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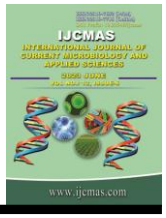

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Biological Activities of Pigments from *Aspergillus nidulans*

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The biological activity shown by fungal pigments is of significance for broadening their applications. In the current research, both cellular and secretory pigments produced by *Aspergillus nidulans* were separated by thin-layer chromatography (TLC) and detected by UV-Visible spectroscopy. The fractions were examined for their biological functions in terms of antimicrobial and antioxidant activities. The findings revealed that the crude cellular pigments had absorption maxima at 458 nm and 521 nm, corresponding to orange and red pigments respectively. The crude secretory pigments showed absorption maxima at 464 nm, representing orange pigments. In TLC, eight pigment fractions were separated in cellular pigment extract whereas nine pigment fractions were observed in secretory pigment extract. The crude cellular pigment extract was exclusively effective against *Bacillus subtilis*. The crude secretory pigment extract presented antibacterial activity on *B. subtilis* and *Staphylococcus aureus*. The crude cellular pigment extract had slight inhibition of *Candida albicans* whereas the crude secretory pigment extract showed inhibition against both *Candida albicans* and *Cryptococcus* sp. In the bioautography, the crude secretory pigment displayed inhibition activity on all the tested fungi whereas the crude cellular pigment extract revealed inhibition activity only against *Curvularia lunata*. Both cellular and secretory pigments showed antioxidant activity in a dose-dependent manner. The potential pigments with biological activity are promising candidates in the food and pharmaceutical industry.

Introduction

Natural source of pigments includes plants, animals, and microorganisms. Microbial producers are preferable for the biotechnological production of pigments, due to their cultural techniques being easily practiced (Aberoumand, 2011). Among various sources, filamentous fungi originating either from marine or terrestrial environments are well known to produce a phenomenon range of biomolecules such as bioactive pigments. These molecules are known to be more stable and water-soluble than plant pigments (Caro *et al.*, 2017). The pigments obtained from the fungi belong to several chemical classes such as carotenoids, flavins, melanins, phenazines, quinones and polyketides (Studt *et al.*, 2012).

The pigments from Ascomycetous fungi have great potential to replace synthetic pigments. People's

interest has increased towards the use of natural sources for colouring products. Hence, the natural pigments demand has also grown significantly over synthetic counterparts (Kumar *et al.,* 2011). Additionally, synthetic pigments are proven to be hazardous. The filamentous fungi belonging to the genera *Aspergillus, Fusarium, Monascus, Neurospora, Penicillium, Talaromyces* and *Trichoderma* have received substantial research interests for the production of pigments (Dufosse *et al.,* 2014; Gmoser *et al.,* 2017). The pigment produced by these fungi are exhibiting different hues from yellow to reddish-purple and are structurally related. They are found both in the mycelium and secreted to the fermentation broth (Caro *et al.,* 2017). Among the filamentous fungi, the genus *Aspergillus* is one of the most ubiquitous genera contributing to fungal natural products (Yang *et al.,* 2020; Carroll *et al.,* 2021). Species of this genus are known to produce diverse groups of secondary metabolites like polyketides, alkaloids, terpenes, steroids, and peptides exhibiting great biological activity (Yang *et al.,* 2020). However, information on *Aspergillus nidulans* producing biologically active pigments are limited. Hence, efforts are made to detect cellular and secretory pigments of *A. nidulans* and determine their biological activities. Here we report the production of pigments in *A. nidulans* and their detection.

Materials and Methods

Isolation of fungi

Soil samples were obtained from the Davangere University campus and dispensed in sterile water. An aliquot of 0.1 ml was inoculated on potato dextrose agar (PDA) medium (pH 6.5±0.2) and incubated the plates at room temperature (30±5 ºC). Every day the plates were checked for the development of fungi for seven days. The pigmented fungal isolate was selected and pure culture was established by sub-culturing on a fresh PDA plate and later transferred to PDA slants. The fungus was identified using standard manuals (Thom and Raper, 1945; Samson *et al.,* 2014).

Extraction of pigments

For the production of pigments, the five-day-old inoculum was prepared in potato dextrose broth (PDB) and inoculated to Roux bottles containing 300 ml of PDB. The bottles were kept in room temperature at 30±5 ºC under stationary condition for seven days.

After the fermentation, the culture filtrate containing secretory pigments was separated from the biomass and extracted using the same volume of ethyl acetate. The cellular pigments present within the mycelium were solubilized in ethyl acetate by soaking overnight under room temperature. The solvent-soluble fraction was recovered by filtration having the pigments from the mycelium.

Separation and detection of pigments

Thin layer chromatography

The pigments produced by the selected fungus were separated by analytical thin-layer chromatography (TLC). The pigments were spotted on a silica gel G plate and placed in the chromatography tank having ethyl acetate:hexane (6:4; v/v) as the mobile phase. The plate was observed in daylight and under UV light.

The R_f value of each separated compound was calculated. Further, the preparative TLC was performed and each compound having a different R_f value was scrapped off and recovered. The recovered silica gel was placed in ethyl acetate and ethanol separately. The extracts were centrifuged and collected the supernatant for further analysis.

UV-Visible spectroscopy

The crude cellular and secretory pigments were dissolved in ethyl acetate. The TLC-separated fractions were individually dissolved in ethyl acetate and ethanol. The UV-Visible spectrum was recorded using a UV-Visible spectrophotometer (LMSP-UV1200, LABMAN) at room temperature from

wavelength range of 200 to 800 nm. The ethyl acetate and ethanol were used as respective control for samples.

Antimicrobial activity by disc diffusion assay

Antibacterial activity was performed against the Gram-positive *Bacillus subtilis* (MCC 2511) and *Staphylococcus aureus* (MCC 2408) as well as Gram-negative *Escherichia coli* (MCC 2246) and *Salmonella enterica* (MCC 3910). The antifungal activity was conducted on *Candida albicans* (MCC 1151) and *Cryptococcus* sp. (MCC 1408) (Microbial culture collection, National centre for microbial resource, Pune, India). The cellular and secretory pigment fractions were loaded onto 5 mm Whatman filter paper 1 disc. The discs were placed over the Mueller-Hinton agar medium swabbed with 24-hour-old bacterial cultures and Sabouraud dextrose agar with 24-hour-old yeast cultures separately. The positive control chloramphenicol was maintained for *E. coli* (MCC 2246) and *Salmonella enterica* (MCC 3910) and Vancomycin for *B. subtilis* (MCC 2511) and *Staphylococcus aureus* (MCC 2408). As a positive control fluconazole was used for antifungal activity. The disc with ethyl acetate only served as a negative control. The discs were placed equidistantly and then incubated at 37 ºC for determining the zone of inhibition after 24 hrs. The standard error for the zone of inhibition was calculated based on the mean values.

TLC-Bioautography for antifungal activity

The cellular and secretory crude pigments of the selected fungus were spotted on the TLC-silica gel G plate. The fluconazole and ethyl acetate was used as positive and negative controls respectively. After air drying, the spore suspension in PDB of target fungi *Alternaria tenuissima, Cladosporium oxysporum* and *Curvularia lunata* were sprayed onto silica gel plates with the help of an automizer in separate plates and incubated in a moist chamber. Inhibition of growth was determined after 24 hrs. The TLC- bioautography was performed for the cellular and secretory crude pigments showing antifungal activity against target fungi using ethyl acetate:hexane (6:4 v/v) solvent system.

Antioxidant activity

By evaluating the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) compound capacity to scavenge free radicals, the antioxidant activity of the pigments was ascertained (Tavares *et al.*, 2018). The crude pigment extracts with different concentrations and their TLC-separated fractions from selected fungus were diluted in methanol. In a dark environment, 100µL of each dilution of the extract was transferred to the microplate wells, and 200µL of the DPPH solution was added at a concentration of 0.004%. The known antioxidant Butylated hydroxyanisole (BHA) was used as the positive control while the negative control used was methanol. The samples were incubated for 30 minutes in complete darkness at room temperature. The absorbance was read using a microplate reader (BIO-RAD) at 517nm. The experiment was carried out in triplicates.

The percentage of inhibition (I%) of DPPH was calculated using the following equation

$$I\% = \frac{\text{A blank - A sample}}{\text{A blank}} \times 100$$

where A blank = absorbance of the DPPH

A sample = absorbance of the tested extracts.

Results and Discussion

Isolation of fungi

The selected fungus was grown well on PDA medium, producing brown conidiophores. The reverse of the colony showed purplish-red pigmentation and becomes dark with age. The fungus was identified as *Aspergillus nidulans* (Eidam) Wint. The fungus was given the Davangere University Microbiology culture collection number (DUMB 14). The soil harbours a diverse group of microorganisms and they are known to synthesize an

extraordinary range of secondary metabolites including pigments (Boonyapranai *et al.,* 2008; Toma *et al.,* 2021). It is known that several Aspergilli synthesize pigments (Teixeira *et al.,* 2012). Direct production of pigments can be visualized by observing the colony and medium around the growth. For the detection of pigment-producing fungi, additional screening procedures may not be essential.

Extraction of pigments

The *Aspergillus nidulans* (DUMB 14) showed pigment production after four days of growth in potato dextrose broth. The biomass and culture filtrate became dark over time, which indicated higher pigment production during growth. The potato dextrose broth favours the production of pigment (Jin *et al.,* 2018; Hasanein *et al.,* 2023). The ethyl acetate extracted cellular and secretory pigments were orange in colour. The average dry weight of mycelia was 7.46 g/L of culture medium**.** The ethyl acetate soluble crude cellular pigment was 34.66 mg/g of biomass. The ethyl acetate soluble crude secretory pigment was 21.48 mg/L of culture filtrate. Both cellular and secretory pigments of *A. nidulans* were soluble in ethyl acetate. A remarkably good amount of pigment fractions was obtained. Previous reports are available on the use of ethyl acetate as a solvent for the extraction of *Aspergillus* pigments (Narendrababu and Shishupala, 2017). The nutrients supplied also going to influence not only fungal growth but also pigment production (Pandey *et al.,* 2018). Hence, it is crucial to standardize media composition and optimize pigment production conditions for each of the fungal isolates.

Separation and detection of pigment

In the cellular pigment extract of *Aspergillus nidulans* (DUMB 14), eight distinct bands with various R_f values were found using TLC (Fig.1.i.A). All the separated cellular pigments also exhibited fluorescence under UV light (Fig.1.i.B). The TLC-separated fractions showed yellow and brick-red colours. The R_f values 0.94 and 0.23 showed yellow colour. The remaining bands showed brick-red colour. The unseparated band had violet colour (Table 1).

The secretory pigment yielded nine bands having different R_f values. These bands were yellow to brown in colour (Fig.1.ii.A). All the secretory pigment fractions also displayed fluorescence (Fig.1.ii.B). The R_f value of 0.94 showed yellow colour. The band has an R_f value of 0.35 and the unseparated fraction showed brown colour. The remaining seven bands showed brick-red colour (Table 2). The results clearly indicate the production of different types of pigments with a range of colours and R_f values. The fluorescence of pigments under UV light also provides a useful parameter for detection. Direct detection of pigment fractions and their colour in TLC provides an added advantage for selection and purification. Many chromatographic techniques are employed in fungal pigment characterization. The separation of three pigment fractions from *Penicillium* sp. using TLC was also reported (Pandey *et al.,* 2018). Different shades of pigments such as yellow, orange and red pigments produced by *Monascus purpureus* were effectively separated by TLC and showed UV fluorescence (Yang *et al.,* 2018; Suraiya *et al.,* 2018). Hence, the detection of pigments by analytical TLC in *A. nidulans* was achieved.

In UV-Visible spectroscopy, the cellular crude pigment showed peaks at 458 nm and 521 nm corresponding to orange and red pigments respectively (Fig. 2). The UV-Visible spectra of TLC-separated cellular pigment fractions dissolved in ethyl acetate and ethanol showed different peaks in the range of 200 to 500 nm (Fig. 3). In cellular pigments, the bands having an R_f value 0.81 and 0.70 showed a peak at 450 nm corresponded to orange pigment. The band having an R_f value of 0.88 showed a peak at 430 nm in ethanol corresponding to yellow pigment whereas no detectable pigment was found in ethyl acetate. Apart from these, no detectable pigments were observed in ethanol-soluble fractions. The ethyl acetate soluble fractions having an R_f value of 0.67 and the

unseparated fraction showed absorption maxima at 450 nm corresponding to orange pigment. The ethyl acetate soluble pigment fractions having an R_f value of 0.23, 0.19 and 0.13 showed peaks in the range of 370 to 400 nm corresponding to yellow pigment. The same fractions also showed orange colour corresponding to 450 nm of absorption maxima (Table1). The absorption maxima at 410, 470 and 510 nm corresponded to yellow, orange and red pigments respectively in *Monascus* spp. (Pereira *et al.*, 2008; Chen *et al.*, 2017). The present results suggest the yellow and orange pigment derivatives with different absorption maxima are available in *A. nidulans*.

The secretory crude pigment showed peak at 464 nm which corresponds to the orange pigment (Fig. 2). The TLC- separated secretory pigment showed various peaks (Fig. 4). The ethyl acetate-soluble fraction having an R_f value of 0.71 showed a peak at 453 nm which corresponded to orange pigment.

Similarly, the ethanol-soluble pigment fraction having an R_f value of 0.81 showed a peak at 470 nm corresponding to orange pigment. The other TLC-separated secretory fractions dissolved in ethyl acetate as well as ethanol did not correspond to any detectable pigment (Table 2). Colour of the pigment fractions may be confirmed by obtaining the absorption spectra of crude and TLC-separated fractions (Brown and Salvo, 1994; Pavia *et al.*, 2007). Hence, spectroscopy is useful in detecting both cellular and secretory pigments of *Aspergillus nidulans*. Comparative analysis was also made in this technique. Specific melanin pigments in *A. nidulans* have been reported (Goncalves *et al.*, 2012; Medeiros *et al.*, 2022).

Antimicrobial activity by disc diffusion assay

The crude cellular pigment inhibited the growth of *Bacillus subtilis* only among the tested bacteria. Among the nine TLC-separated cellular pigment fractions except for fractions C6 to C9, inhibited *B. subtilis* (Table 3). The antibacterial activity was around 30% when compared to the positive control. Selective inhibition of *B. subtilis* from *Aspergillus nidulans* pigment fraction indicated the specific biological activity. All the pigment fractions were not antibacterial which clearly demonstrates the necessity of identifying specific molecule with biological activity (Pandey *et al.*, 2018).

The crude secretory pigment inhibited the growth of Gram-positive bacteria such as *B. subtilis* and *Staphylococcus aureus* showing almost 65 % of activity when compared to positive control whereas no inhibition of growth was noticed against the Gram-negative bacteria. The strongest antibacterial activity was noticed in secretory crude pigment compared to cellular crude pigment. All the TLC-separated secretory pigment fractions showed various levels of antibacterial activity on *B. subtilis* (Table 3). The secretory brick red pigment fraction S3 only showed antibacterial activity over *Staphylococcus aureus*. The maximum antibacterial activity on *B. subtilis* was displayed by fraction S3. However, the antibacterial activity of these fractions was noticeably less compared to Vancomycin (Table 3). The secretory pigment fractions were highly effective antibacterial compounds. The specific fraction may be exploited in pharmaceutical industries to produce antibacterial lotions and/or ointments (Pombeiro-Sponchiado *et al.*, 2017).

The antifungal activity of both crude cellular and secretory pigments was observed against the target fungi. The crude secretory pigment showed slightly higher inhibition of growth against target fungi compared to crude cellular pigment. All the TLC-separated cellular pigment fractions exhibited antifungal activity for target fungi. The TLC-separated secretory pigment fractions, except fractions S6 to S9, showed inhibition activity over *Candida albicans*, whereas all the TLC-separated secretory pigment fractions exhibited antifungal activity on *Cryptococcus* sp. Both extracts exhibit much less antifungal activity than fluconazole at the concentrations tested (Table 4). Antifungal activity of *Aspergillus tamarii* against *Chrysosporium* spp., *Candida albicans* and other fungi have been reported (Saravanan *et al.*, 2020).

Table.1 Detection of TLC-separated cellular pigment fractions from *Aspergillus nidulans* (DUMB 14) by spectroscopy.

Cellular fraction	R_f value	Visible colour	Absorption maxima (nm) in ethyl acetate	Corresponding colour	Absorption maxima (nm) in ethanol	Corresponding colour
C1	0.94	Yellow	323 nm$^{\Phi}$	No colour	322 nm*	No colour
C2	0.88	Brick red	315 nm$^{\Phi}$	No colour	430 nm	Yellow
C3	0.81	Brick red	450 nm	Orange	450 nm	Orange
C4	0.70	Brick red	450 nm	Orange	450 nm	Orange
C5	0.67	Brick red	450 nm	Orange	340 nm*	No colour
C6	0.23	Light yellow	371 nm 400 nm 450 nm	Yellow Yellow Orange	340 nm*	No colour
C7	0.19	Brick red	373 nm 450 nm	Yellow Orange	340 nm*	No colour
C8	0.13	Brick red	396 nm 450 nm	Yellow Orange	340 nm*	No colour
C9	Unseparated fraction	Violet	450 nm	Orange	340 nm*	No colour

* Absorption maxima did not correspond to any pigment.

Table.2 Detection of TLC-separated secretory pigment fractions from *Aspergillus nidulans* (DUMB 14) by spectroscopy.

Secretory fraction	R_f value	Visible colour	Absorption maxima (nm) in ethyl acetate	Corresponding colour	Absorption maxima (nm) in ethanol	Corresponding colour
S1	0.94	Light yellow	315 nm*	No colour	311 nm*	No colour
S2	0.81	Brick red	254 nm*	No colour	470 nm	Orange
S3	0.71	Brick red	453 nm	Orange	262 nm*	No colour
S4	0.68	Brick red	254 nm*	No colour	340 nm*	No colour
S5	0.35	Light brown	254 nm*	No colour	340 nm*	No colour
S6	0.26	Brick red	254 nm*	No colour	310 nm*	No colour
S7	0.20	Brick red	254 nm*	No colour	340 nm*	No colour
S8	0.14	Brick red	254 nm*	No colour	340 nm*	No colour
S9	0.06	Brick red	316 nm*	No colour	335 nm*	No colour
S10	Unseparated fraction	Brown	311 nm*	No colour	238 nm*	No colour

* Absorption maxima did not correspond to any pigment.

Table.3 Antibacterial activity of *Aspergillus nidulans* (DUMB 14) pigment fractions in disc diffusion assay.

Sample	R_f value of the fraction	Zone of inhibition (in mm) ± Standard error			
		***Escherichia coli* MCC 2246**	***Salmonella enterica* MCC 3910**	***Bacillus subtilis* MCC 2511**	***Staphylococcus aureus* MCC 2408**
Positive control		24.5±0.5	22.33±0.33	26±1.5	19.33±0.33
Ethyl acetate		0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Crude cellular pigment		0.0±0.0	0.0±0.0	**8.0±1.0**	0.0±0.0
C1	**0.94**	-	-	7.33±0.66	-
C2	**0.88**	-	-	6.66±0.33	-
C3	**0.81**	-	-	7.33±0.33	-
C4	**0.70**	-	-	6.33±0.33	-
C5	**0.67**	-	-	7.0±0.0	-
C6	**0.23**	-	-	0.0±0.0	-
C7	**0.19**	-	-	0.0±0.0	-
C8	**0.13**	-	-	0.0±0.0	-
C9	**Unseparated fraction**	-	-	0.0±0.0	-
Crude secretory pigment		0.0±0.0	0.0±0.0	**17±1.52**	**16.66±0.88**
S1	**0.94**	-	-	6.66±0.66	0.0±0.0
S2	**0.81**	-	-	8.0±0.57	0.0±0.0
S3	**0.71**	-	-	10±0.57	7.33±0.33
S4	**0.68**	-	-	7.0±0.57	0.0±0.0
S5	**0.35**	-	-	6.33±0.33	0.0±0.0
S6	**0.26**	-	-	7.33±0.33	0.0±0.0
S7	**0.20**	-	-	7.33±0.33	0.0±0.0
S8	**0.14**	-	-	7.66±0.33	0.0±0.0
S9	**0.06**	-	-	6.66±0.33	0.0±0.0
S10	**Unseparated fraction**	-	-	6.66±0.33	0.0±0.0

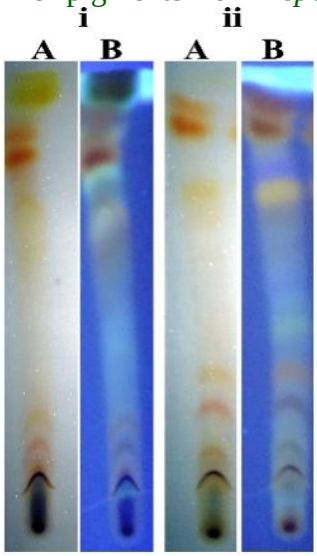
C1 to C9- Cellular pigment fractions separated by TLC.
S1 to S10- Secretory pigment fractions separated by TLC.
(-) Not performed as the crude pigment did not show activity.
Positive control- Chloramphenicol for *Escherichia coli* MCC 2246 and *Salmonella enterica* MCC 3910; Vancomycin for *Bacillus subtilis* MCC 2511 and *Staphylococcus aureus* MCC 2408

Table.4 Antifungal activity of *Aspergillus nidulans* (DUMB 14) pigment fractions in disc diffusion assay.

Treatment	R_f value of the fraction	Zone of inhibition (in mm) ± Standard error	
		Candida albicans MCC 1151	*Cryptococcus* sp. MCC 1408
Fluconazole		27±0.57	27.33±0.33
Ethyl acetate		0.0±0.0	0.0±0.0
Crude cellular pigment		**6.66±0.33**	**6.0±0.0**
C1	**0.94**	7.33±0.33	6.0±0.0
C2	**0.88**	6.66±0.33	6.66±0.33
C3	**0.81**	7.0±0.57	6.33±0.33
C4	**0.70**	6.33±0.33	6.33±0.33
C5	**0.67**	6.66±0.33	7.66±0.33
C6	**0.23**	6.0±0.0	6.66±0.33
C7	**0.19**	6.33±0.33	6.66±0.33
C8	**0.13**	6.66±0.33	7.0±0.0
C9	**Unseparated fraction**	6.0±0.0	6.66±0.33
Crude secretory pigment		**7.33±0.33**	**8.33±0.33**
S1	**0.94**	7.0±0.57	6.66±0.33
S2	**0.81**	7.33±0.33	7.66±0.33
S3	**0.71**	7.33±0.33	7.66±0.33
S4	**0.68**	6.0±0.0	6.33±0.33
S5	**0.35**	6.33±0.33	6.66±0.33
S6	**0.26**	0.0±0.0	6.33±0.33
S7	**0.20**	0.0±0.0	6.33±0.33
S8	**0.14**	0.0±0.0	7.33±0.33
S9	**0.06**	0.0±0.0	6.66±0.33
S10	**Unseparated fraction**	6.0±0.0	6.33±0.33

C1 to C9- Cellular pigment fractions separated by TLC; S1 to S10- Secretory pigment fractions separated by TLC.

Fig.1 Thin-layer chromatogram of pigments from *Aspergillus nidulans* (DUMB 14).



i- Cellular pigment, ii- Secretory pigment, A=Visible light B= Ultraviolet light

Fig.2 UV- Visible spectrum of crude pigments from *Aspergillus nidulans* (DUMB 14).

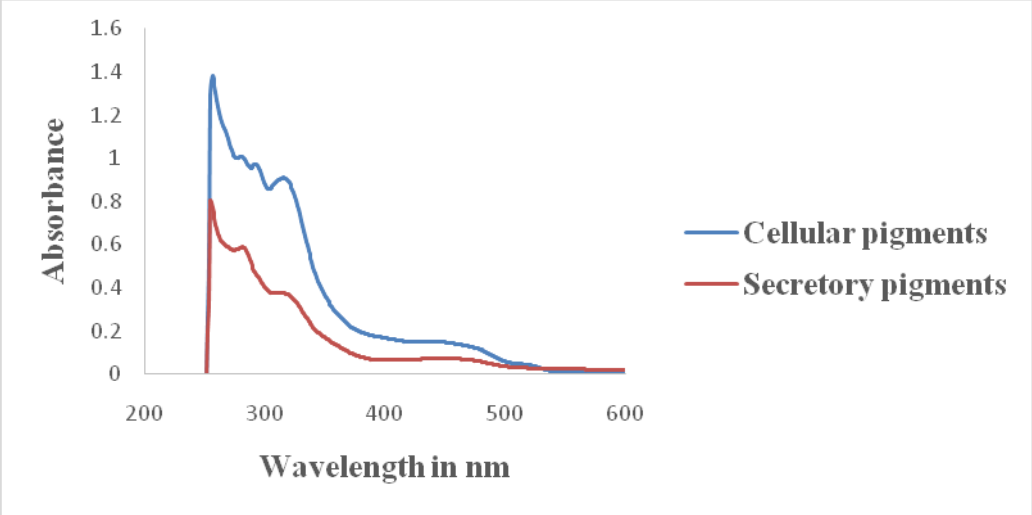
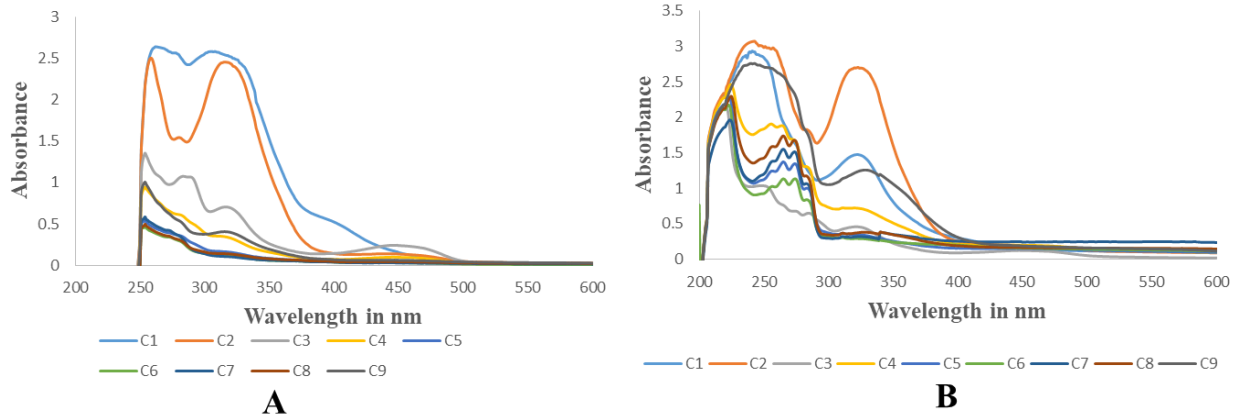


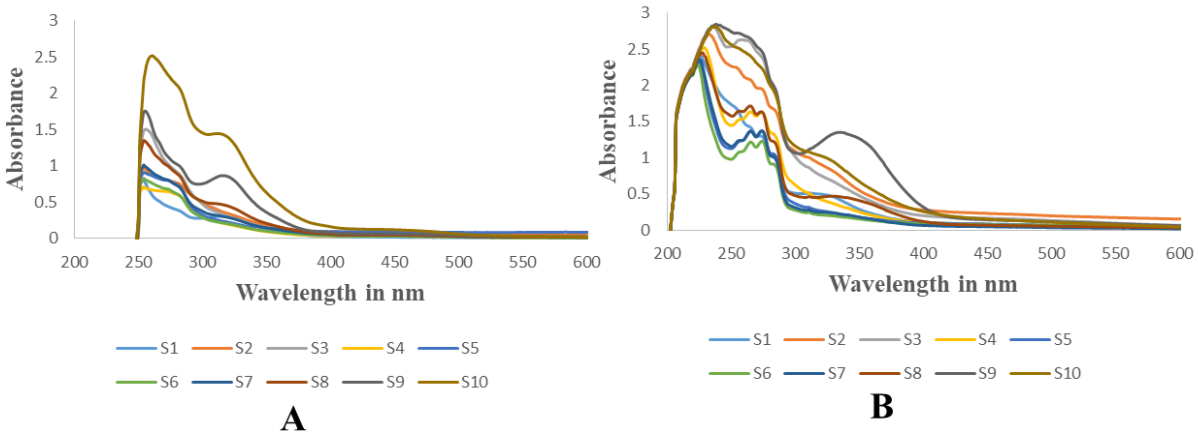
Fig.3 UV- Visible spectra of TLC-separated cellular pigment fractions from *Aspergillus nidulans* (DUMB 14) in different solvents.



Cellular pigment fractions C1 to C9 are separated by TLC.

A. Ethyl acetate soluble fractions, B. Ethanol soluble fractions

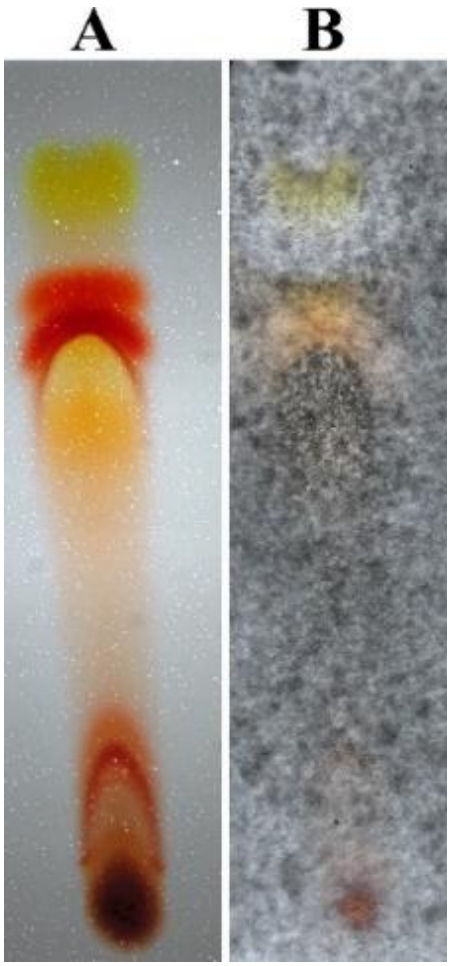
Fig.4 UV- Visible spectra of TLC-separated secretory pigment fractions from *Aspergillus nidulans* (DUMB 14) in different solvents.



Secretory pigment fractions S1 to S10 are separated by TLC.

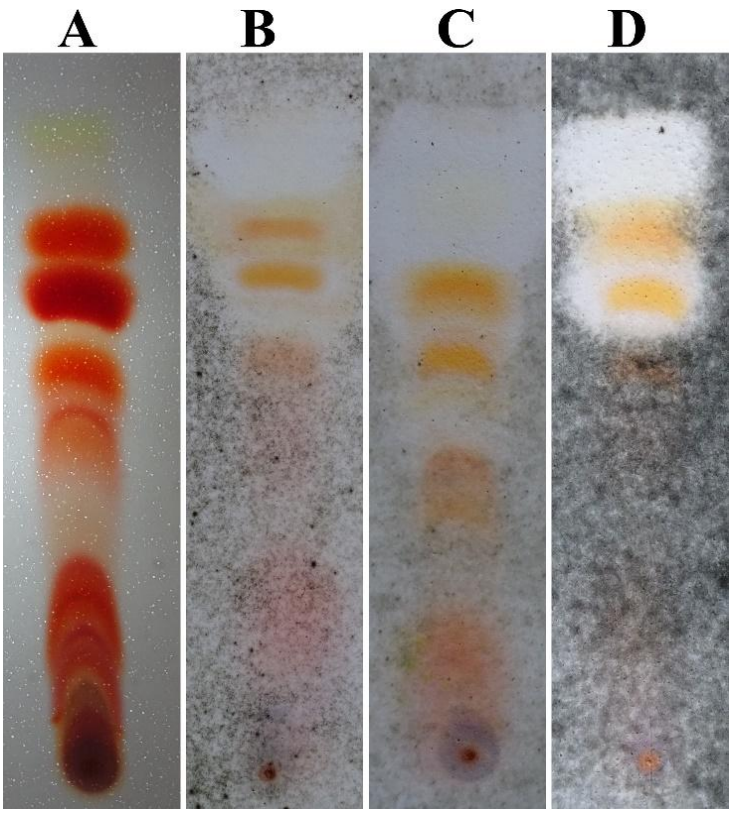
A. Ethyl acetate soluble fractions, B. Ethanol soluble fractions

Fig.5 TLC - Bioautogram of cellular pigment fractions from *Aspergillus nidulans* (DUMB 14).



A - Thin layer chromatogram - Control, B - *Curvularia lunata.*

Fig.6 TLC - Bioautogram of secretory pigment fractions from *Aspergillus nidulans* (DUMB 14).



A - Thin-layer chromatogram - Control, B - *Alternaria tenuissima*,C - *Cladosporium oxysporum*, D - *Curvularia lunata.*

TLC-Bioautography for antifungal activity

The crude cellular pigment of *Aspergillus nidulans* (DUMB 14) showed inhibition activity only against *Curvularia lunata* whereas the crude secretory pigment showed inhibition against all the tested fungi. The TLC-bioautography revealed that the *A. nidulans* (DUMB 14) cellular pigment fraction C4 with orange colour has an R_f value of 0.73 showing 12 mm inhibition zone against the *C. lunata* (Fig. 5).

The secretory fractions having R_f values of 0.94, 0.81 and 0.71 displayed antifungal activity on all the selected fungi. Additionally, the compounds with R_f values of 0.68, 0.35 and 0.26, as well as unseparated fractions displayed antifungal activity only on *Cladosporium oxysporum* (Fig. 6). This bioassay provides an opportunity to detect the antifungal activity of pigment fractions from *Aspergillus nidulans*. Many species of *Aspergillus* are known to produce antifungal compounds (Teixeira *et al.*, 2012; Saravanan *et al.*, 2020). The present study clearly demonstrated antifungal activity in *A. nidulans* as detected by TLC-bioautography. Variations in the antifungal activity against different target fungi also indicate the specific biological activity of *A. nidulans* pigments. These molecules may be exploited to prevent pathogenic fungal infection in plants (Attia *et al.*, 2022).

Antioxidant activity

The crude cellular and secretory pigment of *Aspergillus nidulans* (DUMB 14) displayed free radical scavenging activity in a dose-dependent manner. No much difference in antioxidant activity was noticed between crude cellular and secretory pigments. All the cellular fractions showed different levels of antioxidant activity expressed as percent inhibition of DPPH. Among the nine cellular

fractions, yellow fraction C1 (46.35±2.43) and brick red fraction C2 (46.35±3.34) showed the highest antioxidant activity whereas the lowest was observed in violet fraction C9 (37.52±2.22). All the secretory pigment fractions also showed various levels of antioxidant activity. The secretory brick red fractions S2 (44.82±1.17), S4 (44.41±0.95) and S7 (43.54±2.67) showed the highest antioxidant activity and brown fraction S10 (26.57±3.25) displayed the least amount of antioxidant activity. The pigments produced by the *Aspergillus nidulans* clearly showed antioxidant potential. Pigments of *Aspergillus* sp. were reported to show antioxidant activity (Pandiyarajan *et al.*, 2018; Shanuja *et al.*, 2018). Hence, it must be possible to exploit *A. nidulans* pigment fractions as antioxidants.

Various biological activities of pigments were detected in the fungus *Aspergillus nidulans*. UV-Visible spectroscopy and TLC also provided an advantage to detect pigment fraction. Bioassays developed show the significant antimicrobial properties of fungal pigments. Hence, *A. nidulans* may be exploited further for the production of selective pharmaceuticals.

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Conflict of Interest

The authors have no conflict of interest.

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