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(RESEARCH ARTICLE)

Extraction, purification, and antimicrobial activity of a pigment from *Aspergillus* species isolated from soil

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Abstract

A pigment-producing *Aspergillus* species was isolated from soil samples collected from Ahmednagar College. Brown colored pigment was obtained after extraction. Its phytochemical analysis showed presence of terpenoids, sterols, saponins, and proteins. The pigment showed inhibitory action against Candida, E. coli, & Staphylococcus. It was purified using silica gel column chromatography. The fractions were characterized by UV-Visible spectrophotometry where different peaks were obtained in 200 to 400 nm region

Keywords: *Aspergillus*; Pigment; Secondary Metabolites; Antimicrobial Activity; Silica Gel Column Chromatography; UV-Visible spectrophotometry

1. Introduction

The natural pigments have garnered increasing attention due to their diverse bioactive properties and applications in various industries, including pharmaceuticals, food, and textiles [1,2]. They have antimicrobial, antioxidant [3, 4, 5], anticancer, and cytotoxic activities [6,7,8]. Fungi are a promising source of pigments and can replace the synthetic dyes and chemical preservatives due to their eco-friendly nature and bioavailability [9]. The majority of well-studied fungal pigments are from fungi of four genera: *Aspergillus*, Penicillium, Paecilomyces, and Monascus [10]. Among the filamentous fungi, the genus *Aspergillus* is one of the most ubiquitous genera contributing to fungal natural products [11,12]. Fungal pigments are grouped into carotenoids, melanin, polyketides, and azaphilones [10]. These pigments show different colors like yellow, orange, red, green, purple, brown, blue, etc. It has been reported that pigments from *Aspergillus* species exhibit inhibitory effects against a variety of pathogenic bacteria and fungi, including Candida species, which are responsible for opportunistic fungal infections [13, 14].

Present study aims to isolate an *Aspergillus* species from soil, extract and purify its pigment, perform chemical analysis, and evaluate its antimicrobial activity. This research seeks to explore the role of secondary metabolites such as terpenoids, sterols, saponins, etc. in antimicrobial efficacy and assess the potential of *Aspergillus*-derived pigments as natural antimicrobial agents.

2. Material and methods

2.1. Isolation and cultivation of pigment producing fungi

Soil samples were collected from the area of Ahmednagar College and used for fungal culture. The soil sample was serially diluted and plated on potato dextrose agar (PDA) medium. The plates were incubated at 28°C for 5 days, and

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fungal colonies exhibiting characteristic *Aspergillus* morphology were isolated and sub cultured for further study. *Aspergillus* spores grown on PDA medium were transferred into PD Broth and incubated in a shaker at 23-28°C for 7 days.

2.2. Pigment Extraction

The pigment was extracted according to the method of Narendrababu and Shishupala 2017 [15] with slight modification. The PD Broth culture was filtered to separate the fungal biomass. An equal volume of ethyl acetate was added to the filtrate and transferred to a separating funnel. The mixture was left undisturbed overnight to allow separation. The brown colored pigment settled at the bottom of the separating funnel was collected in a beaker, and dried. It was then dissolved in a minimum amount of ethyl acetate. Brown pigment has also been reported by Kale et al [8] and Toma et al 2021 [16] from *Aspergillus niger*. The phytochemical analysis of the pigment was carried out according to Thorati and Mishra [17].

2.3. Phytochemical Analysis

- Test for Terpenoids: 2 ml extract was mixed with 1 ml of chloroform and shaken gently. Then, 3 ml of concentrated H_2SO_4 was added. The formation of a reddish-brown ring at the interface indicated the presence of terpenoids.
- Test for Sterols (Salkowski's Test): 1.5 ml extract was mixed with chloroform and thoroughly mixed. A few drops of concentrated H_2SO_4 were added. The formation of a golden yellow color indicated the presence of sterols.
- Test for Saponins (Froth Test): 4 ml distilled water was added to 1 ml of extract and shaken vigorously. The formation of foam indicated the presence of saponins.
- Test for Phenols (Ferric Chloride Test): 2 ml extract was mixed with 4–5 drops of ferric chloride solution. The development of a dark green color indicated the presence of phenols.
- Test for Proteins (Xanthoproteic Test): 2 ml extract was mixed with 3 ml of concentrated HNO_3. The appearance of a dark yellow color indicated the presence of proteins.

2.4. Antimicrobial Activity Assay

The antimicrobial activity of the crude extract was assessed using the agar well diffusion method against *Staphylococcus aureus, E. coli, and Candida* species obtained from the Microbiology Department of Ahmednagar College. The bacterial and fungal strains were inoculated onto selective media. Wells were created in the agar, and the extract was added. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured to determine the antimicrobial efficacy of the pigment.

2.5. Purification of crude extract by column chromatography

The pigment was purified using silica gel column chromatography [2, 17,18]. N-hexane was used to prepare a slurry of silica gel (60-120 mesh size) and the column was packed to a height of 25 cm. The crude extract (4 ml) was loaded onto the column, and mobile phases consisting of N-hexane, ethyl acetate, and methanol were used in a gradient manner. Fractions of 5 ml were collected, and the solvent system was changed as required. Yellow-colored fractions were obtained after elution with ethyl acetate and methanol.

The absorbance of collected fractions was measured in between 200 and 800 nm on UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan). [14, 18].

The fractions were then analyzed for presence of phytochemicals and antimicrobial activity similar to the crude extract.

3. Results and Discussion

3.1. Extraction of pigment from isolated *Aspergillus*

Aspergillus was isolated from soil sample and grown in PDB. From this pigment was extracted using ethyl acetate that was brown in color. Narendrababu and Shishupala 2017 [15] have also reported brown pigment from *Aspergillus* isolates DUMB14 grown in PDB extracted with ethyl acetate.

Figure 1 Brown pigment extracted from *Aspergillus* species

3.2. Phytochemical analysis of the pigment

Different tests of the pigment were carried out to find out the presence of proteins, terpenoids, saponins, phenols, etc. The results are reported in table 1.

Table 1 Phytochemical analysis of the pigment

Sr. No.	Chemicals	Observation	Inference
1	Terpenoids	Reddish-brown ring	Present
2	Saponins	Foam formation	Present
3	Sterols	Golden yellow color	Present
4	Phenols	No green color	Absent
5	Proteins	Yellow color	Present

Kale et al [8] reported the presence of glycosides, terpenoids, tannins etc. in the brown pigment from *Aspergillus* niger.

3.3. Antimicrobial Activity Assay

The antimicrobial activities of pigment from *Aspergillus* sp. were tested using a well diffusion assay against Candida, E. coli and Staphylococcus aureus. It was found to be effective against all 3 tested organisms. The zones of inhibition were 14mm, 12mm, and13mm, respectively that indicates more effectiveness against candida.

Kale et al [8] reported antimicrobial activity of *Aspergillus* niger pigment against S. aureus and E. coli with zones of inhibition 17mm and 15mm, respectively.

3.4. Purification and characterization of the pigment

The pigment was purified using silica gel column chromatography. The fractions were analyzed using UV-Visible spectrophotometer between the wavelength 200-800 nm. The absorbance was obtained for fraction numbers 13-15, 17, 18 and 21. The results are given in Figure 2.

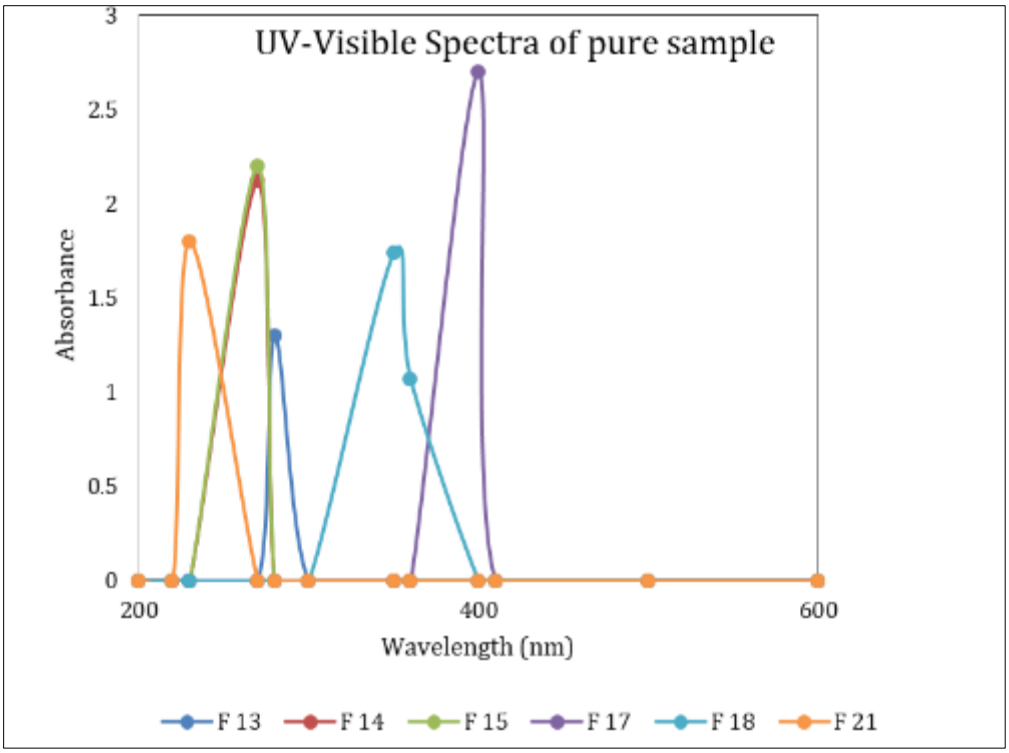

Figure 2 UV-Visible spectra of pure sample

It was carried out between 200 and 800 nm wavelength (in figure shown up to 600 nm only). Maximum absorption was seen in the range 230 to 400 nm that indicates presence of secondary metabolites. Narendrababu and Shishupala [14] reported peaks in the range 200 to 500 nm of the pigment extract from A. nidulans. Toma2021[16] reported 2 peaks one at around 250 nm and another at 295 nm for crude brown pigment from A. niger. The absorption decreased in the visible region also reported by Kale et al [8] and Goncalves et al [19].

3.5. Antimicrobial Activity of Pure Sample

Chemical tests of the fractions indicated presence of terpenoids, sterols, proteins and saponins. Effect of fraction numbers 17 and 18 was checked on test organisms. The observations can be seen in figures 3 and 4.

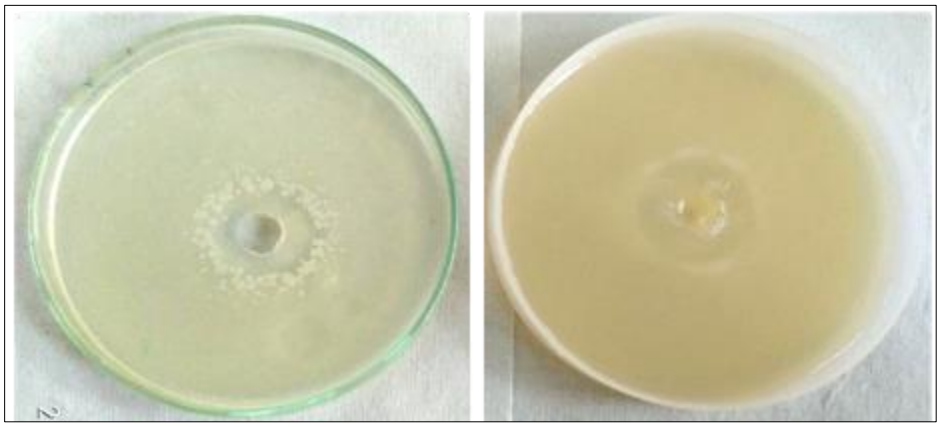

Figure 3 Antimicrobial activity of the 17th fraction against *S. aureus and Candida.* (Zones of inhibition against S. aureus and Candida - 8mm and 11mm, respectively)

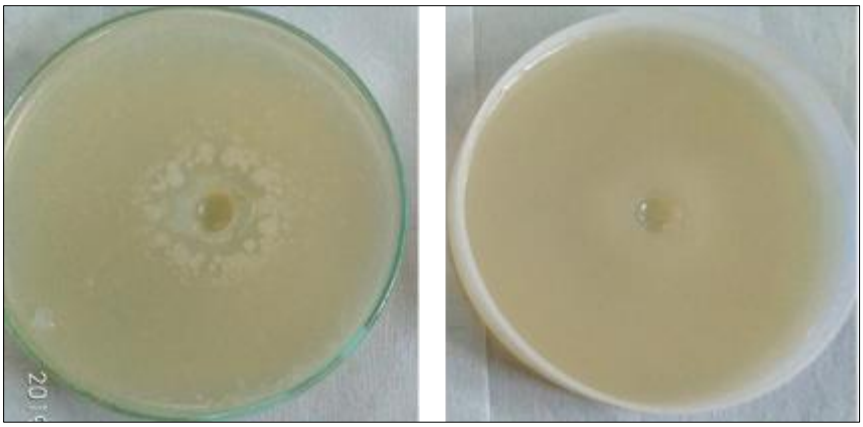

Figure 4 Antimicrobial activity of the 18th fraction against *S. aureus and Candida.* (Zones of inhibition against S. aureus and Candida - 7mm and 10mm, respectively)

4. Conclusion

The study successfully isolated a pigment-producing *Aspergillus* species from soil. Chemical analysis confirmed the presence of terpenoids, saponins, sterols, and proteins in the pigment. The antimicrobial assay revealed significant inhibitory activity against Candida species. The results suggest that *Aspergillus*-derived pigments hold potential as natural antimicrobial agents for pharmaceutical applications. This work paves the way for further research on fungal pigments as eco-friendly and effective antimicrobial agents.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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