Research article

Bioprospecting of *Talaromyces ruber* pigments for antimicrobials

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ABSTRACT

Introduction and Aim: Metabolic flexibility of fungi is unique among organisms. Fungi can produce various pigments of industrial importance. Potential of *Talaromyces ruber* for beneficial pigments needs to be explored. Hence, the present study aims at the detection of pigments from *T. ruber* and determining their antimicrobial properties.

Materials and Methods: A soil isolate of *T. ruber* was tested for pigment production. Cellular and secretory pigments were extracted. They were separated by thin-layer chromatography (TLC) and detected by UV-Visible spectrophotometry. The antibacterial activities of crude pigments were tested by disc diffusion method. The antifungal activity was detected by disc diffusion method, conidial germination inhibition assay and bioautography methods.

Results: The results showed that cellular metabolites yielded seven pigment fractions and secretory metabolites yielded five different pigment fractions in TLC. In UV-Visible spectrometry, the absorption range of visible light with 370 to 412 nm detected yellow pigments and absorption of 500 to 520 nm detected red pigments. Crude cellular and secretory pigment fractions showed inhibition activity only on *Bacillus subtilis*. The antifungal activity of both crude cellular and secretory pigments was observed against *Candida albicans* and *Cryptococcus* sp. The secretory crude pigment showed conidial germination inhibition only against *Alternaria tenuissima*. In bioautography, the cellular and secretory crude pigments showed an inhibition zone for *Cladosporium oxysporum.* Additionally, secretory crude pigment showed similar activity against *Curvularia lunata.* Interestingly, the variations in the antifungals between cellular and secretory pigment fractions are also evident. Production of antimicrobial compounds from *T. ruber* was established after the detection of pigment fractions.

Conclusion: Potential of *T. ruber* to produce yellow and red pigments was realized. Antimicrobial pigments from *T. ruber* were detected providing scope to develop for industrial scale. These pigments may be used in pharmaceutical and nutritional industries.

Keywords: *Talaromyces ruber*; pigments; spectroscopy; bioautography.

INTRODUCTION

Colours are the first parameters one can notice before purchasing any materials. Food colourants chemically prepared are hazardous to health. Natural pigments are produced by microorganisms and plants. Plant pigments are limited. The microorganisms produce a diverse group of pigment molecules, including carotenoids, quinones, flavins, monascins, violacein, indigo etc. (1-3). The pigments from filamentous fungi are considered as great alternatives to synthetic or other natural pigments. Some of these pigments have potential biological effects as they exhibit anticancer, antitumor, antioxidant, and antimicrobial activities (1, 4). These pigments are also used in many industries like pharmacological, food, textile, and leather industry as colouring agents (5, 6). In the past decade, the demand has increased for natural-origin pigments owing to health consciousness.

Potential pigment-producing filamentous fungi are essentially significant to realize their biotechnological applications. Among the microorganisms, fungi are predominant pigment producers. Many fungi such as *Monascus, Paecilomyces, Cordyceps, Talaromyces, Aspergillus,* and *Penicillium* are known to produce various kinds of pigments (7). Many strains of *Talaromyces* species (formerly *Penicillium* sp.) were detected to produce *Monascus*-like polyketide azaphilone (8). Considering the potential of *Talaromyces* spp. the present investigation was made to detect pigments from *Talaromyces ruber* and determine their antimicrobial properties.

MATERIALS AND METHODS

Isolation and identification of pigment-producing fungi

Soil samples from regions of Davangere University, Shivagangothri campus, Davangere, Karnataka were collected. Sterile water was dispersed with soil samples and spread plated on sterile potato dextrose agar (PDA) medium supplemented with chloramphenicol (300 µg/ml) in Petri plates. The plates were incubated for up to seven days at room temperature 25±5ºC. The pigment-producing fungus was isolated and pure culture was established on

PDA slants and identified using standard literature (9, 10).

The colony morphology of pigment-producing fungus was observed on Czapek-Dox yeast autolysate agar (CYA), Saline CYA containing 5% NaCl (CYAS), Malt extract agar (MEA), and Yeast extract sucrose agar (YES) medium (11). The fungus was inoculated onto the media in Petri plates at three points and incubated in darkness for seven days at 25±2ºC. The colony diameter on different media was measured. Colony characters, conidia and conidiophore morphology and size measurements were also determined.

Pigment production and extraction

For pigment production, the selected fungus was inoculated to test tubes having potato dextrose broth (PDB: 5 ml) and kept at room temperature (25±5ºC). Four-day-old inoculum was transferred to Roux bottles containing 300 ml of sterile PDB and incubated for seven days in room temperature for pigment production.

The mycelial biomass from the culture broth was separated through filtration. The cellular pigments were extracted by immersing mycelial mass in ethyl acetate overnight. The ethyl acetate soluble fractions of cellular pigments were collected and remaining biomass was kept in a hot-air-oven at 60ºC for complete dryness and then weighed for biomass estimation. The secretory pigment was extracted by adding ethyl acetate to the culture filtrate at the ratio of 1:1 in a separating funnel. Both the extracts were air-dried and then recorded the dry weight of the crude cellular and secretory compounds.

UV-Visible spectrophotometry

The absorbance of crude cellular and secretory compounds was analyzed by wavelength scan in a UV-Visible spectrophotometer (LABMAN, LMSP-UV1200). The extracts were scanned in the range of 200 to 800 nm wavelength to detect the absorbance of the pigments (12). The ethyl acetate served as a blank for cellular components. The ethyl acetate extract of PDB was employed as control for crude secretory components.

Thin layer chromatography (TLC)

Analytical thin-layer chromatography was carried out by spotting crude extracts on a Silica gel G plate. In the initial trials, eight separate solvent systems were selected. Ideal solvent system Ethyl acetate:Hexane (6:4) was chosen for analysis. After separation, the air-dried plates were visualized under visible light and also in UV light separately. The number of spots and their R_f values were recorded.

Preparative TLC using 20×20 cm Silica gel plates was carried out. The plate was spotted by crude extract as a single streak separately and developed the chromatogram using Ethyl acetate:Hexane (6:4) solvent system. The plates were observed both in visible light and UV light separately. The pigment spots were marked, scrapped, dispersed in ethyl acetate, and then centrifuged. The supernatant was further analyzed.

Antimicrobial activity of the pigments

Disc diffusion assay

Antibacterial activity was conducted on *Escherichia coli* (MCC 2246), *Salmonella enterica* (MCC 3910), *Bacillus subtilis* (MCC 2511) and *Staphylococcus aureus* (MCC 2408). For antifungal activity, the yeasts such as *Candida albicans* (MCC 1151) and *Cryptococcus* sp. (MCC 1408) were used. Pure cultures were procured from a Microbial Culture Collection, National Centre for Microbial Resource, Pune, India.

The crude ethyl acetate extracts of cellular and secretory pigments and their TLC-separated fractions were loaded on the Whatman filter paper disc (5 mm) separately. For *E. coli* (MCC 2246) and *Salmonella enterica* (MCC 3910), chloramphenicol (30 µg) was used as control. For *B. subtilis* (MCC 2511) and *Staphylococcus aureus* (MCC 2408), Vancomycin (30 µg) served as positive control. The disc containing ethyl acetate was negative control. The 24-hour-old bacterial culture grown in Luria Bertani (LB) broth was swab inoculated on sterile Mueller-Hinton agar medium in Petri plates. The pigment-loaded discs were placed equidistantly in the inoculated plates. The plates were kept in an incubator at 37 ºC and observed at 24 hours for growth inhibition.

The 24-hour-old yeast cultures grown in Sabouraud dextrose broth were swab inoculated on Sabouraud dextrose agar medium in Petri plates. The pigment-loaded discs were placed equidistantly in the inoculated plates. Fluconazole (1 mg) containing disc was positive control. The plates were kept in an incubator at 37ºC and observed after 24 hours. For both antibacterial and antifungal activity, duplicates were maintained and the experiment was repeated twice.

Conidial germination inhibition assay

The crude cellular, secretory pigments and TLC-separated fractions were dissolved in 0.1% Dimethyl sulfoxide (DMSO) for determining their antifungal activity. The conidia from the selected fungi such as *Alternaria tenuissima*, *Cladosporium oxysporum*, *Curvularia lunata* and *Fusarium oxysporum* from Departmental culture collection were mixed in cavity slides containing 100 µl of pigment extracts. The target test fungal conidia in 100 µl of sterile water and 0.1% DMSO taken separately as controls. The slides were kept in a moist chamber for up to 20 hours for incubation. The conidia were observed in

different microscopic fields for the germ tube production. In each treatment, a minimum of 100 conidia were considered. Three independent trials were conducted. The conidial germination percentage for each treatment was calculated. Conidial germination inhibition over control was calculated with the following formula.

$$\text{Conidial germination inhibition (\%) over control} = \frac{\text{Germination in control (C)} - \text{Germination in treatment (T)}}{\text{Germination in control (C)}} \times 100$$

The average value of each treatment was considered to calculate the standard error.

Bioautography

The crude extracts of cellular and secretory pigments from selected fungus were spotted on Silica gel G-coated glass plate equidistantly. The fluconazole and ethyl acetate were used as positive and negative controls respectively. The spotted extracts were allowed to air dry. The *Alternaria tenuissima, Cladosporium oxysporum* and *Curvularia lunata* were used as target fungi. The spore suspensions of these fungi were prepared in potato dextrose broth (PDB) separately and sprayed onto Silica gel G-coated glass plates using an automizer uniformly. The plates were placed in a moist chamber for 24 hours for fungal growth. The crude pigment which showed antifungal activity in bioautography against target fungi was subjected to TLC-Bioautography using Ethyl acetate: Hexane (6:4) solvent system.

RESULTS

Isolation and identification of pigment-producing fungi

Various kinds of fungi were grown on a PDA medium placed with soil samples. Among the fungal isolates obtained, the intense diffusible red pigment-producing fungus was selected. The fungus with grayish-green colony showed restricted growth and secreted red colour pigment diffused into the medium (Fig.1).

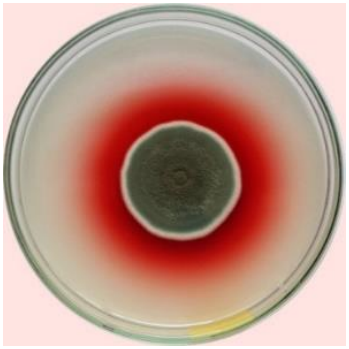

Fig. 1: The pigment-producing fungus *Talaromyces ruber* (DUMB 25) grown on potato dextrose agar medium.

The pigment-producing fungal isolate showed various colony morphology and microscopic characteristics on selected media (Fig. 2). The characteristics shown by this fungus on the individual medium are presented here.

Czapek-Dox yeast autolysate agar (CYA), 25ºC, 7-days: Colonies are 23-25 mm in diameter, Sulcate; margins entire; mycelium white; texture velvety; sporulation moderately dense; soluble pigment weak red; exudates absent; reverse brownish red.

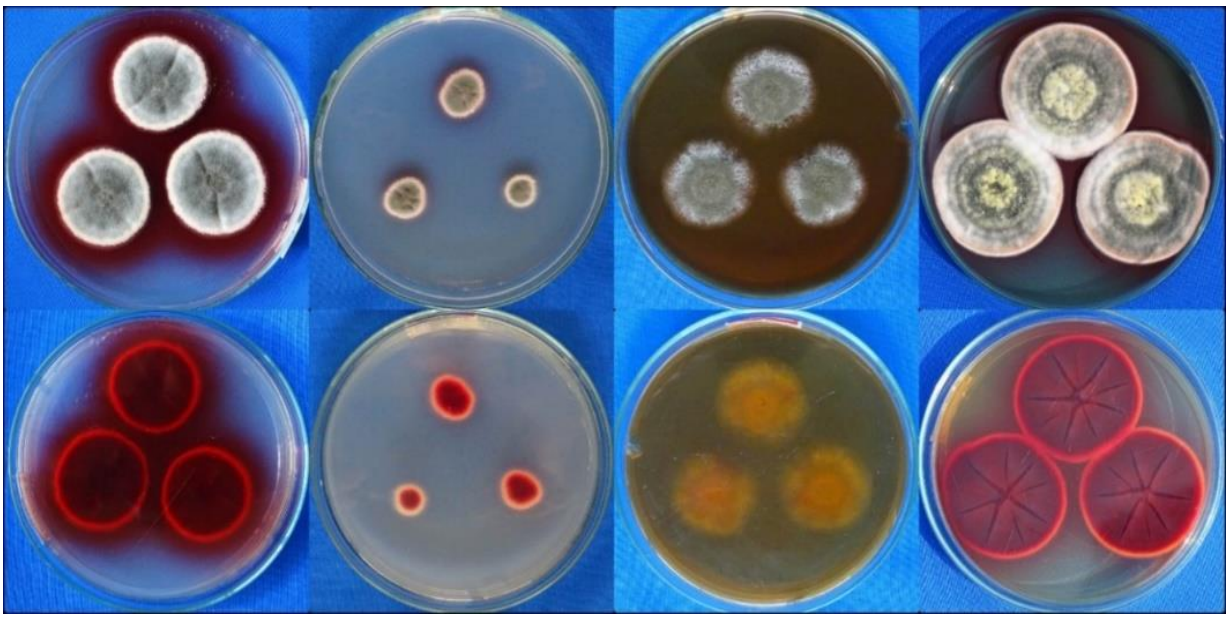

Fig. 2: Growth of *Talaromyces ruber* (DUMB 25) colony on different media. Top row left to right: Colony obverse Czapek-Dox yeast autolysate agar (CYA), Saline CYA containing 5% NaCl (CYAS), Malt extract agar (MEA) and Yeast extract sucrose agar (YES). Bottom row left to right: Colony reverse CYA, CYAS, MEA, and YES.

Saline CYA supplemented with 5% NaCl (CYAS), 25ºC, 7-days: Colonies are 12-17 mm in diameter, raised at the point of inoculation, sulcate; margin entire; mycelium white; texture floccose; sporulation moderately dense; soluble pigment absent; exudates absent; reverse orange.

Malt extract agar (MEA), 25ºC, 7-days: Colonies are 28-29 mm in diameter, plane; margin entire; mycelium white; texture velvety; sporulation dense; soluble pigment absent; exudates absent; reverse brown.

Yeast extract sucrose agar (YES), 25ºC, 7-days: Colonies are 42 mm in diameter, moderately deep, radially sulcate, yellow at the centre; margin entire; mycelium white and yellow; texture velvety; sporulation dense; soluble pigment red; exudates absent; reverse dark red.

Conidia from different media showed olive green to dull green in colour. Conidiophores are biverticillate, smooth walled 103.76 × 2.5 µm; Metulae three to five, 9.12 × 2.36 µm; Phialides acerose, three to six per metulae, 9.56 × 2 µm; Conidia smooth, ellipsoidal, 2.88 × 2.12 µm. Based on these characteristics, the pigment-producing fungal isolate was identified as *Talaromyces ruber* (Stoll) Yilmaz *et al*. It was given with Davangere University Microbial Culture collection number DUMB 25.

Pigment production and extraction

The *Talaromyces ruber* (DUMB 25) showed intense visible red pigment production in Roux bottles containing PDB. The average weight of dry mycelium was 4.03 g/L of the medium. The ethyl acetate extract of cellular pigment was 60.88 mg/g of biomass. The ethyl acetate extract of secretory pigment was 94.31 mg/L of culture filtrate.

Thin-layer chromatography

The ethyl acetate extracts of crude cellular pigment of *Talaromyces ruber* (DUMB 25) yielded seven different fractions in TLC with Ethyl acetate:Hexane (6:4) solvent system (Fig.3.i.A). The crude cellular pigment also contained 10 fluorescing compounds under UV light (Fig.3.i.B). These fractions were different in their colour and R_f value (Table1). The cellular fraction M1, M6 and M7 showed R_f values of 0.97, 0.12 and 0.04 respectively having a visible yellow colour. The fraction M3 having an R_f value of 0.45 and the unseparated fraction showed visible brown colour. The fractions M4 and M5 having an R_f value of 0.31 and 0.29 respectively, showed visible orange colour. Fraction M2 has an R_f value of 0.92 showing visible red colour.

The ethyl acetate extracts of crude secretory pigment of *Talaromyces ruber* (DUMB 25) yielded five different fractions having different colours (Fig.3. ii. A). There were nine UV fluorescing compounds including the pigments (Fig. 3.ii.B). The secretory fraction C1 and C3 having an R_f value of 0.93 and 0.38 showed visible brownish yellow and light brown colour respectively. The fractions C2, C4 and C5 having an R_f value of 0.59, 0.27 and 0.04 respectively showed a visible yellow colour. The fraction C6 which was unseparated showed visible brown colour (Table 2). The TLC results revealed that the number of visible pigment spots obtained in cellular pigment was more than in secretory pigment. Both cellular and secretory pigment fractions were dominated by yellow colour.

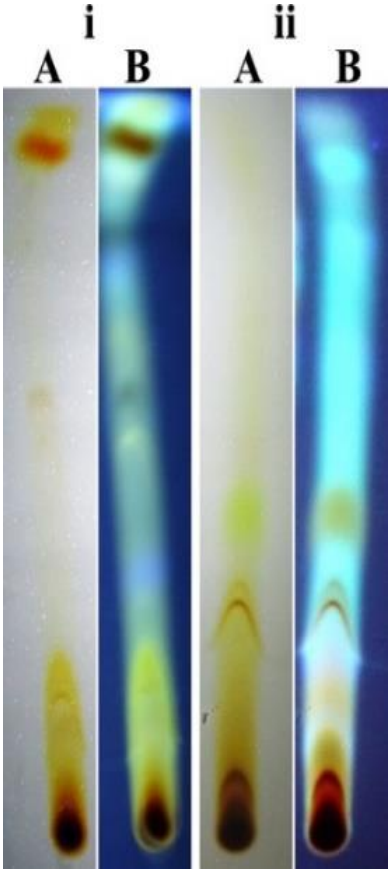

Fig. 3: Thin-layer chromatogram of pigments from *Talaromyces ruber* (DUMB 25).
i - Cellular pigment, ii - Secretory pigment,
A - Visible light; B - Ultraviolet light

UV-Visible spectrophotometry

The UV-Visible spectroscopy resulted in the various numbers of peaks in different compounds. The crude cellular pigment had absorption maxima at 412 nm which corresponded to yellow pigment (Fig. 4A). The TLC-separated cellular pigment fractions with different R_f values showed the presence of compounds with absorption maxima of 260 nm to 275 nm which did not correspond to any pigment. Additionally, the compounds showed absorption maxima in the visible range which corresponded to pigments (Fig. 4B). The cellular pigment fraction except fraction M3 showed peaks in the range of 350 nm to 420 nm which corresponded to yellow pigment. Fraction M3 showed peak at 273 nm which did not correspond to any detectable pigment. Fraction M6 showed an additional peak at 518 nm corresponding to red pigment (Table 1).

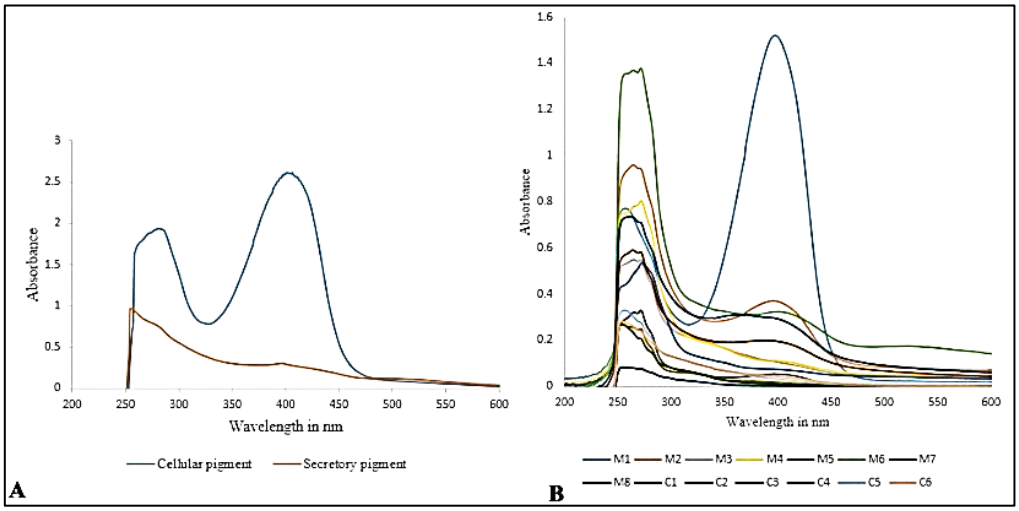

Fig. 4: UV-Visible spectra of pigments from *Talaromyces ruber* (DUMB 25).
A-Crude pigments, B-TLC-separated fractions, M1 to M8-Mycelial fractions, C1 to C6-Culture filtrate fractions.

Table 1: Detection of TLC-separated cellular pigment fractions from *Talaromyces ruber* (DUMB 25) by UV-visible spectroscopy

Thin layer chromatography*			**UV-Visible spectroscopy**	
Cellular fractions^{+}	R_f value	Visible colour	Absorption maxima$^{\#}$	Corresponding colour
M1	0.97	Yellow	404 nm	Yellow
M2	0.92	Red	405 nm	Yellow
M3	0.45	Brown	272 nm$^{\Phi}$	No colour
M4	0.31	Orange	412 nm	Yellow
M5	0.19	Orange	414 nm	Yellow
M6	0.12	Yellow	413 nm; 518 nm	Yellow; red
M7	0.04	Yellow	378 nm	Yellow
M8 (UF)	-	Brown	389 nm	Yellow

UF - Unseparated fraction *Solvent system- Ethyl acetate:Hexane (6:4). $^{+}$ Mycelial mass extracted in ethyl acetate. $^{\#}$ Only absorption maxima in the visible range corresponding to pigments were considered. $^{\Phi}$Absorption maxima did not correspond to any pigment.

Table 2: Detection of TLC-separated secretory pigment fractions from *Talaromyces ruber* (DUMB 25) by UV-Visible spectroscopy

Thin layer chromatography*			**UV-Visible spectroscopy**	
Secretory fractions^{+}	R_f value	Visible colour	Absorption maxima$^{\#}$	Corresponding colour
C1	0.93	Brownish yellow	410 nm	Yellow
C2	0.59	Yellow	392 nm	Yellow
C3	0.38	Light brown	413 nm	Yellow
C4	0.27	Yellow	271 nm$^{\Phi}$	No colour
C5	0.04	Yellow	257 nm$^{\Phi}$	No colour
C6 (UF)	-	Brown	256 nm$^{\Phi}$	No colour

UF - Unseparated fraction *Solvent system- Ethyl acetate:Hexane (6:4). $^{+}$ Culture filtrate extracted in ethyl acetate. $^{\#}$ Only absorption maxima in the visible range corresponding to pigments were considered. $^{\Phi}$ absorption maxima did not correspond to any pigment.

The crude secretory pigment showed absorption maxima at 405 nm and 510 nm which corresponded to yellow and red pigment respectively (Fig. 4A). The TLC- separated secretory pigment fractions with different R_f value showed absorption maxima in the range of 250 nm to 275 nm which represents no detectable pigment. Additionally, some of the fractions showed absorption maxima in the visible range (Fig. 4B). The secretory fractions C1, C2 and C3 showed peaks in the range of 350 to 420 nm corresponding to yellow pigment. The remaining fractions C4, C5 and C6 showed peaks at 272 nm, 260 nm and 256 nm respectively not corresponding to any pigment (Table 2).

Antimicrobial activity of the pigments

Disc diffusion assay

Among the four tested bacteria, the crude cellular and secretory pigments from *Talaromyces ruber* (DUMB 25) exhibited antibacterial activity only on *Bacillus subtilis* (MCC 2511) (Fig. 5). All the cellular pigment fractions showed a zone of inhibition against *B. subtilis* (MCC 2511) (Table 3).

The positive control disc showed a clear zone of inhibition. No zone of inhibition was noticed in disc containing ethyl acetate. The crude secretory pigment fractions showed slightly higher zone of inhibition

compared to crude cellular pigment. All the secretory pigment fractions also showed a zone of inhibition against *B. subtilis* (MCC 2511) (Fig. 5). Among the secretory pigment fractions, the yellow fraction C3 having R_f value 0.38 showed maximum zone of inhibition against *B. subtilis* (MCC 2511) (Table 4).

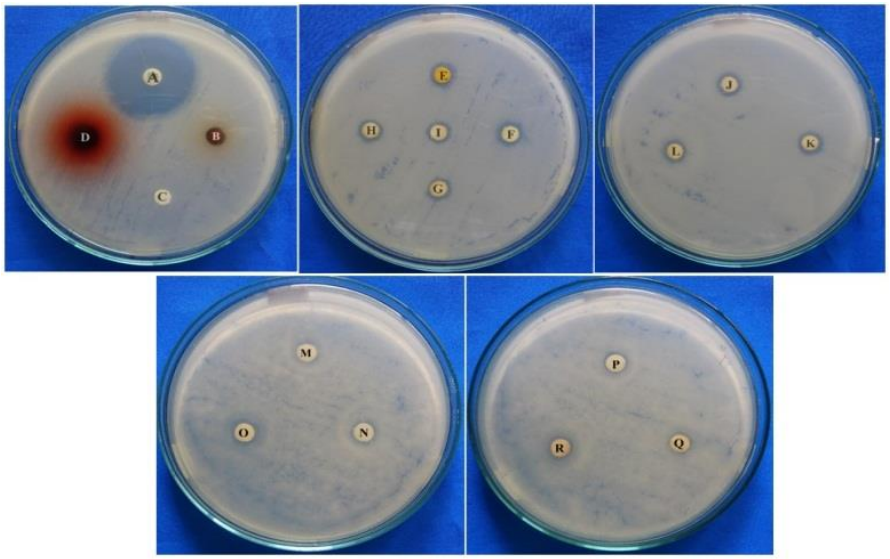

Fig. 5: Antibacterial activity of *Talaromyces ruber* (DUMB 25) pigments against *Bacillus subtilis (*MCC 2511)
A-Vancomycin, B-Crude cellular pigment, C-Ethyl acetate, D-Crude secretory pigment, E to L - 1 to 8 TLC- separated cellular pigment fractions. M to R- 1 to 6 TLC- separated secretory pigment fractions.

The antifungal activity of crude cellular pigment and their separated fractions from *Talaromyces ruber* (DUMB 25) was observed against the *C. albicans* (MCC 1151) and *Cryptococcus* sp. (MCC 1408) in disc diffusion assay (Fig. 6). No major variation was noticed in antifungal activity between the cellular fractions (Table 3). Likewise the crude secretory pigment and their separated fractions displayed antifungal activity over tested fungi in disc diffusion assay (Fig. 6). Among the secretory pigment fractions, insignificant change was noticed against tested fungi (Table 4).

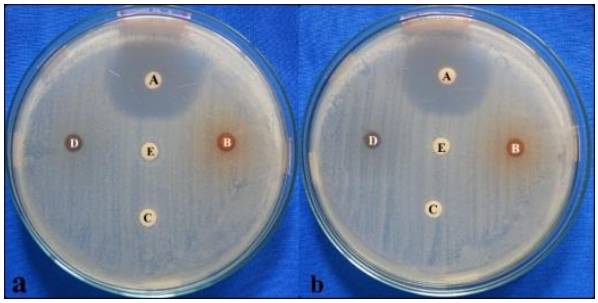

Fig. 6: Antifungal activity of pigment fractions from *Talaromyces ruber* (DUMB 25) against
a-*Candida albicans* (MCC 1151), b- *Cryptococcus* sp. (MCC 1408) in disc diffusion assay.
A-Fluconazole, B-Crude cellular pigment, C-Ethyl acetate, D-Crude secretory pigment, E-Secretory fraction 6.

Conidial germination inhibition assay

Target fungal conidia seeded in sterile water and 0.1% DMSO germinated well by producing germ tubes growing profusely. The crude cellular pigment of *Talaromyces ruber* (DUMB 25) did not show conidial germination inhibition activity against the tested fungi (Table 3). The crude secretory pigment of *T. ruber* (DUMB 25) showed a conidial germination inhibition only against *Alternaria tenuissima*. The secretory yellow pigment fraction C1 having an R_f value of 0.93 showed antifungal activity against *A. tenuissima* by having 43.66 % inhibition (Table 4).

Bioautography

The bioautography results showed the different levels of inhibition zones around the pigment extracts on the tested fungi. The fluconazole showed an inhibition zone and ethyl acetate showed no zone of inhibition over the target fungi. The crude cellular pigment from *Talaromyces ruber* (DUMB 25) had antifungal activity only on *Cladosporium oxysporum* by showing 16.25 mm as zone of inhibition (Fig.7c). None of the separated cellular pigment fractions exhibited antifungal activity on *C. oxysporum* in TLC-bioautography (Table 3).

The crude secretory pigment from *Talaromyces ruber* (DUMB 25) showed a clear zone of inhibition against *Cladosporium oxysporum* and *Curvularia lunata*. The growth of the *C. oxysporum* was inhibited by the crude secretory pigment with an inhibition zone of 33 mm around the pigment (Fig.7). In the case of *Curvularia lunata*, the growth was inhibited only on the secretory pigment to the extent of 8.16 mm. None of the separated secretory pigment fractions revealed antifungal activity on *C. oxysporum* in TLC-bioautography whereas the unseparated secretory pigment displayed antifungal activity on *C. lunata* (Table 4).

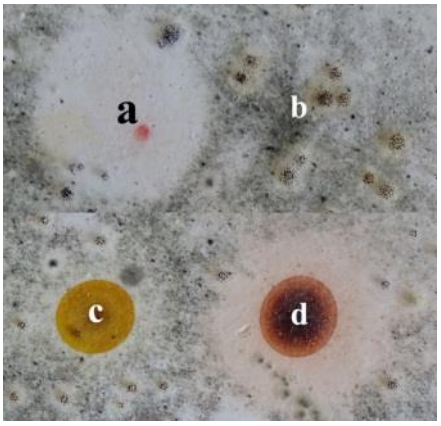

Fig. 7: Bioautogram of cellular pigment fractions of *Talaromyces ruber* (DUMB 25) against *Cladosporium oxysporum*. **a-** Fluconazole, **b-**Ethyl acetate, **c**-Cellular crude pigment**, d**-Secretory crude pigment

Table 3: Antimicrobial activity of cellular pigment fractions from *Talaromyces ruber* (DUMB 25)

Cellular fraction	R_f value	Disc diffusion assay (Zone of inhibition in mm) ± Standard Error						Conidial germination inhibition (%) over control ± Standard Error#				TLC- Bioautography (Zone of inhibition in mm) ± Standard Error		
		Gram-negative		Gram-positive		Yeast								
		EC	SE	BS	SA	CA	CR	AT	CO	CL	FO	AT	CO	CL
Positive control		Chloramphenicol		Vancomycin		Fluconazole						Fluconazole		
		24.5±0.5	22.33±0.33	26±1.5	19.33±0.33	27±0.57	27.33±0.33					30±0	30.25±0.25	30.16±0.16
Crude extract		0.0	0.0	8.66±0.66	0.0	6.66±0.33	6.33±0.33	0.59 ± 0.05	1.53 ± 0.42	0.29 ± 0.08	1.08 ± 0.10	0.0	16.25± 0.75	0.0
M1	**0.97**	-	-	7.66±0.33	-	7.33±0.33	6.66±0.33	-	-	-	-	-	0.0	-
M2	**0.92**	-	-	7.66±0.33	-	6.66±0.33	6.33±0.33	-	-	-	-	-	0.0	-
M3	**0.45**	-	-	7.66±0.33	-	6.33±0.33	6.33±0.33	-	-	-	-	-	0.0	-
M4	**0.31**	-	-	7±0	-	6.33±0.33	6.33±0.33	-	-	-	-	-	0.0	-
M5	**0.19**	-	-	7.33±0.33	-	6.33±0.33	6.66±0.33	-	-	-	-	-	0.0	-
M6	**0.12**	-	-	7.33±0.33	-	7±0.0	6.66±0.33	-	-	-	-	-	0.0	-
M7	**0.04**	-	-	7±0.57	-	6.33±0.33	6.33±0.33	-	-	-	-	-	0.0	-
M8 (UF)		-	-	7±0.0	-	6.33±0.33	6.33±0.33	-	-	-	-	-	0.0	-

UF-Unseparated fraction; EC-*Escherichia coli*, SE-*Salmonella enterica*, BS- *Bacillus subtilis*, SA-*Staphylococcus aureus;* CA-*Candida albicans*, CR-*Cryptococcus* sp. AT-*Alternaria tenuissima*, CO-*Cladosporium oxysporum*, CL-*Curvularia lunata*, FO-*Fusarium oxysporum*. (-) indicates not tested as crude did not show activity. #0.1% Dimethyl sulfoxide (DMSO) was used as the control and the percentage of germination inhibition was calculated over the control.

Table 4: Antimicrobial activity of secretory pigment fractions from *Talaromyces ruber* (DUMB 25)

Secretory fraction	R_f value	Disc diffusion assay (Zone of inhibition in mm) ± Standard Error						Conidial germination inhibition (%)over control ± Standard Error#				TLC- Bioautography (Zone of inhibition in mm) ± Standard Error		
		Gram-negative		Gram-positive		Yeasts								
		EC	SE	BS	SA	CA	CR	AT	CO	CL	FO	AT	CO	CL
Positive control		Chloramphenicol		Vancomycin		Fluconazole						Fluconazole		
		24.5±0.5	22.33 ± 0.33	26±1.5	19.33 ±0.33	27±0.57	27.33 ±0.33					30±0	30.25±0.25	30.16±0.16
Crude extract		0.0	0.0	10.66±0.88	0.0	6.33±0.33	6.66±0.33	100 ±0	1.57 ± 0.34	0.47± 0.10	1.15± 0.16	0.0	33± 0.57	8.16± 0.16
C1	**0.93**	-	-	6.66±0.33	-	6±0.0	6.66±0.33	43.66± 0.57	-	-	-	-	0.0	0.0
C2	**0.59**	-	-	7±0	-	6±0.0	6.66±0.33	0.43 ±	-	-	-	-	0.0	0.0

								0.27						
C3	**0.38**	-	-	8±0.57	-	6±0.0	7.33±0.33	0.14 ± 0.07	-	-	-	-	0.0	0.0
C4	**0.27**	-	-	6.66±0.33	-	6.33±0.33	6.33±0.33	0.15 ± 0.01	-	-	-	-	0.0	0.0
C5	**0.04**	-	-	6.33±0.33	-	6.66±0.33	6.66±0.33	0.16 ± 0.01	-	-	-	-	0.0	0.0
C6 (UF)		-	-	7.33±0.33	-	6.33±0.33	6±0	0.16 ± 0.03	-	-	-	-	0.0	10.0

UF-Unseparated fraction; EC-*Escherichia coli*, SE-*Salmonella enterica*, BS- *Bacillus subtilis*, SA-*Staphylococcus aureus;* CA-*Candida albicans*, CR-*Cryptococcus* sp. AT- *Alternaria tenuissima*, CO- *Cladosporium oxysporum*, CL- *Curvularia lunata*, FO- *Fusarium oxysporum.* (-) indicates not tested as crude did not show activity.

[#]0.1% Dimethyl sulfoxide (DMSO) was used as the control and the percentage of germination inhibition was calculated over the control.

DISCUSSION

Fungi are a unique group of organisms with the potential to produce special metabolites. Pigment-producing *Talaromyces ruber* was isolated successfully from soil and pigments were detected. Different media used for identification indicated the production of both cellular and diffusible pigments (13, 14).

Pigments produced by *T. ruber* were extracted in ethyl acetate and detected by TLC. Pigment fractions were directly visualized on TLC plates which revealed the presence of yellow, red, orange and brown colour. Some of the fractions are capable of showing fluorescence under UV light (15). Secretory pigment fractions ranged from yellow to brown colours. Differences between cellular and secretory pigments were evident in fungi (16). Fluorescent red pigment soluble in water from *Talaromyces amestolkiae* has been reported (17). In the present study too *T. ruber* produced both red and yellow pigments.

Detection of absorption maxima of compounds in the visible range of spectroscopy showed the occurrence of pigments in the extracted samples (12, 16). Out of seven mycelial fractions of *T. ruber*, six showed colours both in TLC and spectroscopy. Differences in visible colour and spectroscopic colour detection depend on the sensitivity and concentration of each fraction (16). Some compounds showing absorption of UV light may not correspond to visible pigments (18). The major peak detected in the cellular crude fraction (yellow) was mainly attributed to the M1 fraction indicating its higher concentration in the sample. The individual pigment fractions concentration may vary among fungal species (16).

Both cellular and secretory fractions of *T. ruber* showed antibacterial activity against *Bacillus subtilis.* The narrow spectrum activity of such pigments may be useful in preventing food-borne infections (19). Pigments with such activity are likely to provide scope to be developed as food colourants and antimicrobials (20, 21). The secreted yellow pigment fraction C3 was found to inhibit *B. subtilis.* The extracellular nature of this fraction makes it ideal for industrial level production (22).

Both cellular and secretory pigment fractions were showing antifungal activity against yeasts. Antifungal compounds available are limited and this finding provided an opportunity to exploit *T. ruber* for novel antifungals (23). In the conidial germination inhibition assay, only secreted yellow pigment fraction showed inhibition to *Alternaria tenuissima* indicating its specificity. Different pigment fractions of *T. ruber* showed antibacterial and antifungal activities with different specificity. Fungal metabolites vary in their specificity and actions (24). Direct bioautography indicated strong antifungal activity of secretory pigment fraction against *Cladosporium oxysporum.* Many of the antifungal compounds may not inhibit conidia but inhibit mycelial growth (25, 26). *Cladosporium oxysporum* is a well-known organism involved in moldy walls and allergies (27). The pigment fractions of *T. ruber* may be used in paints for the prevention of mold growth on walls.

CONCLUSION

Fungal metabolites in the form of pigments with antimicrobial activity may be highly useful in the pharmaceutical and nutritional industries. This study revealed new antimicrobial pigments from *T. ruber* providing scope to develop protocols for industrial-scale production. The compounds produced by *T. ruber* need further characterization to be used as lead molecules in the synthesis of biological pigments.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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