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Research Article

IN VITRO CYTOTOXIC POTENTIAL OF HALOPHILIC BLACK YEAST- *AUREOBASIDIUM PULLULANS* AGAINST HUMAN BREAST CARCINOMA CELL LINES

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ABSTRACT

Solar saltern serves as a hotspot for many hidden microorganisms, where the fungal community seems to be a most unexploited one. Halophilic fungi represent a wide source of yet undiscovered compounds, besides its unprecedented chemical structures, often possess interesting biological activities. This work was designed to study the synthesis of bioactive metabolites from halophilic fungi isolated from Vedaranyam saltpans. Altogether, five halophilic strains with two black yeasts like fungi were observed from the soil sample. Among, crude extracts of halophilic black yeast *Aureobasidium pullulans* exhibited the superlative response to MCF-7 cell lines at the concentration of 300µg/ml with an IC_{50} of 174.29 µg/ml respectively. Witnessing these remarkable preliminary findings, provoking to identify the drug motifs and chemical characteristics nature.

Keywords: *Aureobasidium pullulans*, Cytotoxic activity, Halophilic Fungi, Black yeast

1. INTRODUCTION

The main reasons for studying extremophiles, *i.e.* halophilic microorganisms are to understand their mechanisms involved in stress adaptation and for the biotechnological application of their metabolites capable to act under extreme conditions. Low water activity and high salt concentration of hypersaline environments make these habitats an important source that can provide biological metabolites [1]. In general, fungal communities in hypersaline environments are dominated by *Aspergillus*, *Penicillium* and some of their related teleomorphic genera. Other genera such as *Alternaria*, *Cladosporium*, *Fusarium*, *Chaetomium*, *Wallemia* and *Hortaea* were also reported [2, 3]. Black yeasts are a group of rare extremophilic eukaryotes with different species inhabit various natural extreme niches-solar salterns. According to the newly introduced definition of halophily for fungi [4], *H. werneckii* and *A. pullulans* are true obligate halophilicspecies, which can tolerate 17% NaCl in growth medium. Remarkably, this study encounters these two species in Vedaranyam saltpans that has been reported as admirable bioactive metabolites which has the capacity to shown its anticancer and antibacterial potentials.

2. MATERIAL AND METHODS

2.1. Collection of samples

The Vedaranyam saltern soil sample was collected and brought to the laboratory set ups. Soil suspensions were prepared for physicochemical analysis by mixing 10g of the soil in 50 ml of distilled water.

2.2. Mycological analysis

2.2.1. Isolation and identification of Halophilic Fungi

One gram of collected saline soil sample was diluted in 99ml blank and from the dilution 1 ml was serially diluted to the test tubes containing 9ml of sterile distilled water and dilutions were made up to 10^{-4}. From each dilution, 1 ml of dilution suspension was pipetted and plated on a sterile Potato Dextrose Agar (PDA). Bacterial contamination was inhibited by adding 0.05% of streptomycin in PDA. All plates were incubated at 25°C for 5-7 days. The isolated fungal species were identified up to species level by referring standard mycological manuals and books [5, 6]. The fungal species were maintained on a Sabourad Chloromphenicol Agar (SCA) medium at 27°C for further analysis.

2.2.2. Screening of Secondary metabolites

The isolated halophilic fungi were grown on small scale fermentation medium. A fungal disc (6 mm diameter) of 10 days old cultures was inoculated into 500 ml of flask containing 250 ml of the autoclaved Potato Dextrose Broth (PDB) amended with 20% of NaCl and kept in rotatory shaker at room temperature for 10 days.

2.2.3. Crude Extraction

After the incubation periods, the mycelium was separated from the fermented culture broth by filtration. The culture filtrates were extracted three times with 300 ml of ethyl acetate. The resulting layers were combined and the solvent was eliminated by evaporation using a rotator vacuum distilling apparatus to get the crude extracts.

2.3. Antibacterial activity

Escherichia coli, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Streptococcus pyrogens* were chosen for antibacterial assay by standard disc diffusion method. Based on the results, the crude extracts of potent strain were chosen for further work.

2.4. *In vitro* cytotoxic activity

2.4.1. Cancer Cell line

The human breast cancer cell line (MCF- 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.4.2. MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader [7].

3. RESULTS

3.1. Physico-chemical parameters

During the sampling period (April), the temperature of 26°C was observed in soil sample and pH range was recorded as 7.3. Likely, the salinity was noted in the range of 160‰.

3.2. Mycological analysis

Penicillium chrysogenum, Aspergillus niger, Cladosporium cladosporoides, Fusarium oxysporum and *Aureobasidium pullulans* were isolated and subjected for small scale fermentation (Fig.1). Result shows that the highest percentage of crude extracts (1.3 g) was obtained in *Aureobasidium pullulans* followed by 1g in *Cladosporium cladosporoides* and 0.81 gm in *Fusarium oxysporum* and very least amount (0.50 gm) was observed in both *Penicillium chrysogenum* and *Aspergillus niger*.

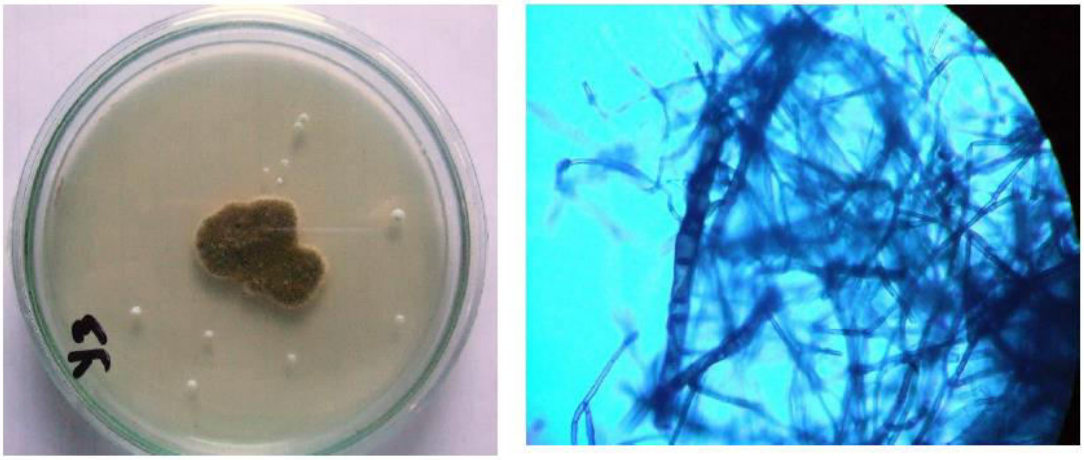

Fig. 1: Colony Morphology and Microscopic observation of black yeast

3.3. Antibacterial activity

The maximum antibacterial activity was observed in the crude extracts of *Aureobasidium pullulans* against *E. coli* (1.9mm). Thus, *Aureobasidium pullulans* was engaged for mass scale production and subjected for anticancer activity (Fig. 2 & Table 1).

3.4. *In vitro* cytotoxic activity (MTT assay)

Results revealed that the crude extracts of *Aureobasidium pullulans* were found to be more effective against MCF-7 cancer cell lines at the maximum cytotoxicity at 300µg/ml concentration (Fig. 3 & 4). IC_{50} value was found to be 174.29 µg/ml (Table 2).

Table 1: Antibacterial activity of Halophilic fungi against Human Bacterial pathogens

Fungi	Pathogen	MIC
Penicillium chrysogenum	*Klebsiella pneumonia* (A)	1
	Klebsilla oxytoca (B)	1.3
	Streptococcus pyogens (C)	1.5
	E. coli (D)	1.5
Aspergillus niger	*Klebsiella pneumonia* (A)	0.8
	Klebsilla oxytoca (B)	1.7
	Streptococcus pyogens (C)	1.4
	E. coli (D)	1.6
Aureobasidium pullulans	*Klebsiella pneumonia* (A)	**1.3**
	Klebsilla oxytoca (B)	**1.7**
	Streptococcus pyogens (C)	**1.8**
	E. coli (D)	**1.9**
Cladosporium cladosporoides	*Klebsiella pneumonia* (A)	1
	Klebsilla oxytoca (B)	1.4
	Streptococcus pyogens (C)	1.5
	E. coli (D)	1.8
Fusarium oxysporum	*Klebsiella pneumonia* (A)	0.9
	Klebsilla oxytoca (B)	1
	Streptococcus pyogens (C)	1.3
	E. coli (D)	1.5

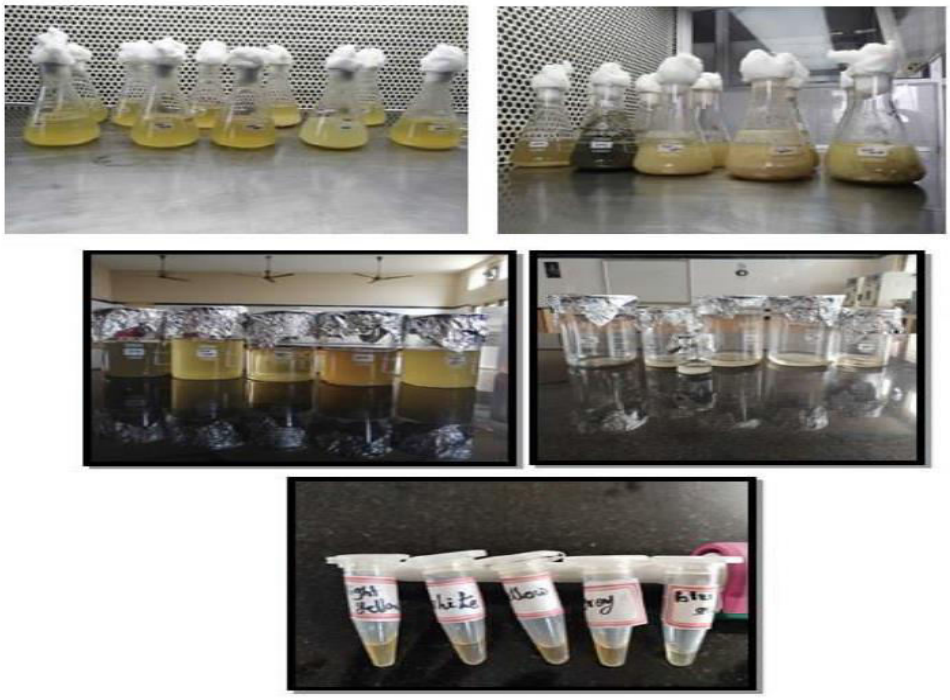

Fig. 2: Extraction of crude compounds

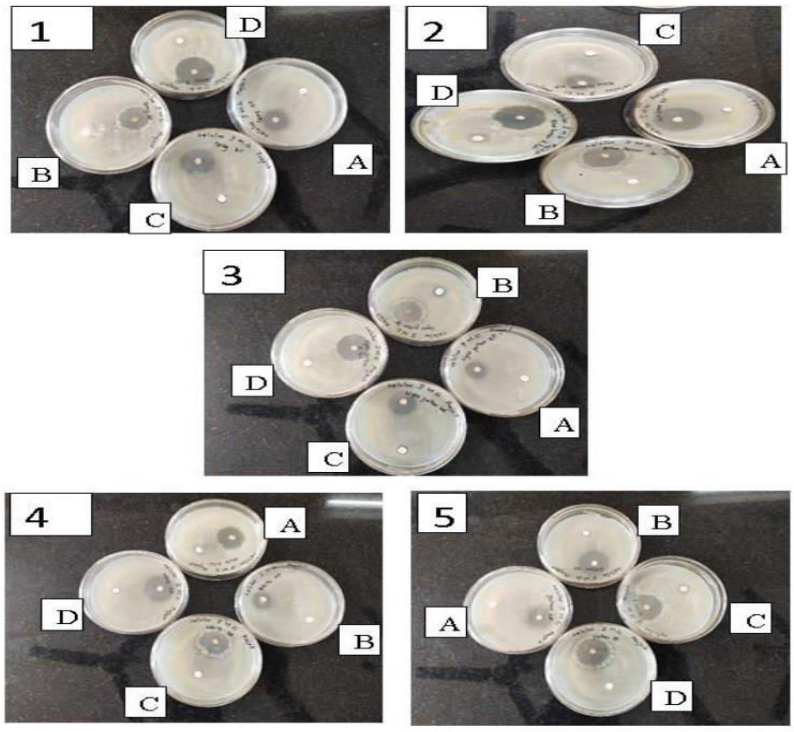

Fig. 3: Minimal Inhibitory Concentration against pathogens

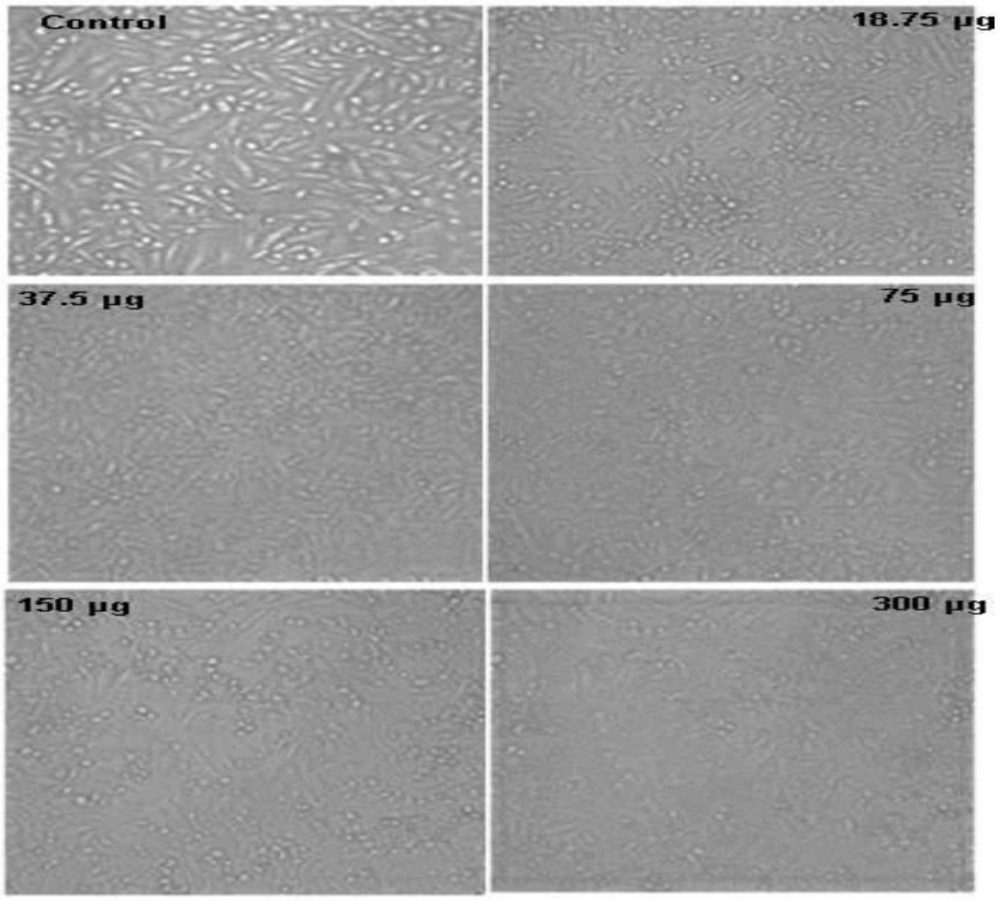

Fig. 4: MTT assay

Table 2: IC_{50} values was plotted in the GraphPad Prism software

Conc	18.75 µg	37.5 µg	75 µg	150 µg	300 µg	Cont
ABS	0.065	0.151	0.244	0.367	0.456	0.781
	0.066	0.151	0.245	0.362	0.457	0.785
	0.067	0.152	0.242	0.363	0.457	0.782
Avg	**0.066**	**0.151333**	**0.243667**	**0.364**	**0.456666667**	**0.782667**
Conc (µg/ml)	**% cell inhibition**				**IC_{50} 174.29**	**µg/ml**
18.75	8.432709					
37.5	19.3356				**R^2**	**0.996**
75	31.13288					
150	46.50767					
300	58.34753					

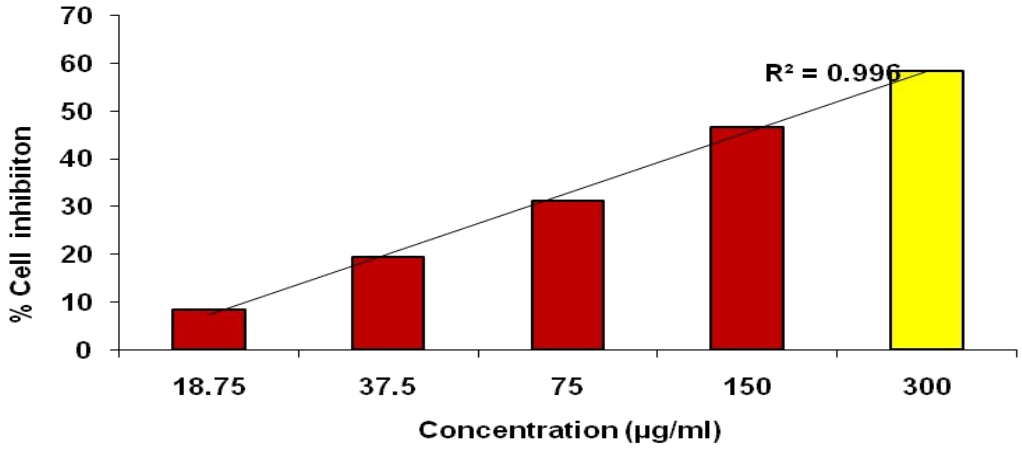

Fig. 5: Graph represents cytotoxic effects

4. CONCLUSION

A number of fungal compounds have been investigated for anticancer activities which are structurally diverse compound and shown to have potential anticancer activity. For instance, Irofulven as a DNA synthesis inhibitor based on the lead compound, Illudin-S, late-phase oncology clinical trials [8]. Lodamin, a new angiogenesis inhibitor based on TNP-470, itself related to Cytochalasin E and originally isolated from *Aspergillusfumigatus* [9]. Later, the compounds were modified using nanotechnology, has shown promising effects in murine models of a number of cancer types [10]. The antitumor active isolates were identified andbelonged to 12 taxa out of which *Alternaria* species, are reported as producers of anticancer compounds taxol and Brefeldin-A [11, 12]. Some new secondary metabolites from halotolerant *Aspergillus variecolor* showed activity against the P388, HL-60, BEL-7402, and A-549 cell lines with IC_{50} values from 70 to 260µg/ml [13]. Ergosterol, Rosellichalasin and Cytochalasin E were isolated from halophilic fungi-*Aspergillus* spand it showed the cytotoxicity activity against human colon cancer cell line RKO with IC_{50} of 3.3±0.5 µM [14]. New bioactive compounds from halotolerant fungi *Penicillium notatum* produce cytotoxic metabolites against *cdc2* mutant cell line [15].

In this present investigation, MCF-7 cell lines were treated with crude extracts of *Aureobasidium pullulans* and showed the cytotoxic activity at maximum concentration of 300µg/ml with IC_{50} value of 174 µg/ml which makes to consider the dosage range in future evaluation. Liamocins (heavy oil) from *A. pullulans* strains RSU 9 and RSU 21 inhibited two human breast cancer cell lines and a human cervical cancer cell line (IC_{50} values of 32.2±1.4 to 63.1±2.4µg liamocins/ml) [16]. In the present study, human breast carcinoma cell line showed the lethality range of 58.34% cell inhibition. Derivatives from β-1, 3-D-Glucan (branch β-1,6) have an anticancer and antimetastatic properties from black yeast *Aureobasidium pullulans* [17]. In conclusion, we observed that halotolerant black yeast

showed the active potentials in both cytotoxic and broad range of antibacterial activity, further investigation is required in connection with synthesis, characterization and drug motifs detections for further biological activities.

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Conflict of interests

There is no conflict of interests regarding the publication of this paper.

6. REFERENCES

1. Satchi-Fainaro R, Puder M, Davies JW, Tran HT, Sampson DA, Greene AK, Corfas G, Folkman *J. Nat. Med.,* 2004; **10(3):**255-226.
2. Oren A. *Environ Technol.* 2010; **31:**825-834.
3. Cantrell SA, Casillas-Martínez L, Molina M. *Mycol Res.* 2006; **110:**962-970.
4. Zalar P, de Hoog GS, Gunde-Cimerman N. *Studies Mycol.* 1999; **43**:38-48.
5. Kohlmeyer J, Kohlmeyer E. Marine Mycology: The higher fungi. Academic press, New York. 1979.
6. Ellis MB, Ellis JP. Micro fungi on land plants: An identification hand book, Croom Helm, London. 1985.
7. Mossman TJ. *Immunol. Methods,* 1983; **65:**55-63.
8. Kelner MJ, McMorris TC, Rojas RK, Estes LA, Suthipinijtham P 2008. *Inv. New Drugs,* **26(5)**:407-415.
9. Xiao L, Liu H, Wu N, Liu M, Wei J, Zhang Y, Lin X. *World Journal of Microbiology and Biotechnology.* 2013; **29(1):**11-17.
10. Strobel GA, Yang XS, Sears J, Kramer R, Sidhu RS, Hess WM. *Microbiology.* 1996; **142:**435-440.
11. Vurro M, Evidente A, Andolfi A, Zonno MC, Giordano F, Motta A. *Plant Science.* 1998; **138:**67-79.
12. Wang WL, Lu ZY, Tao HW, Zhu TJ, Fang FC, Gu QQ, Zhu WM. *J. Nat. Prod.,* 2007; **70:**1558-1564.
13. Wang WL, Zhu TJ, Tao HW, Lu ZY, Fang YC, Gu QQ, et al., 2007. *Chem. Biodivers.,* **4**:2913-2919.
14. Xiao L, Liu H, Wu N, Liu M, Wei J, Zhang Y, Lin X. *World Journal of Microbiology and Biotechnology.* 2013; **29(1):**11-17.
15. Xin ZH, Wang WL, Zhang YP, Xie H, Qun Q, Zhu WM. *The Journal of Antibiotics.* 2009; **62:**225-227.
16. Manitchotpisit P, Watanapokasin R, Neil P J Price, Kenneth M Bischoff, Tayeh M, Teeraworawit S, Kriwong S, Timothy D Leathers. *World J Microbiol Biotechnol.* 2014; **30(8):**2199-2204.
17. Kusaka M, Sudo K, Matsutani E, Kozai Y, Marui S, Fujita T, Ingber D, Folkman J. *Br. J. Cancer.* 1994; **69(2):**212-216.