

Infra-red Spectral analysis of Taxol Produced by Different Species of *Pestalotiopsis*

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Abstract

Twenty species of *Pestalotiopsis* were isolated from different hosts and screened for the production of an anti-cancer drug taxol. Taxol production was confirmed in all the 20 fungi by ultra violet (UV) spectroscopic analysis, and Infra-red Spectrum analysis (IR). The taxol produced by the fungi was compared with that of standard taxol. The taxol from the selected fungi were identical with authentic taxol.

Keywords: Taxol; Anti-cancer; Fungi; *Pestalotiopsis*; IR – Spectrum

Introduction

Coelomycetes group were mainly isolated from the tropical and temperate regions, less so from the Antarctic and Arctic but they are capable of growing, reproducing and surviving in a wide variety of ecological situations. They are commonly found and recovered from different cultivated and uncultivated soils of different types, leaf litter and other organic debris from both natural [1]. There are reports of some members of Coelomycetes like *Pestalotiopsis microspora* [2,3] which possess anti cancerous drugs, and *Pestalotiopsis* sp. has been reported to be anti-diabetic [4].

Taxol

Among the various anticancer drugs of plant origin, taxol is considered as the most important chemotherapeutic agent discovered so far. Taxol was approved in 1992 by the US Food and Drug Administration for the treatment of refractory ovarian and breast cancer. It was shown to be active against a variety of cancers such as lung, gastrointestinal, neck and head as well as malignant melanoma [5].

Taxol, the world's first billion-dollar anticancer drug

The most important member of the clinically useful natural anticancer agent is paclitaxel (Taxol), which was first extracted from the bark of western yew (*Taxus brevifolia*) [6]. This compound is the world's first billion-dollar anticancer drug, and it is used to treat a number of other human tissue-proliferating diseases as well.

Taxol from fungi

By the early 1990's, however, fungi had been isolated from many of the world's representative yew species. After several years of effort, the search for novel sources of Taxol has led to the isolation of a novel endophytic fungus (*Taxomyces andreanae*) colonizing the inner bark of the yew tree (*Taxus brevifolia*) which is capable of producing Taxol and other taxanes *de novo* when grown in semi-synthetic liquid medium [7]. However, the yield of taxoids from *T. andreanae* is very low (24-50 ng/l). This study also suggests that improved culture techniques, addition of activators / elicitors and application of genetic engineering methods may result in enhanced production of taxoids by this endophytic microbe and permit commercialization of *T. andreanae* for Taxol production.

Strobel *et al.* [8] (1996) studied Taxol production from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*, and reported

that some of the most commonly found endophytes of the world's yews are *Pestalotiopsis* spp. Li *et al.* [9] (1996) also reported that one of the most commonly isolated endophytic species is *Pestalotiopsis microspora*. Strobel *et al.* [10] (1996) isolated *Pestalotiopsis guepini* from extremely rare and previously thought to be extinct, Wollemi Pine (*Wollemia nobilis*), which has been proved to produce taxol. Subsequently, Li *et al.* [9] (1996) isolated an endophytic fungus *Periconia* sp. from *Torreya grandifolia*. Also, quite surprisingly, a rubiaceaceous plant – *Maguireothamnus speciosus*, yielded a novel fungus *Seimatoantlerium tepuiense* that produces taxol.

In recent years, other investigators have also made observations on taxol production by endophytes. Caruso *et al.* [11] (2000) detected taxol production from 15 out of 150 fungal strains isolated as endophytes from the internal tissues of woody branches, shoots and leaves of different plants belonging to the genus *Taxus* spp. The production of taxanes in a few cases reached 50-100 ng/l. Wang *et al.* [12] (2000) studied taxol production from *Tubercularia* sp. isolated from Southern Chinese yew (*Taxus mairei*) in the Fujian province of southeastern China. In addition to that, Shrestha *et al.* [13] (2001) showed a new report in the production of taxol from three different endophytic fungi isolated from the Himalayan yew *Taxus Wallachiana* viz., *Sporormia minima*, *Trichothecium* sp. and an unidentified dimorphic fungus were confirmed by different analytical and immunoassay methods. It can be inferred that fungi more commonly produce greater quantity of Taxol than higher plants; and the distribution of those fungi producing Taxol is worldwide and not confined to endophytes specific host are from specific geographic location. Thus, it may be thought that taxol had its origin in certain fungi and ultimately, if there is any lateral gene transfer, it may have been in the direction of the microbe to the higher plants [8] (Strobel, 1996). *In vitro* taxol production from *Pestalotiopsis breviseta*, Coelomycetous fungi, first reported by Kathiravan and

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Sriraman (2010) [14,15], was confirmed by Ultra Violet, Infra-red, High performance liquid chromatography, Nuclear magnetic resonance and LC MASS spectroscopy [16]. The presence of Taxol was identical with that of authentic taxol. Similar work was carried out by Kathiravan and Muthumary, 2009 [17]; Kumaran *et al.*, 2008 a, b [18,19]; Kathiravan *et al.*, 2013 [20]; Gowri *et al.*, (2011) [21].

Materials and Methods

The general laboratory techniques followed in the course of the present investigation were as outlined by Booth (1971) [22]. The test fungi used in the present study were grown in 2L Erlenmeyer flasks containing 500 ml MID medium supplemented with 1 g Soytone L⁻¹ for taxol production. Three mycelial agar plugs (0.5 cm) were used as inoculum. The organisms were grown at 24 ± 2°C under static condition for 3-4 weeks [23].

Isolation methods

The samples were placed on suitable agar growth media like PDA and Oatmeal Agar amended with antibiotics. Plates were incubated at a temperature close to that of the collection site. The length of incubation depends on the growth rate of the fungal propagules. Fungal species were isolated when separate colonies appeared. Appendage and sheaths were observed using differential interference or phase contrast microscopy. Measurements of the various structures were made on fresh mounts. Dimensions of these structures were taken from at least 100 individuals. All the cultures were deposited in Madras University Botany Laboratory (MUBL). Microbial Type Culture Collection, Chandigarh (MTCC).

Culture media [8] (Strobel *et al.*, 1996)

MID medium was supplemented with Soytone - 1.0 g, Sucrose - 30.0 g/l, Ammonium tartrate - 5.0 g, Yeast extract - 0.5 g, Ca₂ (NO)₃ - 280 mg, KNO₃ - 80 mg, KCl - 60 mg, MgSO₄ - 360 mg, NaH₂PO₄ - 20 mg, H₃BO₃ - 1.4 mg, MnSO₄ - 5.0 mg, ZnSO₄ - 2.5 mg and KI - 0.7 mg.

Extraction of taxol

Extraction of taxol was performed according to Strobel *et al.* [8] (1990). After incubating the culture for 3-4 weeks the culture filtrate was passed through four-layered cheesecloth. In order to avoid fatty acid contamination of taxol, 0.25 g of NaCO₃ was added to the filtrate. The culture fluid was extracted with two equal volumes of methylene chloride and the organic phase was evaporated to dryness under reduced pressure at 35°C.

Column chromatography

A 1.5 × 30 cm column of silica gel was loaded with the crude sample dissolved in methylene chloride. Elution of the sample was done in a stepwise manner with solvent system as 70 ml of 100% methylene chloride, 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 and 1:1 v/v methylene chloride : ethylacetate. Fractions having the same mobility as that of the authentic Taxol were combined and evaporated to dryness. The residue was subjected to thin layer chromatography.

Thin layer chromatography (TLC) analysis

TLC analysis was carried out on Merck 1 mm (20 × 20 cm) silica gel plate developed with 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) and solvent E (Methylene chloride : Methanol : Dimethylformamide, 90:9:1, v/v/v) respectively. The area of the plate containing putative taxol was carefully removed by scraping off the silica at the appropriate R_f and eluted with acetonitrile. Taxol was detected with 1 % w/v vanillin / sulphuric acid reagent after gentle heating. It appeared as a bluish spot that faded to dark grey after 24 h.

Ultra Violet (UV) spectroscopic analysis of taxol

The purified sample of taxol was analyzed by UV absorption, dissolved in 100 % methanol at 273 λ_{max} and compared with authentic taxol.

Infer Red spectroscopic analysis of taxol

The purified TAXOL was ground with IR quality potassium bromide (1:10) pressed into discs under vacuum using spectra lab Pelletiser and the spectrum was recorded (4000– 500 cm⁻¹ nm) in a Burker 17S 85 FTIR Spectrophotometer.

Results and Discussion

Colony morphology

Cultures were growing faster on the Potato dextrose agar , (Plate 1 and Table 1) Czpek Dox agar and Oate meal agar medium and the diameter of colony reached 80 mm after 5 days incubation at 30 ± 2°C. The colony was flat and velvety to woolly and was covered by brown to white hyphal area, short and long. The surface was light brown to white. Reverse side was typically pale brown to black due to melanin production. Out of 20 (a, f, h, and n) which are light brown others produced white colonies.

Growth rate

Among the three medium Potato Dextrose Agar, Czapek Dox Agar and Oat Meal Agar used. PDA medium was good for producing mycelia, followed by OMA and CDA, respectively. After 5 days of growth, among the twenty species, only three species showed maximum of growth (80 mm) in PDA, 70 mm growth in OMA and CDA was showed minimum growth (Table 1).

Infer Red – Results (Tables 2 & 3)

Pestalotiopsis acacia / *P. clavispora* (Figure 1a-t)

An intense band was observed at 3379.1 cm⁻¹, which is due to the absorption of H-OH. The sharp band at 2927.7 cm⁻¹ is due to the absorption of sp³ hybridized carbon and the band at 2858.3 cm⁻¹ is due to the absorption of symmetric stretching vibration of methylene group. Bands observed at 2364.6 cm⁻¹ and 2337.6 cm⁻¹ regions were due to the absorption of -COOH and enols. The band at 1720.4 cm⁻¹ was due to the absorption of ketone and band at 1654.3 cm⁻¹ was due to the absorption of weak combinations and overtones. Symmetrical stretching of Carboxylic acid gave rise to a band at 1384.8 cm⁻¹ and C-O stretching in esters gave rise to a band at 1261.4 cm⁻¹. An intense at 1068.5 cm⁻¹ due to the unconjugated C-N linkage bands coupled with the stretching of adjacent bands.

P. adusta / *P. coangae*

A highly intense band was observed at 3363.6 cm⁻¹ which was broad; this band was due to the absorption of H-OH. A peak with less intensity was observed at 2939.3 cm⁻¹, which was due to the absorption of sp³ hybridized carbon. There was no band observed at 2858.3 cm⁻¹ and 1720.4 cm⁻¹. This indicates the absence of methylene group and

overtone. Band found at 2360.7 cm^{-1} was due to $-\text{COOH}$ and enols. The bands at 1631.7 cm^{-1} and 1404.1 cm^{-1} indicate the presence of weak combinations of overtones and cyclopropane, respectively. The bands at 1307.6 cm^{-1} , 1261.4 cm^{-1} and 1215.1 cm^{-1} were observed due to C-N stretching and C-O stretching of esters and ketone. An intense band at 1064.6 cm^{-1} was observed which was due to the unconjugated C-N linkage bands coupled with the stretching of adjacent bands.

S.No	Fungal Name	MUBL NO/ MTCC NO	Growth medium and mycelia growth (mm)		
			PDA	OMA	CDA
1	<i>Pestalotiopsis acaciae</i>	453	65	60	54
2	<i>P. adusta</i>	469/4166	80	78	62
3	<i>P. breviseta</i>	470	80	77	61
4	<i>P. calabae</i>	471	67	58	50
5	<i>P. clavispora</i>	455,	62	56	52
6	<i>P. coangae</i>	454	69	60	58
7	<i>P. coffeae</i>	472/4241	71	65	62
8	<i>P. conigena</i>	456	68	60	58
9	<i>P. eriobotryofolia</i>	457	60	52	48
10	<i>P. foedaris</i>	459	67	54	48
11	<i>P. fibricola</i>	458/3445	69	56	50
12	<i>P. glandicola</i>	460	73	68	60
13	<i>P. japonica</i>	461	80	78	64
14	<i>P. matildae</i>	462	75	68	60
15	<i>P. oleandri</i>	463	72	64	59
16	<i>P. paeoniae</i>	464	73	66	60
17	<i>P. paciseta</i>	465	76	68	56
18	<i>P. taxica</i>	467	74	69	58
19	<i>P. torulosa</i>	466	71	64	59
20	<i>P. zalbrukneriana</i>	468	70	66	59

Table 1: Fungal growth on different media.

Chemical group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Methyl group	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methylene group	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+
Aromatic group	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-
Amide Group	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	-
Alcohol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ester	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Ketone	+	-	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+
Carboxylic acid	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-
Cyclic group Cyclic propane	-	+	-	-	-	-	-	-	+	-	+	+	-	+	+	+	+	-	+	+
C-N Linkage	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Combination and Overtone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+

Table 2: Infrared – spectroscopy analysis of Chemical groups of 20 Different *Pestalotiopsis* spp.

P. breviseta

A band at 3776.4 cm^{-1} was observed which increased with increase in intensity. The peak at 3409.9 cm^{-1} was broad and intense due to the absorption of H-OH. There were no band observed at 2858.3 cm^{-1} , 2337.6 cm^{-1} , 1720.4 cm^{-1} and 1261.4 cm^{-1} indicating the absence of methylene group, ketone group and esters. This intensity of the peak at 2353 cm^{-1} increased considerably and appeared sharp. The bands at 1647.1 cm^{-1} , 1384.8 cm^{-1} , and 1065.9 cm^{-1} were broad due to the absorption of weak combinations and overtone, symmetrical stretching (carboxylic acid) and unconjugated C-N linkage bands coupled with the stretching of adjacent bands. The band at 2920 cm^{-1} was due to the absorption of sp³ hybridized carbon. A band at 840.9 cm^{-1} was due to the aromatic C-H out of plane bending.

P. calabae

There was a band at 3787.9 cm^{-1} with increase in intensity. A band at 3394.5 cm^{-1} was broad and intense due to the absorption of H-OH. The band at 2923.9 cm^{-1} was less intense due to the absorption of sp³ hybridized carbon. There were no bands observed at 2858 cm^{-1} and 1261 cm^{-1} indicating the absence of methylene group and esters. There was a sharp band at 2353.0 cm^{-1} . A band at 1651 cm^{-1} was due to the absorption of weak combinations overtones. Slightly prominent bands were observed at 1454.2 cm^{-1} and at 1384.8 cm^{-1} due to the C-H deformation and symmetrical stretching (carboxylic acid), respectively. The band at 840.9 cm^{-1} was due to the absorption of aromatic C-H out of plane bending.

P. coffeae

The broad intense peak at 3382.9 cm^{-1} was due to the absorption of H-OH. The band at 2923.9 cm^{-1} with decreased intensity was due to the absorption of sp³ hybridized carbon. There was a slight twist in the peak at 1731.9 cm^{-1} which was due to the absorption of ketone. The band at 2349.1 cm^{-1} was highly intense. The band at 1654.8 cm^{-1} was due to the absorption of weak combinations and overtones. The bands at 1465.8 cm^{-1} and 1380.9 cm^{-1} were due to the C-H deformation and symmetrical stretching (carboxylic acid). The band at 1045.3 cm^{-1} was due to the un-conjugated C-N linkage; coupled with the stretching of adjacent bands. There was a band at 844.8 cm^{-1} due to the absorption of aromatic C-H out of plane bending.

P. conigena

A sharp peak at 3776.4 cm^{-1} (non-H bonded OH stretching which was found to the left of the broad band at 3467.5 cm^{-1} . The broad band

was intense. A less intense peak at 2935.4 cm⁻¹ was due to the absorption of sp³ hybridized carbon. There was a sharp intense peak at 2350 cm⁻¹ and the prominent peak was found at 1731.9 cm⁻¹. The peak is due to the absorption of ketone. The intensity of the peak at 1654.8 cm⁻¹ has been increased. This peak is due to the absorption of weak combination and overtones. The very prominent peak appears at 1458.1cm⁻¹ due to the C-H deformation. Another peak at 1377.1 cm⁻¹ was observed due to

the symmetrical stretching (carboxylic acid). A band at 1050 cm⁻¹ was observed due to the unconjugated C-N linkage bands coupled with the stretching of adjacent bands. A peak was observed at 848.8 cm⁻¹ due to C-H aromatic out of plane bending. The peak at 663.5 cm⁻¹ was intense due to the absorption of bromide and iodide. This may be due to the solvent effect.

P. eriobotryofolia

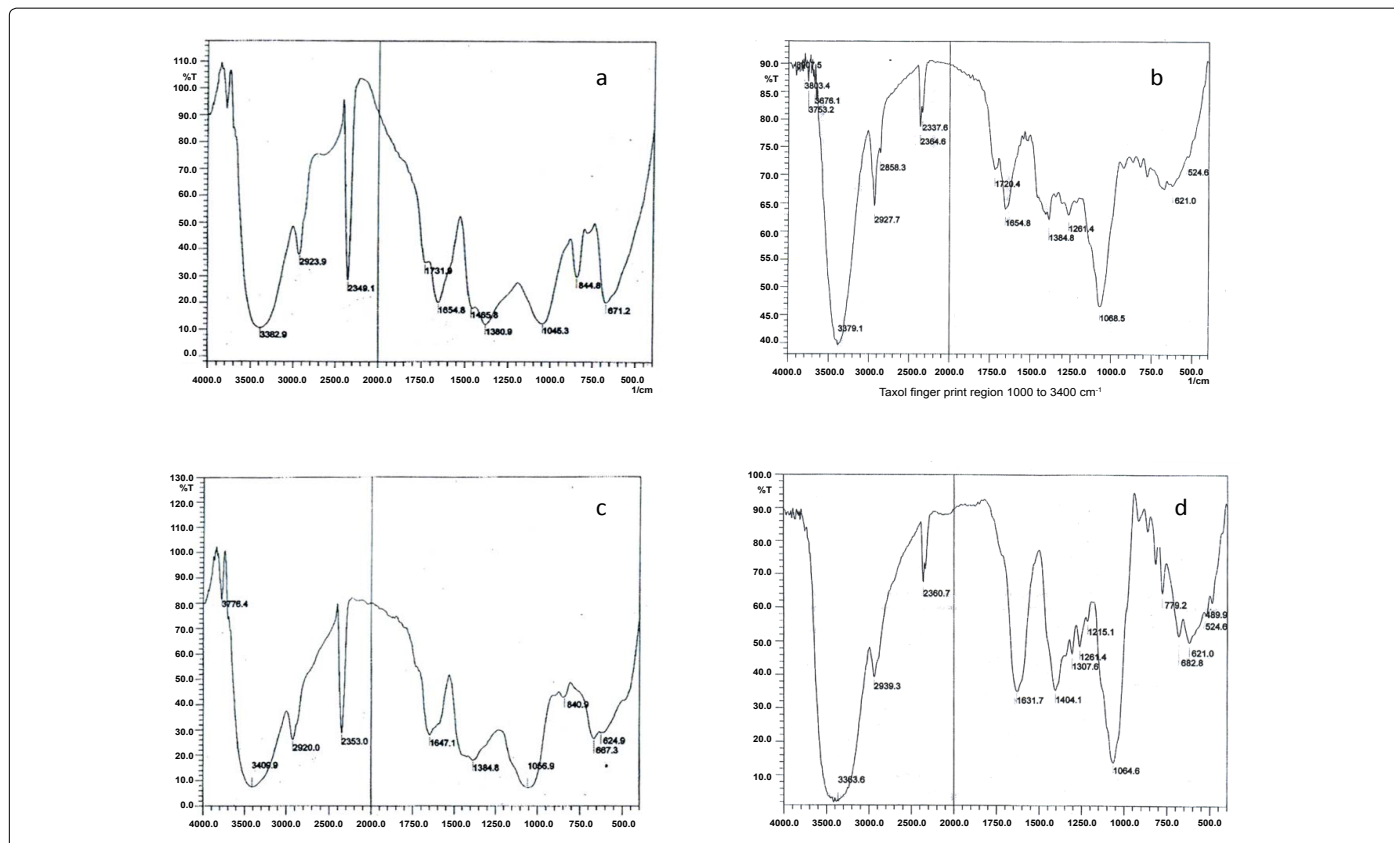
A highly intense band at 3409.9 cm⁻¹ was observed due to the absorption of H-OH. The bend at 2925 cm⁻¹ is due to the absorption of sp³ hybridized carbon. The absorption bend at 2350 cm⁻¹ was sharp and intense. This is due to the absorption of - COOH and enols. A least prominent band was observed at 1775 cm⁻¹, which is due to the absorption of C=O carboxylic acid. The band at 1654.8 cm⁻¹ is due to the absorption of weak combinations and overtones. The absorption band due to unconjugated C-N linkage bands coupled with the stretching of adjacent bands, at 1045.3 cm⁻¹. A band was observed at 848.8 cm⁻¹ due to C-H aromatic out of plane bending. There was no band observed at 1261 cm⁻¹ indicating the absence of C-O stretching in esters.

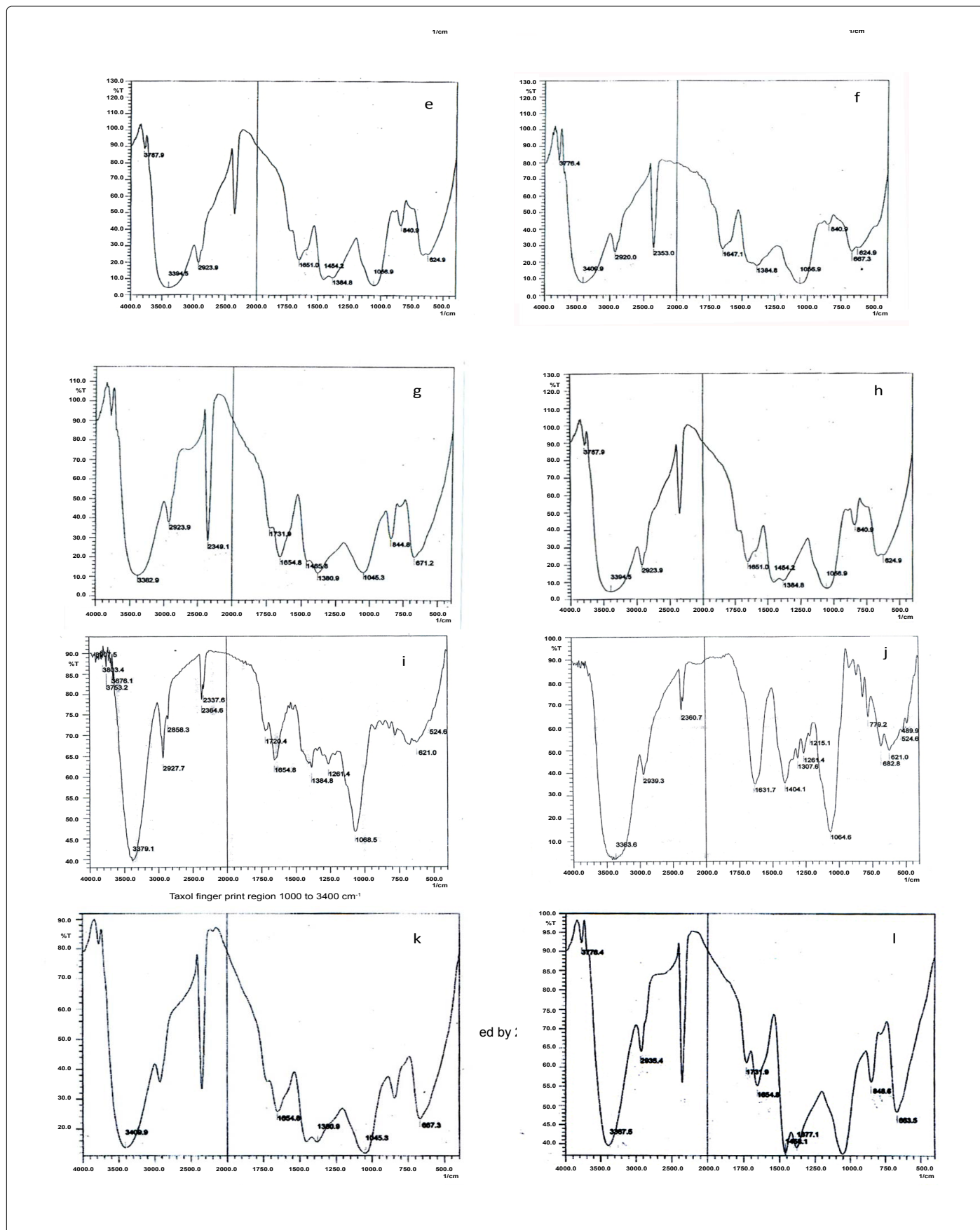
P. foedaris

The intensity of broad peak at 3375.2 cm⁻¹ was due to the absorption of H-OH. The peak at 2927.7 cm⁻¹ was less intense due to the absorption of sp³ hybridized carbon. No peak was observed at 2858.3 cm⁻¹ which is due to the absence of symmetric stretching methylene group. The peak at 2349.1 cm⁻¹ was sharp and intense. This peak was due to absorption of -COOH and enols. The less intense peak at 1735.8 cm⁻¹ was due to the absorption of acetone and another peak at 1654.8 cm⁻¹ due to absorption of weak combinations and overtone. The band at 1388.7 cm⁻¹ was due to symmetrical stretching (Carboxylic acid). A medium band

S.No	Fungal Name	Taxol content µg/L
1	<i>Pestalotiopsis acacia</i>	65
2	<i>P. adusta</i>	67
3	<i>P. breviseta</i>	64
4	<i>P. calabae</i>	52
5	<i>P. clavispora</i>	56
6	<i>P. coangae</i>	58
7	<i>P. coffeae</i>	62
8	<i>P. conigena</i>	53
9	<i>P. eriobotryofolia</i>	54
10	<i>P. foedaris</i>	57
11	<i>P. fibricola</i>	59
12	<i>P. glandicola</i>	53
13	<i>P. japonica</i>	68
14	<i>P. matildae</i>	42
15	<i>P. oleandri</i>	53
16	<i>P. paeoniae</i>	45
17	<i>P. paciseta</i>	47
18	<i>P. taxica</i>	66
19	<i>P. torulosa</i>	64
20	<i>P. zalbrukneriana</i>	47

Table 3: Species showing taxol production.





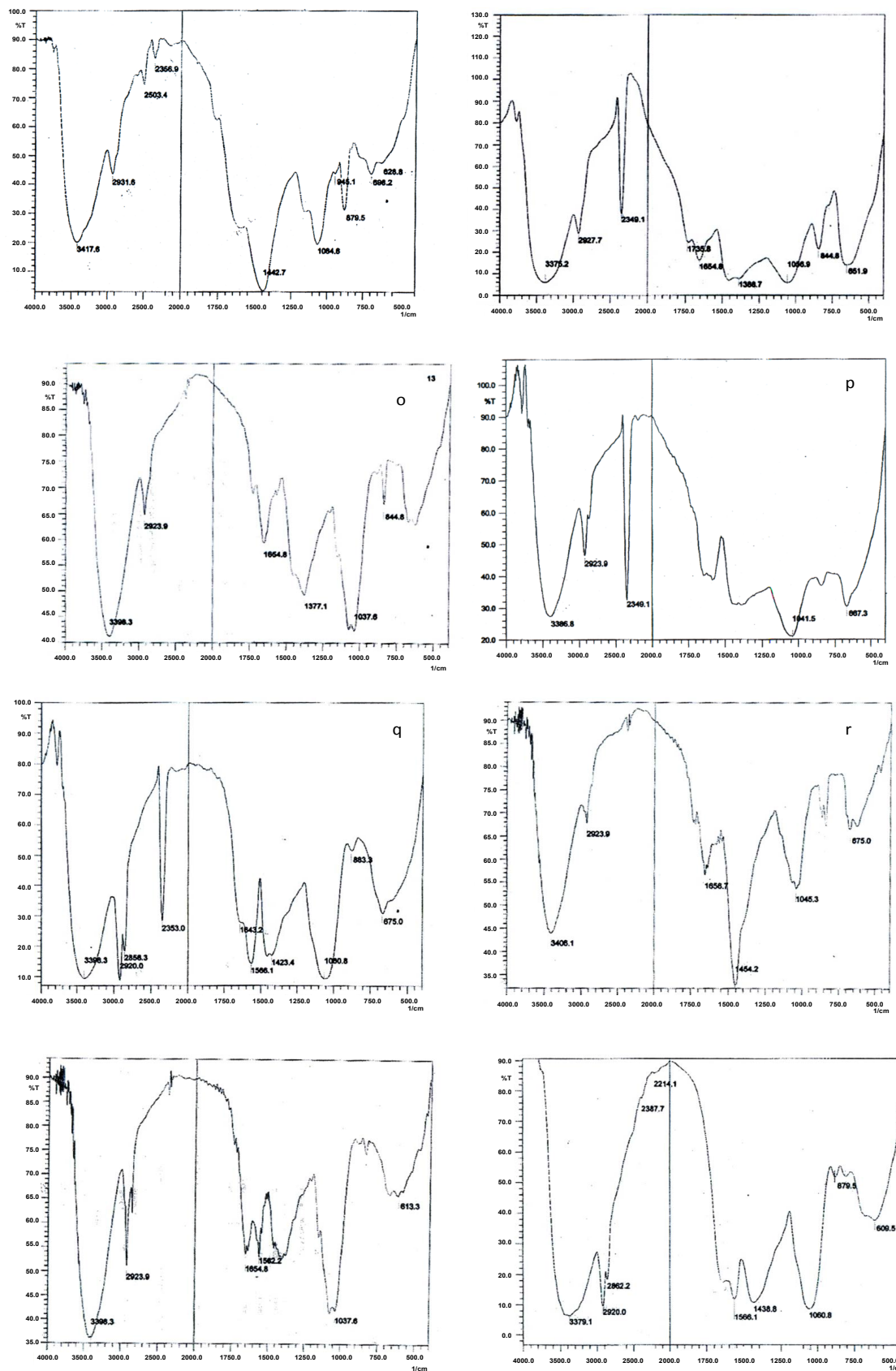


Figure 1a-t: Infra-red Spectral analysis of taxol produced by 20 different species of *Pestalotiopsis*.

was observed at 1056.9 cm^{-1} due to the absorption of unconjugated C-N linkage bands coupled with the stretching of adjacent bands. A band was observed at 844.8 cm^{-1} due to C-H aromatic out of plane bending. The band at 651.9 cm^{-1} was intense and appeared due to absorption of bromide and iodide. This may be due to the solvent effect. No band was observed at 1261.4 cm^{-1} indicating the absence of C-O stretching in esters.

P. fibricola

The band found in the region 3417.6 cm^{-1} was intense and it was found due to the absorption of H - OH. A less intense band at 2931.6 cm^{-1} was found due to the absorption of sp^3 hybridized carbon. No band was observed at 2858 cm^{-1} due to the absence of symmetric stretching methylene group. A less intense band at 2503.4 cm^{-1} , 2356.9 cm^{-1} were observed due to the stretching of O - H (strongly H-bonded) and often overlaps the C-H absorption. The absorption of bands at 1750 cm^{-1} and 1650 cm^{-1} were widened due to the absorption of ketonic group and weak combinations and overtones. The very intense band at 1442.7 cm^{-1} was due to cyclopropane. This was a strong band. The absorption occurred due to the unconjugated C-N linkage. Bands coupled with the stretching of adjacent bands were found at 1664.6 cm^{-1} . A weak band was observed at 945.1 cm^{-1} due to the absorption of O-H bending (out of plane bending). The absorption band at 879.5 cm^{-1} was due to

C-H aromatic out of plane bending. No peak was observed at 1654 cm^{-1} indicating the absence of combinations and overtones.

P. glandicola

The intense peak was observed at 3396.3 cm^{-1} due to the absorption of H-OH. The less intense peak than the previous peak was due to the absorption of sp^3 hybridized carbon. No peaks were observed at 2858.3 cm^{-1} , 2364 cm^{-1} and 2337 cm^{-1} representing the absence of symmetrical stretching vibration of methylene group. Ketonic group was not present because there was no band absorbed at 1720 cm^{-1} . The band at 1664.8 cm^{-1} represents the presence of weak combinations and overtones. The band at 1377 cm^{-1} was due to the absorption of symmetrical stretchiry (Caboxylic acid). No band was observed at 1200 cm^{-1} indicating the absence of ester. The band at 1037.6 cm^{-1} occurred due to the absorption of CN linkage bands coupled with stretching of adjacent bands. There is a sharp peak observed at 844.8 cm^{-1} due to C-H aromatic out of plane bending.

P. japonica

The intense peak at 3396.3 cm^{-1} was due to the absorption of H-OH. The sharp peak at 2920 cm^{-1} , and 2858.3 cm^{-1} is recovered back indicating the presence of sp^3 hybridized carbon and symmetrically stretching vibration of methylene group respectively. A very sharp peak was observed at 2353.0 cm^{-1} . A less intense peak at 1643.2 cm^{-1} was observed due to the weak combination and overtones. The band at 1566.1 cm^{-1} and 1423.4 cm^{-1} were due to cyclopropane. An intense band observed at 1050.8 cm^{-1} was due to the absorption of CN linkage bands coupled with the stretching of adjacent bands.

P. matildae

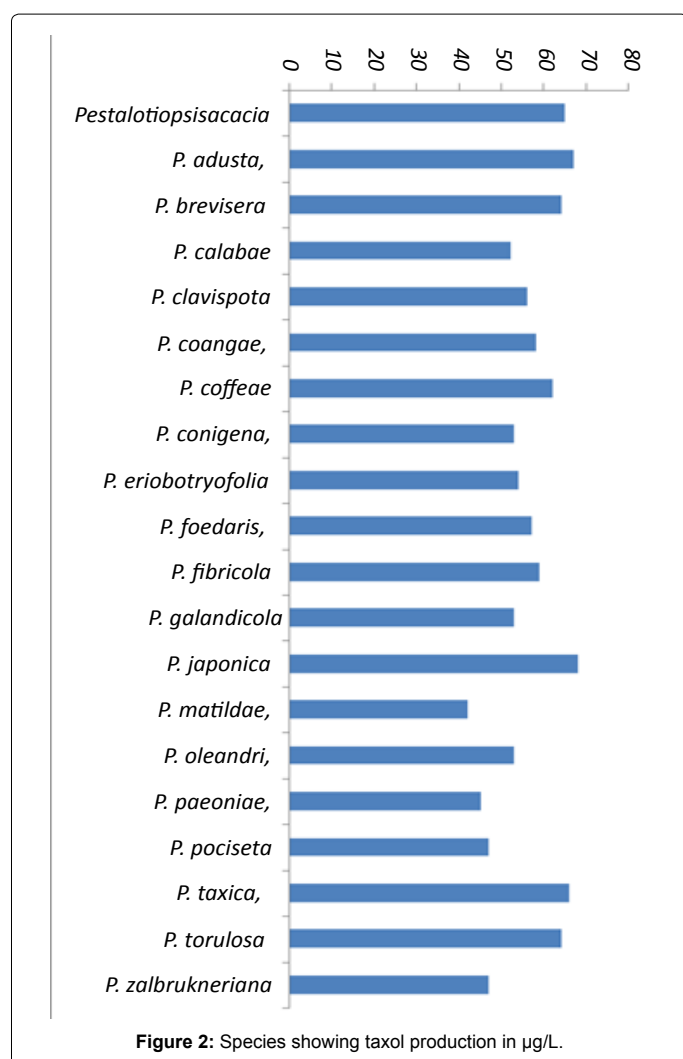
An intense band at 3386.8 cm^{-1} was observed due to the absorption of H-OH. The less intense band at 2923.9 cm^{-1} was due to the absorption of sp^3 hybridized carbon. A very sharp band observed at 2349.1 cm^{-1} was prominent variation in the band. A prominent variation in the band was observed at 1200 cm^{-1} and 1800 cm^{-1} indicating the presence of cyclopropane and overtones. An intense band at 1041.5 cm^{-1} was observed due to the absorption of unconjugated C-N linkage bands coupled with the stretching of adjacent bands.

P. oleandri

A highly intense band at 3398.3 cm^{-1} was observed due to the absorption of H-OH. A sharp band observed at 2923.9 cm^{-1} was observed due to the absorption of sp^3 hybridized carbon and there was another sharp band at 2858.3 cm^{-1} which is due to the absorption of symmetrical stretching vibration of methylene group. The bands at 2364 cm^{-1} and 2337 cm^{-1} were much less intense and another band was observed at 1654.8 cm^{-1} . A sharp band at 1562.2 cm^{-1} was due to the bending of secondary amide. The band at 1037.6 cm^{-1} was intense which occurred due to the un-conjugated C-N linkage bands coupled with the stretching of adjacent bands. No absorption were observed at 1384 cm^{-1} and 1261 cm^{-1} indicating (carboxylic acid) and C-O stretching in ester respectively.

P. paeoniae

An intense band at 3406.1 cm^{-1} was observed due to the absorption of H-OH. The band at 2923.9 cm^{-1} was found to have the least intensity, which was due to the absorption of sp^3 hybridized carbon. The band at 1720.4 cm^{-1} was due to the absorption of ketone and the band at 1654.8 cm^{-1} was due to the absorption of weak combinations and overtones. The most intense band in this spectrum was observed at 1454.2 cm^{-1} which is due to cyclopropane. The band at 1045.3 cm^{-1} is due to the



absorptions of C-N linkage bands coupled with the stretching of adjacent bands. No bands were observed at 1384 cm⁻¹ and 1261 cm⁻¹ indicating the absence of symmetrical stretching (carboxylic acid) and C-O stretching in ester.

P. paciseta

An intense peak was observed at 3396.3 cm⁻¹ due to the absorption of H-OH. A well prominent band observed at 2920.0 cm⁻¹ and 2858.3 cm⁻¹ which were due to the absorption of sp³ hybridized carbon and due to the absorption of symmetric stretching vibrations of methylene group, respectively. These peaks were very intense. A sharp band was observed at 2353.0 cm⁻¹. There was no band observed at 1720.4 cm⁻¹ indicating the absence of ketonic group. The band at 1643.2 cm⁻¹ is due to the weak combinations and overtones. An intense band at 1566.1 cm⁻¹ was observed which is due to the bending of secondary amide. The band at 1423.4 cm⁻¹ was observed due to the cyclopropane. An intense band at 1060.8 cm⁻¹ was due to the C-N linkage bands coupled with the stretching of adjacent bands.

P. taxica

An intense band at 3379.1 cm⁻¹ was due to the absorption of H-OH. The bands at 2920.9 cm⁻¹ and 2862.2 cm⁻¹ were observed due to the absorption of sp³ hybridized carbon and symmetrical stretching vibrations of methylene group respectively. In this spectrum the bands at 2387.7 cm⁻¹ and 2212.1 cm⁻¹ have the least intensity. There was no absorption at 1720 cm⁻¹ indicating the absence of ketonic group. The band at 1566.1 cm⁻¹ was due to the bending of secondary amides. A band was observed at 1438 cm⁻¹ due to cyclopropane, and 1060.8 cm⁻¹ due to the C-N linkage bands coupled with the stretching of adjacent bands. There were no absorptions at 1384 cm⁻¹ and 1261 cm⁻¹ representing the absence of symmetrical stretching carboxylic acid and C-O stretching in esters.

P. torulosa

The intense band at 3421.5 cm⁻¹ was due to absorption H-OH. The less intense band at 2935.5 cm⁻¹ is due to sp³ hybridized carbon. There was no peak at 2858.3 cm⁻¹ indicating the absence of symmetrically stretching vibration of methylene group. The band 2506.4 cm⁻¹ is sharp and due to the stretching of O-H and often overlaps the C-H absorption. A very less intense band was observed at 2360.7 cm⁻¹. A very intense band was observed at 1442.7 cm⁻¹ due to cyclopropane. A band was observed at 1276.8 cm⁻¹ which is due to the absorption of C-O stretching in esters. There was another band at 1064.6 cm⁻¹ representing the C-N linkage coupled with the stretching of adjacent bands. A slight sharp peak was observed at 879.5 cm⁻¹ due to the absorption of aromatic C-H out of plane bending. There were no bands observed at 1720 cm⁻¹, 1658 cm⁻¹ and 1384 cm⁻¹ indicating the absence of ketone, weak combinations and overtones, symmetrical stretching (carboxylic acid) respectively.

P. zalbrukneriana

An intense band was observed at 3336.6 cm⁻¹ which is due to the absorption of H-OH. The less intense band at 2939.3 cm⁻¹ is due to sp³ hybridized carbon. There was no band observed at 2858.3 cm⁻¹ indicating the absence of methylene group. There was a very small twist in the band at 2364.6 cm⁻¹ and 2337.6 cm⁻¹ when compared. No bands were observed at 1720 cm⁻¹ and 1260 cm⁻¹ indicating the absence of ketone and ester. The bands at 1635.5 cm⁻¹ and 1384.8 cm⁻¹ indicate the presence of weak combinations, overtones and symmetrical stretching (carboxylic acid) respectively. The band at 1064.6 cm⁻¹ is due to the

absorption of C-N linkage band coupled with the stretching of adjacent bands.

Conclusion

Out of 20 species tested for taxol 6 species showed above 60 µg/L of taxol production 9 species produced 50-60 µg/L, and others produced 40-50 µg/L (Figure 2).

Among the three medium Potato Dextrose Agar, Czapek Dox Agar and Oat Meal Agar used. PDA medium was good for producing mycelia, followed by OMA and CDA, respectively.

All the 20 samples were aromatic derivative consisting of sp³ hybridized C-H stretching alkyl substitutions and carbonyl groups as it or amide and few samples as esters. All the 20 samples contain the C-N linkage except Sample 3. Hence, the IR spectra analysis and correlation of 20 different *Pestalotiopsis* spp., samples clearly indicate the existence of important functional groups in the aromatic derivative which beautifully coincides with the standard *pestalotiopsis* spp.

From all the 20 different *Pestalotiopsis* it is evident except for a few samples; all samples show the existence of aromatic system. All 20 samples contain a methyl group and carbon – nitrogen linkage. It can be concluded from IR spectra that the OH group (Alcohol group) is also present in all the 20 test samples. A few samples *viz* sample 1, 5, 6, 8, 9, 14, and 20 show the existence of a keto group. This keto group is present as carboxylic acid in samples 1,5,6,7,8, 10 and 18 respectively. The existence of Carbonyl (C=O) group is present as amide in sample 13, 15, 16 and 19 respectively. The existence of aromatic system is further confirmed by presence of overtones and combination bands in almost all samples except sample 16, 17, and 19. One or two samples contains methylene group also.

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