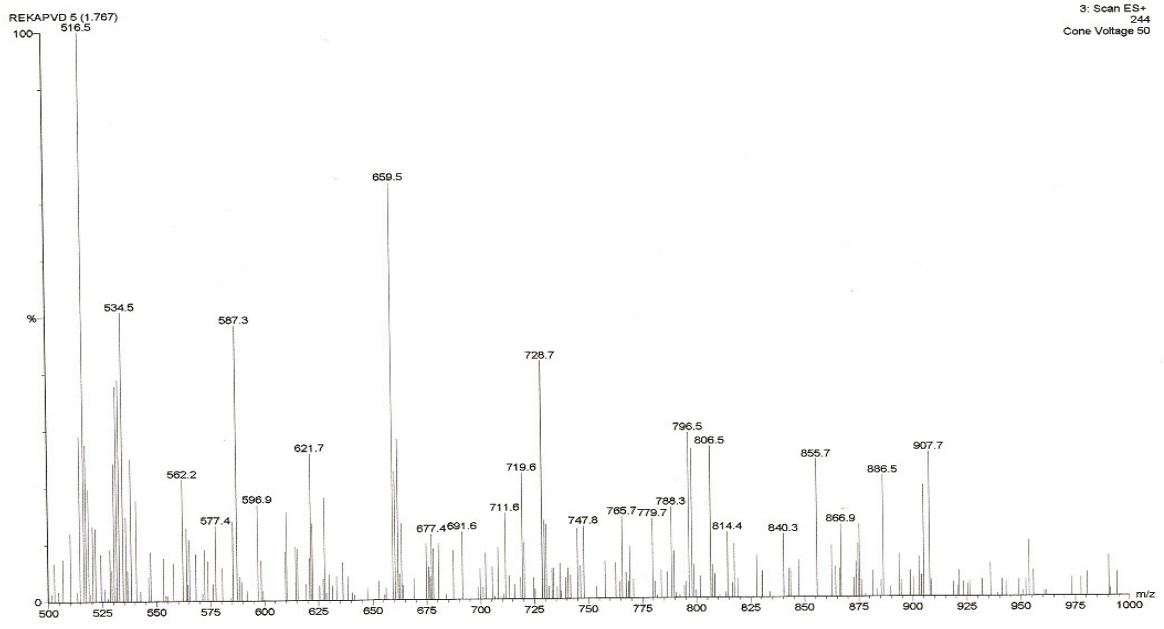


capable of producing taxol at the rate of 50 mg/L would represent an inexhaustible source of the drug. From the present investigation it is understood that the fungi *P.menezesiana, P. uvicola* and *B. theobromae* produced 274.9, 118.7 and 118.4 µg/L of taxol respectively. Probably improved culture techniques and addition of activators to the medium might improve the production. Genetic engineering methods to isolate a better strain of these fungi would also be possible in future.

It was observed that along with the peaks at 854.5 and 876.5 m/z, there were other prominent peaks at 563.8, 562, 924, 813.8 and 986 m/z (Table 3). These could possibly be other taxane derivatives. Taxol is a member of a group of compounds possessing a 4 membered oxetane ring and a complex ester side chain in their structures, both of which are essential for the antitumor activity. 10 deacetylbaccatin III (10DABIII) and Baccatin have been efficiently transformed into taxol. On comparison with the mass to charge values of the standard taxane mixture (Georgios *et al*., 1999), it is evident from our investigation that *P. menezesiana* shows the presence of Paclitaxel (855.7 m/z), 7 Epitaxol (855.7 m/z), 10 DAB III (562.2 m/z), 10 deacetyltaxol (814.4 m/z) and 7 Epi 10 deacetyltaxol (814.4 m/z). *P. uvicola* shows the presence of Paclitaxel (854 m/z), 7 Epitaxol (854 m/z), 10 DAB III (563.8 m/z) and *B. theobromae* shows the presence of taxol (877.6 m/z), 10 DAB III (563.7 m/z), 7 xylosyl 10 deacetyltaxol B (924 m/z), 10 deacetyltaxol (813.8 m/z), 7 xylosyltaxol (986 m/z) and 7 Epi 10 deacetyltaxol (813.8 m/z).

Docetaxel (Taxotere) is a side chain analogue of taxol, which has been produced by semi synthesis from 10 DAB III. It has improved water solubility and as tested clinically against breast and ovarian cancers. Possible option may be the semi synthesis of taxol from active analogues from more abundant natural compounds (Lavell *et al*., 1995). Gangadevi and Muthumary studied the effect of cytotoxicity of fungal taxol isolated from fungal endophytes by apoptotic assay (Gangadevi & Muthumary, 2007a, b). The fungal taxol isolated from the organic extract of six endophytic coelomycetous cultures viz., *Bartalinia robillardoides, Botryodiplodia theobromae, Colletotrichum gloeosporioides, Pestalotiopsis pauciseta, P. terminaliae* and *Phomopsis arnoldiae* had strong cytotoxic activity towards BT 220, H116, Int 407, HL 251 and HLK 210 human cancer cells *in vitro*, tested by Apoptotic assay. An endophytic fungus was isolated from *Taxus chinensis* var. *mairei*, and it was observed to produce taxol (Guo *et al*., 2006). Besides Taxol, it could also yield taxane baccatin III, which was an important intermediate for Taxol and semi-synthesis of Taxol in industry. Similar to other synthetic methods, fungi do not represent rapid, significantly large supply of taxol.

Fig. 3c. Mass spectral analysis of Pestalotiopsis menezesiana



Continued research may improve this. Amending the medium with precursors and improved culture techniques can enhance the production of taxol. Of the fifteen fungi analyzed, *P. menezesiana*, *P. uvicola* and *B. theobromae* can be selected as good candidates for further research studies for enhancement of taxol production.

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