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Study of phylloplane fungi isolated from *Lagerstroemia speciosa* and their biochemical screening for alkaloid production

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ABSTRACT

Phylloplane fungi are microbes that reside on the leaf surface without showing any disease symptoms. Plants produce several compounds to protect themselves from the continuous attack of naturally occurring pathogens, insect pests and environmental stresses. Presence of phylloplane mycoflora and their associations with the leaf helps as potential defence and in enhancing the plant growth and productivity. The present paper focuses on the isolation and documentation of phylloplane fungi from the leaf surface of Lagerstroemia speciosa. The presence of fungi was confirmed by SEM analysis. *Chaetomium globosum, Curvularia pallescens* and *Myrothecium verrucaria*, the dominant isolates were selected to study the biochemical analysis. Leaf extract and the three fungal extracts in Methanol, DCM and Ethyl acetate were screened for secondary metabolites like alkaloids, flavonoids, phenols and terpenoids using phytochemical qualitative techniques. The presence of the compounds was confirmed with UV-Vis and FTIR. The biochemical results of the fungal extracts were compared with that of the leaf.

Keywords: Phylloplane fungi, FTIR and alkaloid.

INTRODUCTION

Fungi are achlorophyllous organism found everywhere. Biodiversity of these organism in a particular ecosystem differs.The phylloplane represents an important terrestrial habitat that harbors a wide range of microbial populations. Phylloplane microflora plays an important role affecting the plant - microbe interactions on leaf surface and thereby contribute significantly for beneficial plant growth and disease suppression (Amarjyoti Tanti et. al.). Plant leaf surface serves as a

suitable environment inhabitating a larger proportion of microbial resources.

Lot of investigations have been carried out on the phylloplane flora of leaf surfaces of several plants growing in garden or cultivated in many parts of the world by several researchers (Abdel-Fattah, et al., 1977; Abdel-Hafez, 1981, 1984, 1985; Abdel-Hafez, et al., 1995; Eicker, 1976; Khallil, and Abdel- Sater., 1993; Mazen, et al., 1985; Nagaraja, 1991; Sharma, 1974). EI-Said (2001). Many physical, chemical and biological factors bring about causative changes in composition of aeromycoflora of an area and different fungal species are restricted to that particular areas with specific environmental conditions (Bajwa et al., 1997; Verma, 1990).

Fungi provide a plentiful and diverse source of unique and often bioactive metabolites, and they have produced a number of medicinally important compounds, including penicillin, mevinolin (Lovastatin) (Gloer, 2007), fingolimod (Strader et al., 2011) and caspofungin (Keating and Figgitt, 2003).

In the present investigation, ***Lagerstroemia speciosa*** leaves were examined for phylloplane fungal flora. Phylloplane fungi may be residing on the leaf surface without any disease symptoms or may be casually present. They were further investigated for the alkaloid production.

MATERIALS AND METHODS

Collection of Plant material :
Fresh leaf of plant specimen was collected from Western Ghats of SGNP, Borivali, India. The plant specimen was identified as ***Lagerstroemia speciosa*** (L.) Pers. (**Fig 2**,) by Blatter Herbarium, St. Xaviers College, Mumbai, Fort.
Leaves were examined for fungi on the surface by the following methods.

Direct method
Leaf section: The leaf was cut with sterile blade. The sections were mounted in lactophenol blue. The blue stained hyphae or spores shows the presence of phylloplane fungi on the epidermal cell wall.

Nail paint impession (Masurovsky and Jordan,1960), technique was performed in which transparent nail polish was applied on the surface of the leaf. The coating of the nail paint was gently peeled off after drying. This peeling was mounted in lactophenol and observed under compound microscope for fungal presence on the leaf surface.

Cellotape impression method (Edward and Hartman, 1952)**,** was carried out in which strips of cellotape was pressed gently against the surface of the leaf. After impression, the strips were stained in cotton blue and was observed in microscope for fungal presence.

Leaf impression :
The leaves of the plant were washed in distill water and allowed to dry to remove the surface contaminants and soil particles. The leaves were surface sterilized and pressed on PDA media in a petri-plate for about five minutes. The plates were kept in at room temperature for 7 days and observed. The different colo nies formed were sub-cultured. The pure isolates were stored as master slants at 4°C. The dominant phylloplane cultures were authenticated and deposited at Agarkar Research Institute, Pune. Among these, three cultures *Chaetomium globosum, Curvularia pallescens* and *Myrothecium verrucaria*, were selected to study the biochemical analysis.

Scanning electron microscopy :
The dried small leaf segments (2 x 10 mm) were mounted ventral side up on aluminum stub mounts using 12-mm carbon adhesive tabs coated with carbon-conducting glue and sputter coated with 6 nm of platinum using a Hummer 6.2 sputtering system (SAIF-IIT, Bombay, Powai). Images were obtained in high-vacuum mode with accelerating voltages at or around 2.0 kV.

Biochemical analysis :
The leaves were washed with water, shade dried and ground to powder using an electronic blender, sieved and the fine powder was stored in air tight container for further study.

Preparation of leaf extract :
100 gram of powder was subjected to methanolic extraction by hot percolation method through Soxhlet

apparatus. The extract was filtered through Whatmann filter paper no. 1. This leaf extract was concentrated using rotary evaporator at 40°C and dried.

Preparation of fungal extract :
The three fungal isolates were cultured on PDA broth and incubated for 21 days at room temperature.

Extraction of fungal cultures :
The culture filtrate was extracted in ethyl acetate and DCM. Culture filtrate was then was evaporated to dryness under vacuum in rotary evaporator. The dried organic extract was reconstituted with 10ml of the same solvents. The culture mats were weighed before and after drying. These mats were then extracted in methanol and dried in rotary evaporator. The dried extracts of leaf and the fungal cultures was analyzed for alkaloid production by phytochemical study, UV & FTIR.

Phytochemical analysis :
Preliminary phytochemical screening for bioactive compound of the above extracts was carried out using standard qualitative methods ([Kotate et al, 2010; Harborne 1998; Egwaikhide and Gimba, 2007; Savithramma et al, 2011).

Detection of Alkaloids (Evans, 1997)
Mayer's test (Evans, 1997) To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive.

Wagner's test (Wagner, 1993) –
To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Detection of Flavonoids
Few drops of dilute NaOH solution was added to the extract, an intense yellow or pink colour was observed. Further on addition of dilute acid it becomes colourless. Thus, indicating presence of flavonoids. To a small quantity of extract dilute H_2SO_4 was added. Appearance of orange colour indicated the presence of flavonoids.

Detection of Phenolic compounds
Ferric chloride test (Mace, 1963) - The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution is added. A dark green or deep blue colour indicates the presence of phenolic compounds.

Lead acetate test
The extract dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Alkaline reagent test
An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Detection of Terpenoids

Salkowski test - The extract was mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (1ml) was carefully added to form a layer. A reddish violet coloration in the interface indicates positive result for the presence of terpenoids.

Libermann's reaction –
The extract was mixed with equal amount of acetic acid. It was heated & colled. Then few drops of concentrated H_2SO_4 was added. Formation of bluish green rings indicates the presence of terpenoids.

UV - Vis analysis
To detect the UV-Vis spectrum profile, the leaf and fungal extracts were scanned in the wavelength ranging from 200-1100 nm on Lambda 25 UV-Vis spectrophotometer - 01 Perkin Elmer model at CIL LAB SPPTM, MUMBAI. The characteristic peaks were detected which confirms the presence of alkaloid in all the extracts. The peak values of the UV-Vis were recorded.

FTIR analysis
The leaf extract, the crude fungal extracts of methanolic, DCM and ethyl acetate were studied under FTIR. A drop of the liquid leaf and fungal crude extract was placed between the two NaCl cells circular and transparent in nature. These liquid samples were scanned from range 400 to 4000cm^{-1} with a resolution

of 4cm^{-1} in FTIR spectroscopy (Spectrum v.5.3.1 Perkin Elmer) at CIL LAB SPPTM, MUMBAI.. The results of FTIR peak values and functional groups are represented in **Table 3** . The FTIR analysis suggest the presence of alkaloid in all extracts.

RESULTS AND DISCUSSION

Plant description :

Lagerstroemia speciosa, (**Fig 1 and 2**) belonging to family Lythraceae is commonly called as the Queens crape myrtle "PRIDE OF INDIA" in English and Arjuna in Hindi. (Flora of Maharashtra).

Direct observations:

The direct observation of the leaf of *Lagerstoemia speciosa,* was seen under stereoscope (**Fig 3 & 4**) and light microscope.

Nail paint and cello tape impression methods

The phylloplane fungal diversity was studied by the presence of mycelial forms impressed on nail paint strips (**Fig 5**) and cello tape strips (**Fig 6**).

Fig 1: Lagerstroemia speciosa(L.)

Fig 2: Specimen identified.

Fig 3: Leaf surface

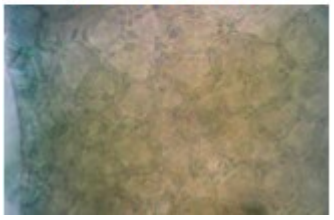


Fig 4: Leaf stained in cotton blue

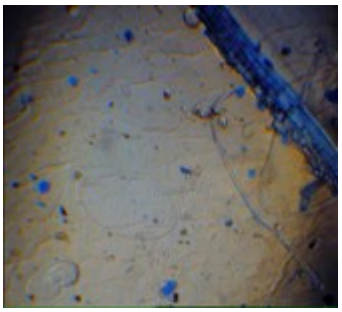


Fig 5: Nail paint impression

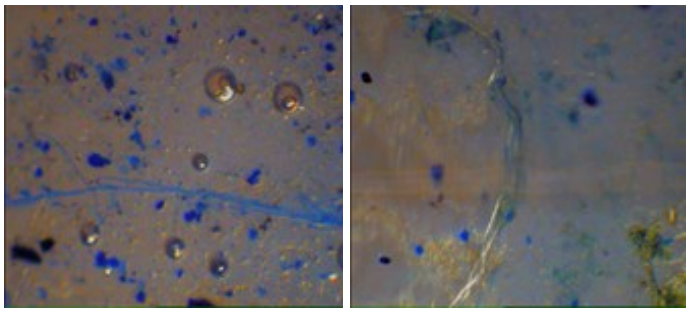


Fig 6: Cello tape impresssion

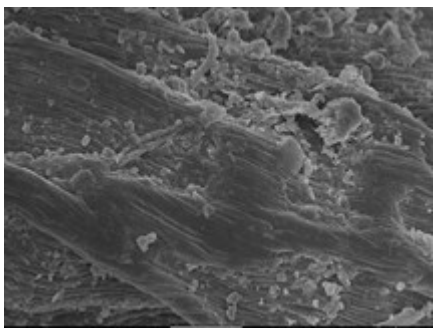


Fig 7: SEM Image of *Lagerstoemia speciosa (magnification X 2,000, bar indicates 1µm)*

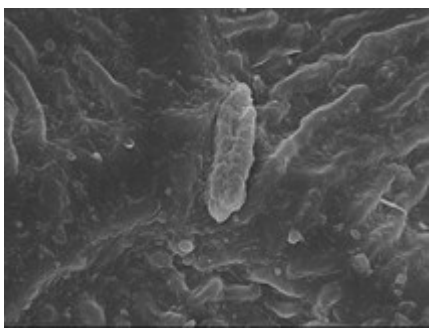


Fig 8: SEM Image of *Lagerstoemia speciosa (magnification X 5,000, bar indicates 1µm)*

The presence of phylloplane fungi was further confirmed by Scanning Electron Microscopy where mycelial forms and spore were observed on the surface of the leaf of Lagerstroemia speciosa (**Fig 7 & 8).**

Leaf impression showing fungal colonies

The fungal colonies (**Fig 9 &10**) were seen on 7th day on the PDA medium. These colonies were isolated into pure cultures and stored in slants at 4°C.

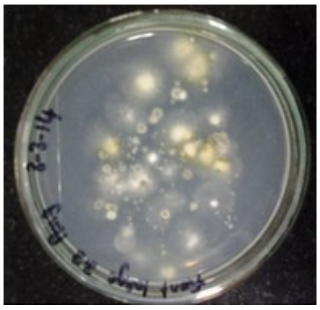


Fig 9: surface view

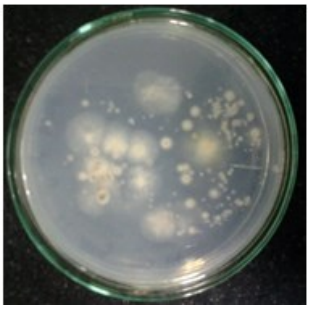


Fig 10: reverse view

Phylloplane fungi

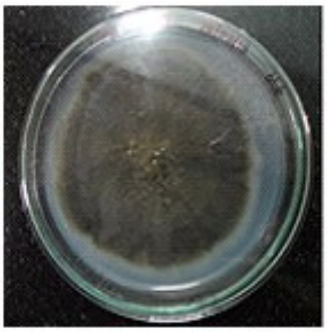
A total number of 14 phylloplane fungi were isolated from surface sterilized leaf impression of *Lagerstoemia speciosa.*

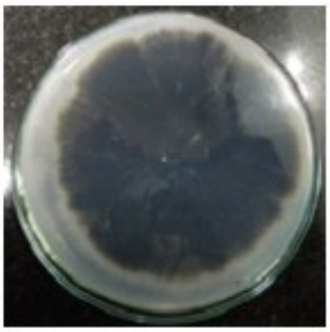
Phylloplane fungi on *Lagerstroemia speciosa*

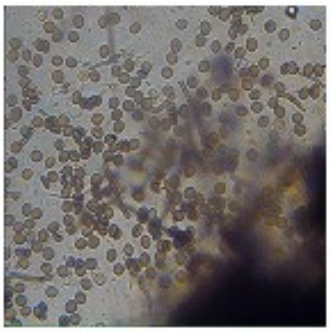
The following were the colonies identified,

Aspergillus sp, Alternaria alternate, Chaetomium globosum, Cuvrularia lunata, Curvularia pallescens, Dematicious sp, Fusarium oxysporum, Gleosporium sp, Myrothecium verrucaria, Mycelia sterilia, Paecilomyces cerevisae, Phoma sp, Rhizopus sp,Torula sp.

Out of these phylloplane fungi, 3 fungal isolates namely *Chaetomium globosum* (**Fig 11**) *Curvularia pallescens* (**Fig 12)** and *Myrothecium verrucaria* (**Fig 13)**, were found to be dominant.







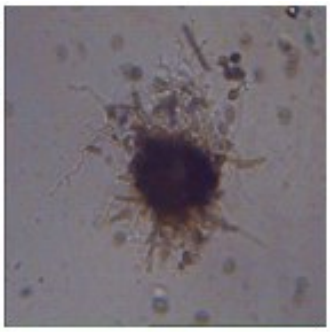


Fig11: Surface Reverse Ascospores Perithecia

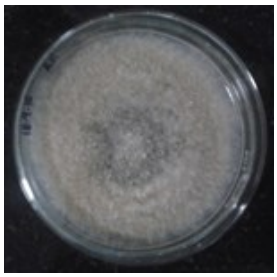
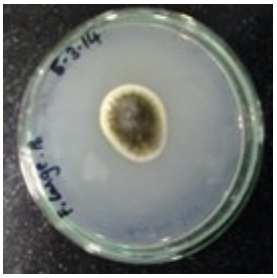
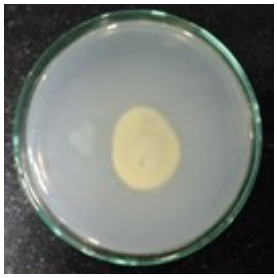


Fig12: Colony Conidia





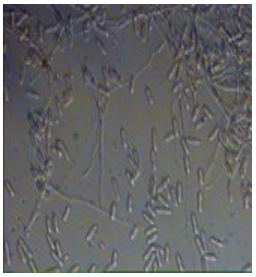


Fig13: Surface Reverse conidia

***Chaetomium globosum* Kunze (NFCCI accession no 4111, family- Chaetomiacea)**

Colony brownish grey and dark black at reverse, growing moderately about 4cm in 5 days. Perithecia ostiolate, sub globose, somewhat elongated with a bluntly pointed base, producing short cirrhi, with dark brown colour rhizoids. Terminal hairs numerous, drooping., slender., septate with spines. Ascospores filled with several large mature dark, brown, ovate & lemon shaped with apiculate 9-13 u in length and 7 u broad.

***Curvularia* aff. *pallescens* Boedijn (NFCCI accession no 1602, family-*Pleosporaceae*)**

Colony velvety creamish, moderate in growth of diameter 7 cm in 4 days, reverse dark brown. Mycelium partly immersed, septate, branched, pale brown 3-4 um wide. Conidiophore mononematous,

nodose, septate, branched, thick walled, dark brown, 40-90um long .Conidia solitary, dry, curved, ellipsoidal, rounded at both ends, thick-walled, smooth, dark brown, 3-septate, end cells paper than central cells.

***Myrothecium verrucaria* @ (Alb & Schwein) (NFCCI accession no 4106, family *Incertae sedis*)**

Slow growing upto 2cm in 12 days. Colony brown in centre and margin creamish in colour. Reverse cream in colour. Sporodochia cushion like, sometimes with marginal hyaline setae; conidiophores subhyaline to coloured, repeatedly branched, bearing conidia terminlly. Philiades cylindrical, densely aggregated, conidia fusiform 1 celled or cylindrical, forming dark, often greenish-black masses.

Biochemical analysis

The three phylloplane isolates were further studied for their biochemical presence of alkaloid by performing phytochemical tests, Uv and FTIR analysis.

The phytochemical tests (Indian Pharmacopia) showed the presence of Alkaloid in all the solvent extracts of leaf and fungi by Wagners tests. The presence of flavonoid was detected in methanol extracts of leaf, *Chaetomium globosum* and *Curvularia pallescens.* Whereas, flavonoid was present in ethyl acetate extract of *Myrothecium verrucaria.* The phenolic compounds were present in methanol extract of leaf and *chaetomium globosum* by ferric chloride and lead acetate test respectively. Terpenoid was only present in ethyl acetate extract of *Chaetomium globosom.* (**Table 1**).

UV-Vis analysis [Harborne, 1998]

The UV spectrum of leaf and all three phylloplane fungi showed sharp peaks by proper baseline correction which confirms the presence of Alkaloids at 234.676nm (Nandha Kumar. et al, 2015) (**Table 2**).

Table 1: Phytochemical analysis

Phytochemical test	Methanolic leaf extract of Lagerstroemia speciosa	Crude fungal extracts of *Chaetomium globosum*			Crude fungal extracts of *Curvularia pallescens*			Crude fungal extracts of *Myrothecium verrucaria*		
		DCM	Ethyl acetate	Methanol	DCM	Ethyl acetate	Methanol	DCM	Ethyl acetate	Methanol
Alkaloid test	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	-	-	+	-	-	+	-	+	-
Phenolic compounds	+	-	-	+	-	-	-	-	-	-
Terpenoids	-	-	+	-	-	-	-	-	-	-

Table 2: UV spectrum data of methanol leaf extract and three fungal extracts in DCM,

Solvents	**leaf extracts of *Lagerstroemia speciosa***	**Crude fungal extracts of Chaetomium globosum**	**Crude fungal extracts of *Curvularia pallescens***	**Crude fungal extracts of *Myrothecium verrucaria***
DCM	-	403.21 470.92	236.80	380.88
Ethyl acetate	-	363.38	394.84	323.70 244.47
Methanol	324.83 329.17 470.92	342.18	243.75	381.61

Table 3: FTIR spectrum data of methanol leaf extract and three fungal extracts in DCM, Ethyl acetate and methanol.

Leaf sample and Phylloplane fungi	FTIR peak values for alkaloids presence of crude extract of different solvents		
	DCM	Ethyl acetate	Methanol
Lagerstroemia speciosa	-	-	1651.72
Chaetomium globosum	1729.57	1742.84	1650.73
Curvularia palles	1645.50	1841.82	1654.70
Myrothecium verrucaria	1648.35	1743.37	1657.53

FTIR analysis

From the above observation (**Table 3**) it is evident that alkaloids are present in the leaf and the fungal extracts in the range of 1850 - 1620cm-1 IR radiation region (Robert M Silverstein, 2014) in similar traces, which may be potentially defensive, detection of specific alkaloids needs to be further confirmed by TLC and HPTLC analytical techniques for better understanding their biological activities for future needs.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Harborne JB (1998) Phytochemical Methods: A guide to modern techniques of plant analysis, third ed. Chapman and Hall, London.

Kokate CK, Purohit AP and Gokhale SB (2010) Pharmacognosy, Nirali prakashan, Pune, pp.6.15-6.36.

Kumar Nandha S and Nivetha Thampi (2015) Phytochemical screening and characterization of the bioactive compounds from the leaves of *Hyptis suaveolens* and *Spathodea campanulata*. *Journal of Chemical and Pharmaceutical Research*, 7(7):840-850.

Padmavathy S et al. (2014) Phytochemical evaluation of Musa acuminata bract using screening, FTIR and UV-Vis spectroscopic analysis. *Journal of international academic research for multidisciplinary* vol. 2, issue 1.

Lee Olive HK and Kevin D Hyde (2002) Phylloplane fungi in Honk Kong mangroves: evaluation of study methods. *Mycologia*, 94(4), pp. 596-606.

Ellis MB (1976) More Dematiaceous Hyphomycetes, (Common wealth mycological Institute, Kew, Surrey, England), 1976, 507.

Sharma Tanu and Sidhu MC (2017) Cytomorphological and preliminary phytochemical screening of Eclipta alba (L.) hassk. *International journal of green pharmacy*, 11(1) S23.

Mishra RR and Srivastava VB (1973) Leaf surface microflora of Hordeum vulgare L. *Acta societatis botanicorum poloniae* Vol. XLIII, nr 2.

Papitha R et al. (2016) Phytochemical screening, FTIR and gas chromatography mass spectrometry analysis of Tinospora cordifolia (Thunb.) miers. International journal of pharmacognosy and phytochemical research 8(12); 2020-2024.

Salman A et al. (2010) FTIR spectroscopy for detection and identification of fungal phytopathogenes. Spectroscopy 24 261-267.

Knoll D and Schreiber L (1998) Influence of epiphytic micro-organisms on leaf wettability. New phytol, 140, 271-282.

Willaim P Scherer, DPM, MS and Michael David Scherer, BS (2004) Scanning electron microscope imaging of onychomycosis. *J Am podiatr med assoc* 94(4): 356-362.

Pathan AK et. al (2009) Sample preparation for SEM of plant surface. *Electron microscopy special issue* vol. 12.

Amarjyoti Tanti et. al (2016) Diversity of phylloplane microflora in certain tea cultivars of Assam, North-East India. *European journal of biological research*; 6 (4): 287-292.

Ahmed M Emam et al. (2008) Furocoumarin and quinolone alkaloid with larvicidal and antifeedant activities isolated from Ruta chalepensis leaves. *Journal of natural products*, Vol. 2: 10-22.

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