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# Isolation and Identification of an Anticancer Drug, Taxol from *Phyllosticta tabernaemontanae*, a Leaf Spot Fungus of an Angiosperm, *Wrightia tinctoria*

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***Phyllosticta tabernaemontanae*, a leaf spot fungus isolated from the diseased leaves of *Wrightia tinctoria*, showed the production of taxol, an anticancer drug, on modified liquid medium (M1D) and potato dextrose broth (PDB) medium in culture for the first time. The presence of taxol was confirmed by spectroscopic and chromatographic methods of analysis. The amount of taxol produced by this fungus was quantified using high performance liquid chromatography (HPLC). The maximum amount of taxol production was recorded in the fungus grown on M1D medium (461 μg/L) followed by PDB medium (150 μg/L). The production rate was increased to $9.2\times10^3$ fold than that found in the culture broth of earlier reported fungus, *Taxomyces andreanae*. The results designate that *P. tabernaemontanae* is an excellent candidate for taxol production. The fungal taxol extracted also showed a strong cytotoxic activity in the *in vitro* culture of tested human cancer cells by apoptotic assay.**

***Keywords*:** anticancer drug, leaf spot fungus, *in vitro* cytotoxicity test, *Phyllosticta tabernaemontanae*, taxol production

Taxol is a highly functionalized, diterpene anticancer drug widely used in hospitals and clinics. It is especially targeted to treat breast, lung, and ovarian cancers and was originally isolated from the inner bark of Pacific yew, *Taxus brevifolia* (Wani *et al*., 1971). This compound is the world's first billion dollar anticancer drug and it is also used to treat a number of other human tissue-proliferating diseases as well. Taxol differs from other cytotoxic drugs by its unique mode of action. It interferes with cell division by manipulating molecular regulation of cell cycle. The primary target of taxol is the microtubule, which is vital for mitosis, mortality, secretion, and proliferation. Taxol promotes the assembly of tubulin and stabilizes the resulting microtubules (Horwitz *et al*., 1993). Thus, the division of the cell in two equal daughter cells is interrupted (Horwitz, 1992; Jordan *et al*., 1993).

Taxol is found extremely in low amount in the needles, bark, and roots of yews (*Taxus* spp.). Presently, all taxol in the world's market has originated from *Taxus* spp. Although complete chemical synthesis of taxol has been achieved, the process is too expensive for commercialization. The cost makes it unavailable to many people worldwide. Ultimately, to lower the price of taxol and make it more available, a fermentation process involving microorganisms would be the most desirable and alternate source of supply (Yuan *et al*., 2006). The search for novel sources of taxol from the tree has led to the isolation of an endophytic fungus, *Taxomyces andreanae* colonizing the inner bark of the yew tree which is capable of producing taxol and other taxanes *de novo* when grown in semi-synthetic medium (Stierle *et al*., 1993). The discovery of *T. andreanae* was the first demonstration that any organism other than *Taxus* spp. could produce taxol. According to the report, the production of taxol using *T. andreanae* in culture broth yield about 24∼50 ng/L. A diversity of endophytic fungal species isolated from *Taxus* spp. has also been reported to produce taxol (Strobel *et al*., 1996a). However, the yield of taxol and taxanes were found to be low. Over the last 15 years, there has been a great deal of interest in finding endophytic fungi isolated from *Taxus* spp. and few other gymnosperm plant species. Specifically, these species alone were given importance towards the isolation of fungal taxol. In the present study, an attempt was made to screen taxol from the fungus *P. tabernaemontanae* associated with the leaf spot of *W. tinctoria*, an angiosperm (flowering plant), for the first time.

## Materials and Methods

### Fungal isolation

*Phyllosticta tabernamontanae* (Syd.) Aa was isolated from the infected leaves of *Wrightia tinctoria* (Roxb.) R. Br. (Apocyanaceae) collected from Yercaud, Tamilnadu, India. The leaf samples were thoroughly washed in running tap water and the infected regions alone were cut into small fragments (approximately 0.5 cm square pieces) with the aid of a flame-sterilized razor blade. Then the leaf fragments were surface sterilized by immersion in 70% ethanol

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for 5 sec, followed by 4% sodium hypochlorite for 60 sec, and then rinsed three times in sterile distilled water for 10 sec each. The excess moisture was blotted on a sterile filter paper. The surface sterilized leaf segments were evenly spaced in petri dishes (9 cm diameter) containing potato dextrose agar (PDA) medium (amended with chloramphenicol 150 mg/L). The petri dishes were sealed using Parafilm™ and incubated at 24±2°C in a light chamber with 16 h of light, followed by 8 h of dark cycles. The petri dishes were monitored every day to check the growth of fungal colonies from the leaf segments. The hyphal tips, which grew out from the leaf segments were isolated and sub-cultured onto PDA and brought into pure culture. Photomicrographs of conidia were taken with the help of Carl Zeiss Axiostar plus-Photomicroscope (bright field) with Nikon FM 10 Camera using Konica film. The isolated fungus was identified as *P. tabernamontanae* by using standard monographs (Van der Aa, 1973). The fungal culture was deposited at Madras University Botany Laboratory (MUBL) culture collection, University of Madras. The Coelomycetous fungus, *P. tabernamontanae* (MUBL No. 626), was screened for taxol production.

**Submerged fermentation and taxol isolation**

The fungus was grown in a 3 L Erlenmeyer flask containing 1,000 ml of modified M1D liquid medium supplemented with soytone (Pinkerton and Strobel, 1976). The potato dextrose broth (PDB) was also prepared for screening the test fungus for taxol production. The discs of 3 agar plugs (5 mm diameter) containing mycelia were used as inoculum. The organisms were grown at 24±2°C under still condition for 3 weeks in a light chamber with 16 h of light, followed by 8 h of dark cycles. The blank cultures (uninoculated sterile medium) were also maintained. After 3 weeks, the culture fluid was passed through four layers of cheese cloth to remove solids. Extra-cellular taxol was extracted from the culture medium by using dichloromethane (Strobel *et al*., 1996b). The solvent was then removed by evaporation under reduced pressure at 35°C in a rotary vacuum evaporator. The solid residue was dissolved in 1 ml of dichloromethane and placed on a 1.5×30 cm column of silica gel (40 μ). Elution of the column was performed in a step-wise manner starting with 70 ml of 100% dichloromethane, followed by methylene chloride:ethylacetate at different proportions (viz., 20:1 v/v, 10:1 v/v, 6:1 v/v, 3:1 v/v, 1:1 v/v). Fractions with same mobility as the standard taxol were combined and evaporated to dryness. The residue was subjected to chromatographic and spectroscopic analyses. The solvents used for the analyses were high performance liquid chromatography (HPLC) grade and the standard taxol (Paclitaxel) for reference purposes was purchased from Sigma Co.

**Chromatographic and spectroscopic analyses**

The thin layer chromatographic (TLC) analysis was carried out on Merck 1 mm (20×20 cm) silica gel pre-coated plate developed in a solvent A, Chloroform:Methanol, (7:1, v/v) followed by solvent B, Chloroform:Acetonitrile (7:3, v/v); solvent C, Ethyl acetate:2-propanol (95:5, v/v); solvent D, Methylene chloride:Tetrahydrofuran (6:2, v/v); solvent E, Methylene chloride:Methanol:Dimethylformamide (90:9:1, v/v/v), respectively. Taxol was detected with 1% (w/v) vanillin in sulphuric acid reagent after gentle heating (Cardellina, 1991). It appeared as a bluish spot fading to dark grey after 24 h. The area of the plate containing putative taxol was carefully removed by scraping off the silica and eluted with acetonitrile. The ultra-violet (UV) absorption of the samples were carried out with methanol at 273 nm (Wani *et al*., 1971) in a Backman DU-40 spectrophotometer. Samples were ground with infra-red (IR) grade potassium bromide pressed into discs under vacuum using spectra lab pelletiser and the spectrum was recorded in a Bruker Optics Vertex 80v FT-IR spectrometer.

Study on HPLC was conducted on HP1100 series using $C^{18}$ reverse phase column (Alltech Econosil, 250 mm×4.4 mm×10 μm) with an isocratic mobile phase consisting of methanol:water (80:20) at flow rate of 1 ml/min. Each sample of 10 μl was injected with the help of a micro syringe. Registration of peak and retention time was recorded on UV at 254 nm. Based on the HPLC analysis, fungal taxol was quantified by comparing the peak area of the samples with that of the taxol standard. The liquid chromatography-mass spectroscopic (LC-MS) analysis was also carried out on samples dissolved in methanol:water (9:1 v/v). Each sample was injected in Varian LC/MS 1200L Single Quadrupole MS with a spray flow of 2 μl/min and a spray voltage of 2.2 kV by the loop injection method. The proton nuclear magnetic resonance ($^1$H NMR) spectroscopic analysis was also performed in order to confirm the taxol structure by using Varian Unity Inova Spectrometer at 23°C (operating at 400 MHz with 16 scans and 298 real points). Samples dissolved in $CDCl_3$ (Sigma) were used for the analysis. Proton spectrums were assigned by comparison of chemical shifts and coupling constants with those of related compounds. Chemical shifts were reported as δ-values relative to tetramethylsilane (TMS) as internal reference and coupling constants were reported in Hertz.

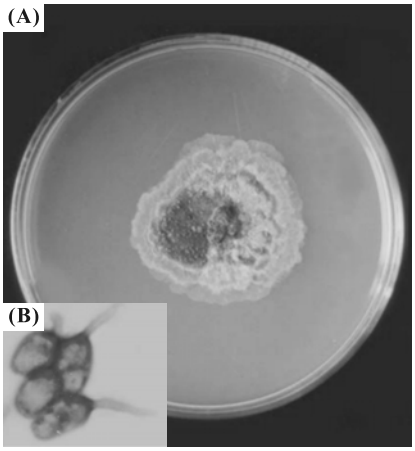


**Fig. 1.** Culture morphology of the taxol producing fungus, *Phyllosticta tabernaemontanae*. (A) A 10-day-old culture on PDA medium showing the mycelial growth with the formation of globular fruit bodies called pycnidia. (B) Conidial cells (×400) were viewed under bright field microscopy showing apical appendage stained with modified Leifson's flagella stain.

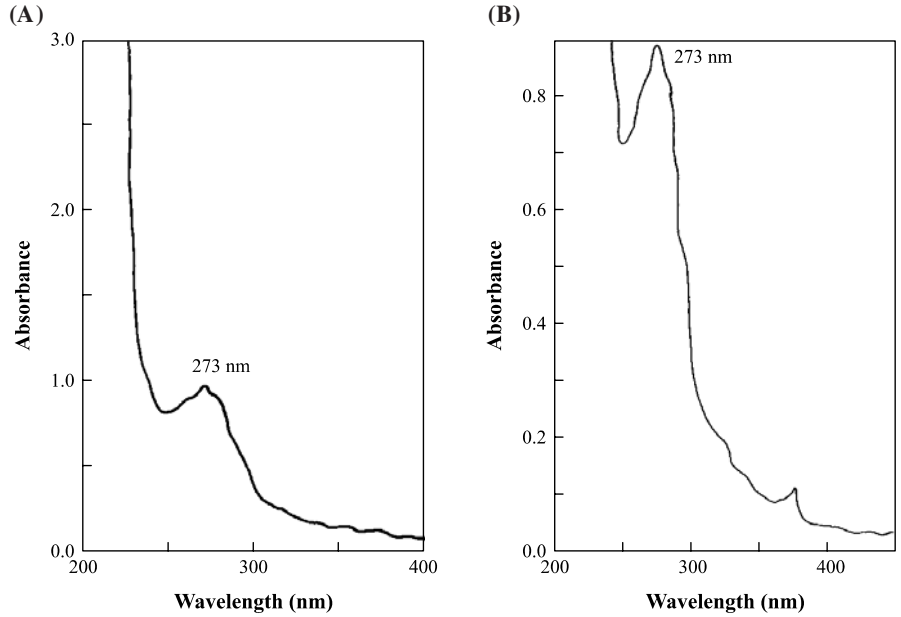


**Fig. 2.** UV absorption spectrum of standard taxol (A) and fungal taxol (B) showing the maximum absorption at a wavelength of 273 nm in methanol. Fungal taxol obtained form *P. tabernaemontanae* were found to be identical in comparison with standard taxol.

## Cytotoxicity test for taxol

The cytotoxic effect of fungal taxol isolated from the culture filtrate of M1D and PDB, was detected and quantified by using *in vitro* apoptotic method of assay (Ruckdeschel *et al*., 1997) on various cancer cells, at various concentrations. The human cancer cell lines (HLK210, H116, Int407, HL251, and BT220) used in the present study, were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The morphological changes of the cancer cells which were treated with different concentrations of fungal taxol ranging between 0.005 μM and 5 μM were incubated for 48 h. The cells were then stained (DNA staining) with 0.5 mg/ml propidium iodide in phosphate buffered saline (PBS) for 15 min and destained in PBS solution. After treatment with

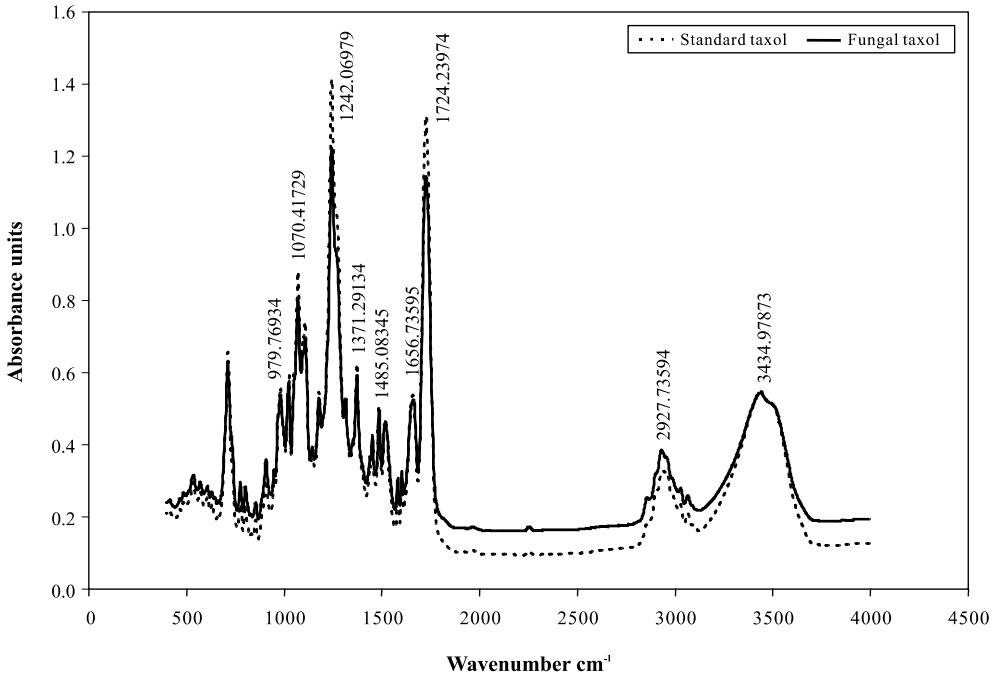


**Fig. 3.** IR spectrum of standard taxol (---) and fungal taxol (—) showing finger print region between 1000 and 3500 $cm^{-1}$. Fungal taxol finger prints were found to be similar in comparison with standard taxol.

different concentrations of fungal taxol, the cell morphology was determined by light microscopy. In all, five different fields were randomly selected for counting at least 500 cells. The percentage of apoptotic cells was calculated for each experiment. Cells designated as apoptotic were those that displayed the characteristic morphological features of apoptosis, including cell volume shrinkage, chromatin condensation, and the presence of membrane bound apoptotic bodies. For each experiment, 500 cells were counted. All cytotoxicity data shown are the means of at least three independent experiments. The cells in the apoptosis were calculated by using the following formula.

$$\text{Percentage of apoptotic cells} = \frac{\text{Number of apoptotic cells}}{\text{Total number of cells}} \times 100$$

## Results and Discussion

### Identification of the fungus

*P. tabernaemontanae* is a foliicolous Coelomycetous fungus. The fungal colony grew well in PDA with an initial pale gray colour, which later becomes ash-gray with a dark wavy

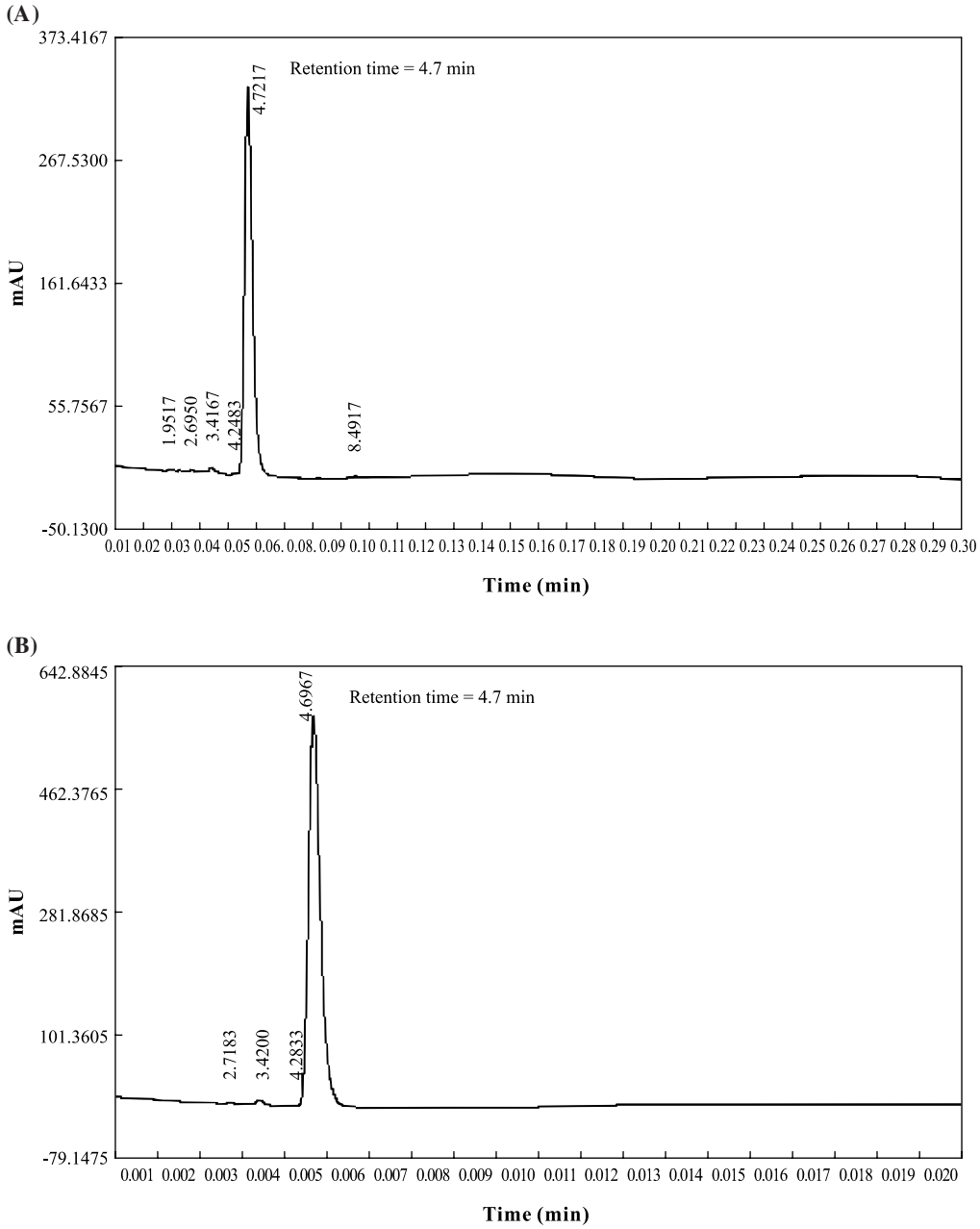


**Fig. 4.** HPLC analysis of standard taxol (A) and fungal taxol (B). The mobile phase was methanol/water (80:20, v/v), with the flow rate of 1.0 ml/min. The registration of peak and retention time was recorded on UV at 254 nm. Fungal sample showing a peak with retention time of 4.7 min, which was found to be identical in comparison with standard taxol.

margin (Fig. 1A). A good sporulation was observed during 10~15 days of incubation. Pycnidial bodies measured about 55~100 μm diameter in size and papillate with an irregular pore. Conidial spores were found to be one-celled, subspherical to obovoidal in shape and size measuring about 8~11×3~7 μm (Fig. 1B). Each conidium was enclosed by a slime layer with an apical appendage measuring 7~14 μm in size. In the earlier studies, *P. tabernaemontanae* is reported from the infected leaves of *Tabernaemontana pandacaque* (Van der Aa, 1973), whereas in the present study, it was isolated from the leaf spot of *W. tinctoria*, a new host which was not reported before.

## TLC, UV, and IR analyses

Presence of taxol in the fungal samples was confirmed by TLC, showing a blue-gray colour reaction with the vanillin/sulfuric acid reagent. The compound displayed chromatographic properties similar to that of standard taxol, giving colour reaction with the spray reagent and exhibiting a Rf value identical with the taxol standard (Cardellina, 1971). The UV absorption at 273 nm (Fig. 2A and B) also authenticates the presence of taxol in the fungal sample, in comparison with standard taxol (Wani *et al*., 1971). The IR spectrum showed a broad peak at 3434.99 $cm^{-1}$, which was assigned for the presence of O-group in the parent com-

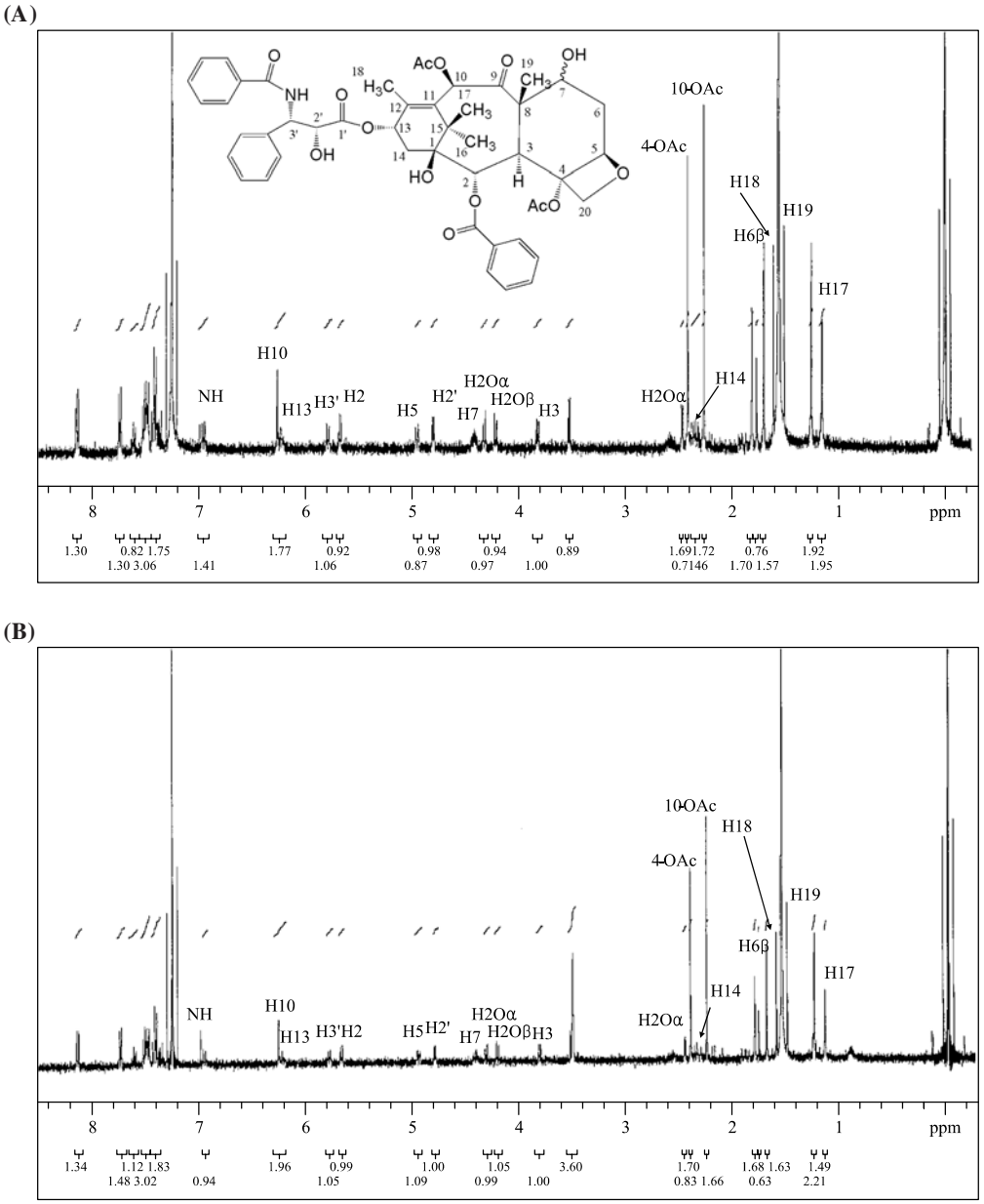


**Fig. 5.** $^{1}H$ NMR spectrum of standard taxol (A) and fungal taxol (B) in $CDCl_3$ at 400 MHz. All the signals were well resolved and distributed in the region between 1.0 and 8.5 ppm. The chemical shifts in ppm with high frequency from TMS. The structure of taxol is shown as an insert. In comparison with standard taxol, fungal taxol also produced an identical spectrum.

pound, and it was evident by its OH stretch. The aliphatic CH stretch was observed at 2927.74 $cm^{-1}$. The C=O (keto group) stretch was positioned at 1724.24 and 1656.74 $cm^{-1}$. The registration peak observed at 1485.08 and 1450.37 $cm^{-1}$ was due to NH stretching frequency. The COO stretching frequency was observed at 1371.29 and 1242.07 $cm^{-1}$. The peaks in the range between 1070.42 and 979.77 $cm^{-1}$ were due to the presence of aromatic C and H bends. Fungal taxol was further confirmed by IR finger prints recorded between 1000 and 3500 $cm^{-1}$, which was also identical in comparison with standard taxol (Fig. 3). Therefore, it was evident that this fungus showed positive results for taxol production in both M1D and PDB medium.

## HPLC separation analysis

Results of HPLC analysis showed the presence of taxol by

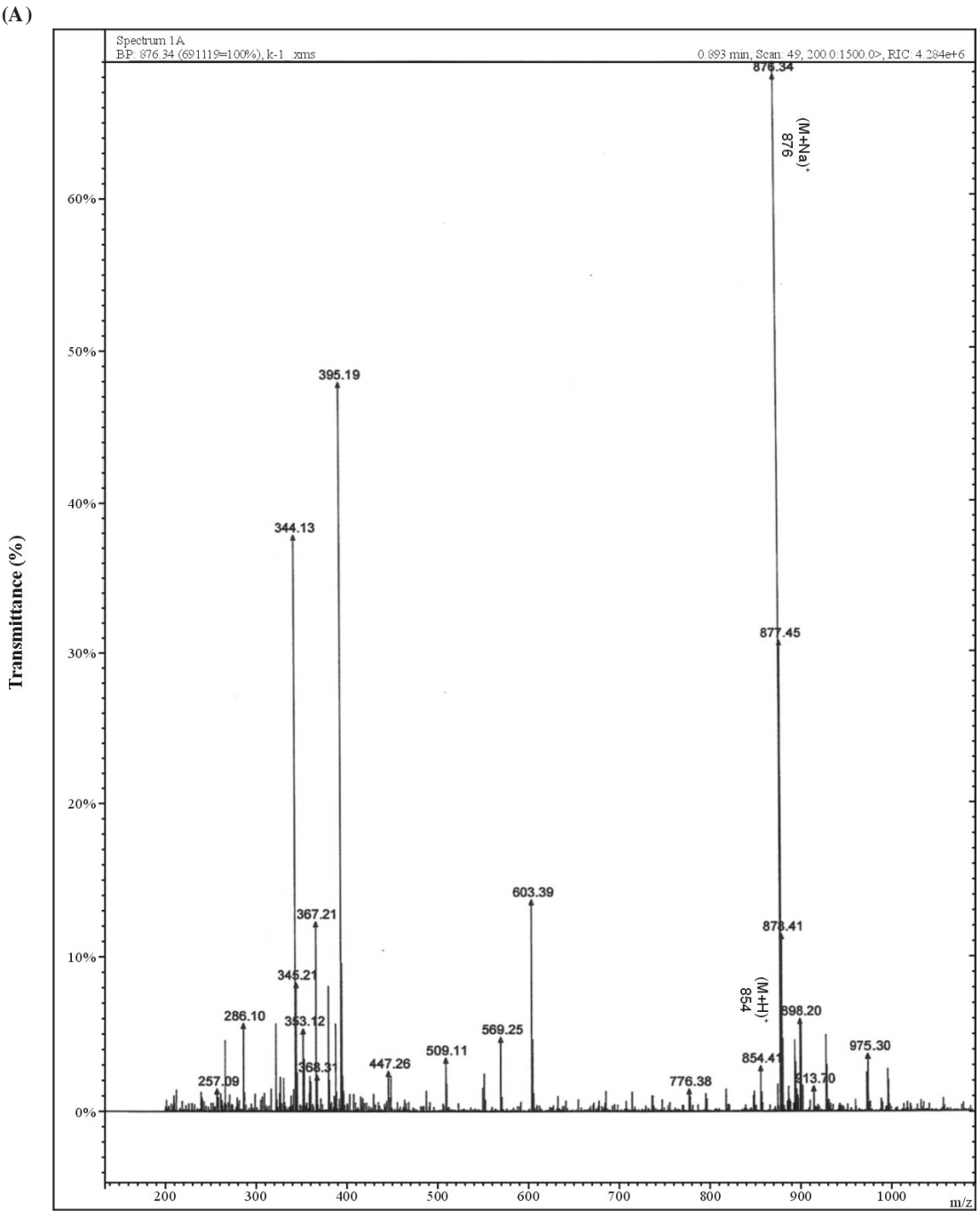


**Fig. 6.** LC-MS analysis of standard taxol (A) and fungal taxol (B). Mass spectrum of the fungal extracts showing a $(M+H)^+$ peak at molecular weight 854 and a $(M+Na)^+$ peak at molecular weight 876 was identical in comparison with standard taxol. Sample was dissolved in methanol/water (9:1, v/v) and injected with a spray flow of 2 µl/min with a spray voltage of 2.2 kV.

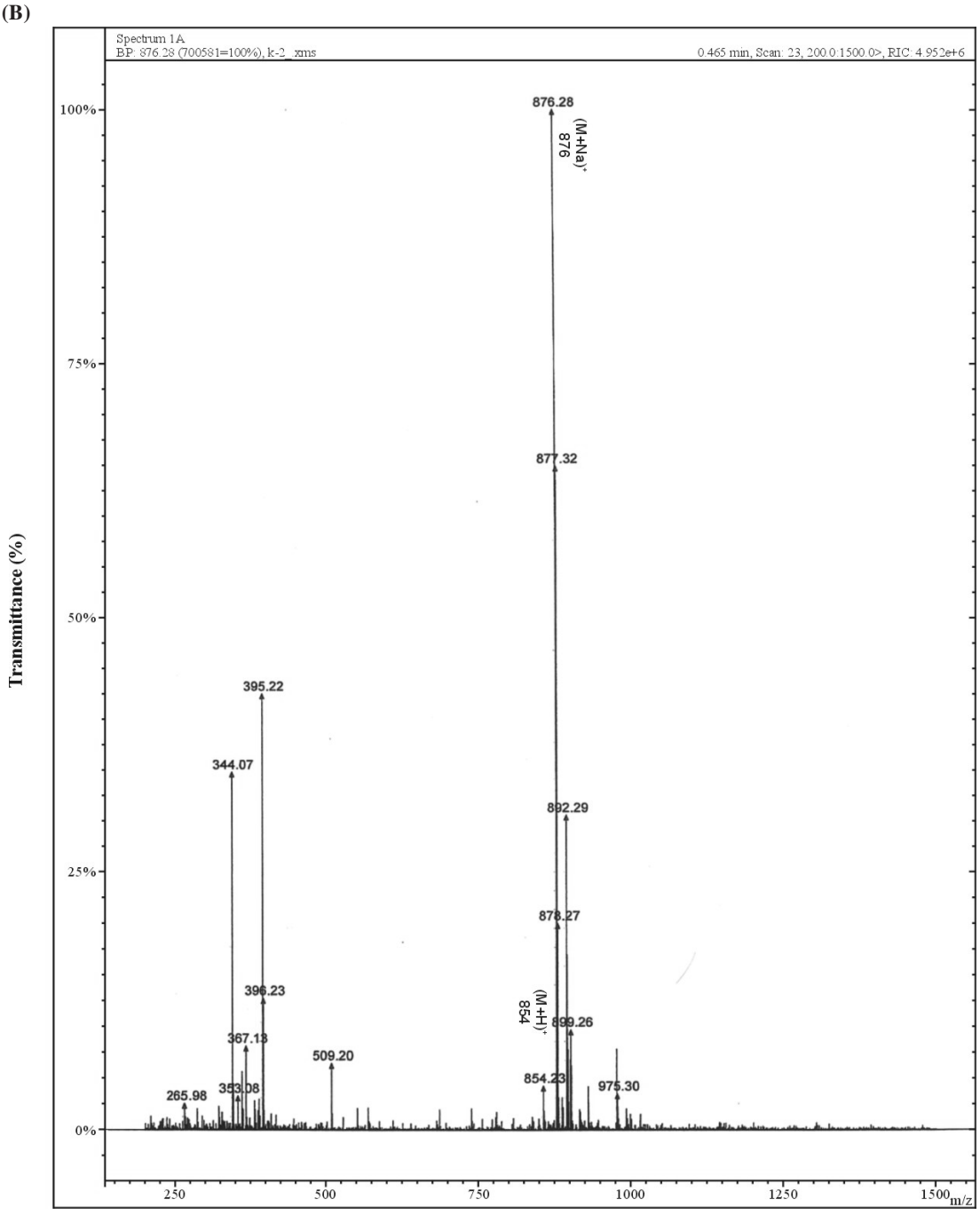


**Fig. 6.** Continued

showing a peak with a retention time of 4.7 min (Fig. 4A and B). *P. tabernaemontanae* produced a high content of taxol in M1D (461 μg/L) when compared with PDB (150 μg/L). Taxol detection was not observed in the blank culture samples, in which they showed negative results in all the analyses. The fungal taxol yield was easily quantified with HPLC analysis since the production was found to be higher (in micrograms). Where as, in the earlier reports it was quantified with the aid of immunoassay since the yield level was recorded to be low (in nanograms) (Li *et al*., 1996; Strobel *et al*., 1996a; Strobel *et al*., 1997). The biggest problem of using fungi in fermentation is the low level of yield with unstable taxol production. Taxol yield of such reported fungi diverges from 24 to 70 ng/L (Stierle *et al*., 1993; Strobel *et al*., 1996a). Although the amount of taxol produced by the endophytic fungi associated with yew trees is relatively small, when compared with the host trees, the short generation time and high growth rate of fungi will make it worthwhile to continue our investigation on *P. tabernaemontanae* isolated from *W. tinctoria*.

## $^1$H NMR and LC-MS analyses

In $^1$H NMR spectroscopic analysis, almost all the signals were well resolved and distributed in the region between 1.0 and 8.5 ppm (Fig. 5A and B). The strong three-proton signals caused by the methyl and acetate groups lie in the region between 1.0 and 2.5 ppm, together with multiplets caused by certain methylene groups. Most of the protons in the taxane skeleton and the side-chain were observed in the region between 2.5 and 7.0 ppm, and the aromatic proton signals caused by C-2' benzoate, C-3' phenyl, and C-3' benzamide groups appeared between 7.0 and 8.3 ppm. The $^1$H NMR spectrum of the fungal taxol was found to be identical in comparison with standard taxol. The characteristic chemical shifts of taxol are shown in Fig. 5. The taxol assignments obtained in the present investigation were also confirmed with the earlier report (Chmurny *et al*., 1992). Further convincing evidence for the identity of taxol was obtained by LC-MS spectroscopic analysis (Fig. 6A and B). Characteristically, standard taxol yielded both a $(M+H)^+$ peak at molecular weight 854 and a $(M+Na)^+$ peak at molecular weight 876. On comparison, fungal taxol also produced peaks $(M+H)^+$ at *m/z* 854 and $(M+Na)^+$ at *m/z* 876 with characteristic fragment peaks at 344, 367, and 395. Major fragment ions observed in the mass spectrum of taxol are placed into three categories, which represent the major portion of the taxol molecule (McClure and Schram, 1992). The peaks analogous to taxol exhibited mass-to-charge (*m/z*) ratios corresponding to the molecular ions $(M+H)^+$ of the standard taxol (854), confirming the presence of taxol in the fungal extracts. It was evident that the diterpene taxol was much more complex since its molecular weight from high-resolution mass spectrometry is 854, corresponding to the molecular formula of $C_{47}H_{51}NO_{14}$ as reported earlier (McClure and Schram, 1992).

Analytical methods acquired clearly suggested that the fungal compound is taxol, which was produced in both M1D and PDB in comparison with standard taxol. The blank cultures did not detect the presence of taxol. Early reports on the isolation of endophytic fungi from *Taxus* spp. (Fungal species: *Monochaetia* sp., *Fusarium lateritium*, *Pestalotia bicilia*, *Alternaria* sp., *Pestalotiopsis microspora*, and *Pithomyces* sp.) and few other gymnosperm plants (Fungal species: *Pestalotiopsis* spp., *P. microspora*, *P. guepinii*, *Periconia* sp., and *Alternaria* sp.) showed limited production of taxol (in nanogram level) in the artificial culture media (Li *et al*., 1996; Strobel *et al*., 1996a; Strobel *et al*., 1997; Li *et al*.,

**Table 1.** Taxol-induced apoptosis by the endophytic fungi, *P. tabernaemontanae* in various human cancer cell lines

| Sl. No. | Cell lines | Taxol Conc. (μM) | % of Apoptotic cell | | |
|---|---|---|---|---|---|
| | | | Taxol Standard | Taxol form M1D | Taxol form PDP |
| 1 | HLK 210 (Leukemia) | 0 | 0 | 0 | 0 |
| | | 0.005 | 25.3 (±2.32) | 24.2 (±2.32) | 23.7 (±2.44) |
| | | 0.05 | 65.6 (±3.42) | 63.7 (±4.58) | 62.8 (±3.54) |
| | | 0.5 | 78.7 (±6.81) | 78.5 (±5.68) | 77.4 (±5.42) |
| | | 5 | 37.4 (±3.12) | 34.7 (±3.24) | 34.2 (±2.68) |
| 2 | HL 251 (Lung) | 0 | 0 | 0 | 0 |
| | | 0.005 | 20.1 (±1.90) | 18.5 (±1.23) | 18.2 (±1.84) |
| | | 0.05 | 27.3 (±2.12) | 65.7 (±2.96) | 63.6 (±4.56) |
| | | 0.5 | 79.8 (±5.22) | 77.4 (±4.22) | 76.3 (±6.48) |
| | | 5 | 37.4 (±3.95) | 35.6 (±2.34) | 34.2 (±2.95) |
| 3 | Int 407 (Intestine) | 0 | 0 | 0 | 0 |
| | | 0.005 | 21.4 (±2.13) | 19.3 (±1.15) | 18.7 (±1.92) |
| | | 0.05 | 67.2 (±4.35) | 65.4 (±2.85) | 63.5 (±4.27) |
| | | 0.5 | 74.2 (±5.43) | 76.5 (±4.34) | 74.9 (±6.74) |
| | | 5 | 24.2 (±2.62) | 22.5 (±1.56) | 21.7 (±1.68) |
| 4 | H 116 (Colon) | 0 | 0 | 0 | 0 |
| | | 0.005 | 18.5 (±1.94) | 15.7 (±0.67) | 15.4 (±1.78) |
| | | 0.05 | 62.4 (±4.52) | 60.5 (±3.85) | 58.5 (±2.97) |
| | | 0.5 | 73.2 (±5.38) | 74.8 (±6.54) | 73.4 (±6.35) |
| | | 5 | 30.4 (± 3.12) | 32.4 (±1.80) | 32.3 (±2.47) |
| 5 | BT 220 (Breast) | 0 | 0 | 0 | 0 |
| | | 0.005 | 20.7 (±1.82) | 18.8 (±0.85) | 18.2 (±1.58) |
| | | 0.5 | 79.3 (±4.32) | 78.3 (±4.37) | 77.6 (±5.95) |
| | | 5 | 84.6 (±6.72) | 83.7 (±6.44) | 81.7 (±6.91) |
| | | 5 | 23.5 (±2.72) | 24.8 (±1.20) | 24.3 (±2.48) |

1998a, 1998b; Kim *et al*., 1999; Guo *et al*., 2006). In present investigation, the total amount of taxol produced per litre in M1D, was 461 μg, and found to be the highest in the fungi reported so far. This was three orders of magnitude (i.e. $9.2\times10^3$ fold) more than that produced by *Taxomyces andreanae* (Stierle *et al*., 1993). The taxol production rate was increased to 7 fold than that found in the culture filtrate of *P. microspora* (Strobel *et al*., 1996b). And it was also found to be increased approximately double the rate of production when compared with the early reported fungi, *P. citricarpa* and *P. spinarum* (Kumaran *et al*., 2008a, 2008b). Most of the earlier reported fungal taxol producers are isolated as endophytic forms especially from yews (*Taxus* spp.) rather than other plant groups. Whereas, in the present study, *P. tabernaemontanae* is a leaf spot fungus isolated from the infected leaves of *W. tinctoria*, and exhibited extracellular taxol production in M1D and PDB medium for the first time. Also, the production of taxol by a phytopathogen, *P. tabernaemontanae* was found to be recorded here for the first time. Some possible evidence from the earlier reports proved that the plant pathogens in the environment might have originated from the endophytic forms in the host plants (Carroll, 1988). Many innocuous fungal endophytes are quiescent phytopathogens which may cause infectious symptoms when the host plant is aged or stressed (Tan and Zou, 2001). Thus, taxol-producing fungi may be found not only in yews, but from other plant species that share the same environmental requirements with the yew. The genetic origin of fungal taxol production has been speculated to have arisen by horizontal gene transfer from host plant to its endophytes (Strobel *et al*., 1996b; Strobel *et al*., 1997). Little documentation exists for gene transfer from a higher plant to an endophyte or parasite. Alternatively, of course, fungi may be an independently evolved system for taxol production (Strobel *et al*., 1996a). Fungi are, obviously, rich and reliable sources of bioactive and chemically novel compounds with huge medicinal and agricultural potential.

**Cytotoxic evaluation of taxol on human cancer cells**

Cytotoxicity effect of fungal taxol from *P. melochiae* was further tested using apoptotic assay on various human cancer cells viz. leukemia cell HLK210, lung cell-HL251, intestine cell-Int407, colon cell-H116, breast cell-BT220. It is indicated that with the increase of taxol concentration from 0.005 μM to 0.05 μM, taxol induced increased cell death through apoptosis. With further increase of taxol concentration from 0.05 μM to 0.5 μM, the taxol-induced cell death through apoptosis only increased slightly. When the taxol concentration was increased from 0.5 μM to 5 μM, the taxol-induced cell death through apoptosis decreased significantly (Table 1). In the present investigation, it was observed that at low to medium concentration (0.005 to 5 μM), the efficacy of fungal taxol was relatively dependent on the specific cell type. This is in concurrence with the results of earlier report (Yeung *et al*., 1999). It has been reported that taxol at low concentrations (nM) induces cell apoptosis and the efficacy of taxol is fairly dependent on the specific cell type. This also supports the earlier findings of other groups that at low concentration, taxol inhibits cell proliferation by blocking mitosis.

In conclusion, taxol production from the fungi reported so far are isolated as endophytic forms from gymnosperm plants. Where as, in this investigation, production of taxol was recorded by a leaf spot fungus, *P. tabernaemontanae*, isolated from *W. tinctoria*, an angiosperm plant, for the first time. In the present study, it is confidently evident that the spectroscopic and chromatographic estimates are close to reality, given fact that the fungal taxol and standard taxol give identical results. It also indicates that the formation of taxol by the fungus, *P. tabernaemontanae*, was found to be the highest, and suggests that the fungus can serve as a potential species for genetic engineering to enhance the production of taxol.

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