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Extraction and bio evaluation of extracellular pigment produced by four species of *Penicillium sp*

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Abstract
The present study was carried out on 4nos. of pigment-producing fungi for their enability to produce pigments in different media and antimicrobial activity. To this effect, the fungi were mass cultured and treated for solvent extraction and partial purification through column chromatography. The different extracts were collected and treated against 2 nos. of bacterial strain (Gram–ve Bacterial strain -I and Gram–ve Bacterial strain-II) and 2nos. of fungi *Aspergillus sp.* and *Penicillium sp.* Data recorded on bioactivity against fungi and bacteria exhibited the importance of pigments, and their solubility in methanol and ethanol. Some water-soluble pigments having antibacterial activity were observed in *Penicillium sp.-4* also. Results obtained through their preliminary study exhibited the influence of pigments as a bioactive agent which may be exploited as food preservatives in future.

Keywords: Antimicrobial, *Aspergillus sp*., Bacteria, *Penicillium sp.*, Pigment

Introduction
Fungi are reported well for pigment production and widely used for large-scale production due to simple culture method, and cost-effective, stability and easy production and extraction processes [1]. Some of the pigments derived from fungi included black coloured Melanin isolated from *A. bridger* [2], black and brown-coloured Aspergillin derived from the fungi *A. Niger* [3], yellow-coloured pigment called Neoaspergillic acid derived from *A. Sclerotiorum* [4] and red coloured pigment derived from *A. Versicolor* [5] were having antioxidant activity and antimicrobial activity. Some of the pigments isolated from *F. oxysporum* producing pink/violet coloured pigment called anthraquinone [6], *F. verticillioides* producing a yellow pigment called naphthoquinone [7] were responsible for antibacterial activities. Another pigment-producing fungi *Monascus sp.* was producing orange-red coloured pigment Rubropuntatin responsible for anticancer activity [8]. Some *Penicillium sp.* producing pigments such as *P. herquei* producing a yellow pigment Atronenetin having antioxidant activity [9], *P. sclerotiorum* producing a yellow to orange pigment Sclerotiorin having antimicrobial activity [10, 11, 12]. Pigment-producing fungi *T. virens* synthesized a yellow coloured pigment viridolvirone [13, 14] and *T. viride* synthesized a yellow green brown pigment called viridin, both were having antifungal activities [15, 16].

Materials and Methods
Four nos. of fungi were screened through plate culture method for extracellular pigment production by growing them (5 days at 30℃) in 21 different types of Nutrient media of two different pH 4.5 and 8.5 [17]. The 4 selected pigment-producing fungi were tested for the same in liquid culture condition. All the pigment-producing fungi were tested against 2 nos. of bacterial strain (Gram-veBacterial strain -I and Gram-ve Bacterial strain -II) and 2nos. of fungi *Aspergillus sp.* and *Penicillium sp.* for their antimicrobial properties by following co-inoculation method. The extracellular metabolite was separated and partially purified through solvent extraction and subsequent column chromatography process by using polar to non-polar solvents. Prepared extracts were tested for antimicrobial activity against 2 nos. of bacterial strain (Gram –ve Bacterial strain -I and Gram–ve Bacterial strain -II) and 2nos. of fungi *Aspergillus sp.* and *Penicillium sp.* by following the agar cup diffusion method[18]. The data was recorded for zone of inhibition after 48 hours at 37 ºC for bacteria and 72 hours for the growth of fungi at 30 ºC.

Results and Discussion

All four pigment-producing fungi were grown in 21 different types of nutrient media in order to observe the production of diffusible pigments on Agar culture plates. Among them, *Penicillium sp*. 1 was produced orange pigment in Pikovskya's media of 8.5 and others produced yellow pigment in Sabouraud dextrose media of 4.5 pH. All fungal cultures were mass cultured in liquid culture media separately, culture filtrate was treated with Ethyl acetate in 1:2 ratio in order to separate secondary metabolites. DMSO and Ethanol dissolved crude extracts were partially purified through silica gel column chromatography by using polar to nonpolar solvents and aliquots were evaluated for antifungal and antibacterial properties (Table-1). The bioactive metabolites obtained through Ethanol and Methanol in all fungal sources exhibited positive antibacterial activity against (Gram–ve Bacterial strain -I and Gram-ve Bacterial strain -II) and antifungal activity against *Aspergillus sp.* and *Penicillium sp*. The ethanolic samples of all fungi exhibited positive antibacterial activity against Gram–ve Bacterial strain–II only, whereas the sample was not effective against Gram–ve Bacterial strain -I. The present study expressed the dissolution of bioactive metabolites from all four fungal sources in ethanol and methanol. Partially purified metabolite from *Penicillium Sp*.-4 extracted using DMSO, Ethanol and dissolved in water have their bioactivity against Gram–ve Bacterial strain -II. This is a preliminary attempt to purify and evaluate the pigments against bacteria and fungi which provides clue for the presence of polar metabolites in the form of extracellular pigments produced by the fungi. Results obtained an antibacterial and antifungal properties make these more suitable as food colorants and preservative agents [19].

Fig 1(a, b): Orange pigment produced by *Penicillium sp-1*

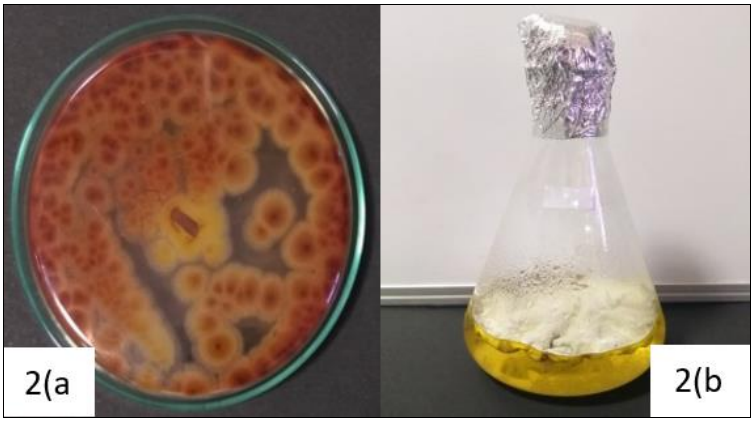

Fig 2 (a, b): Yellow pigment produced by *Penicillium sp-2*

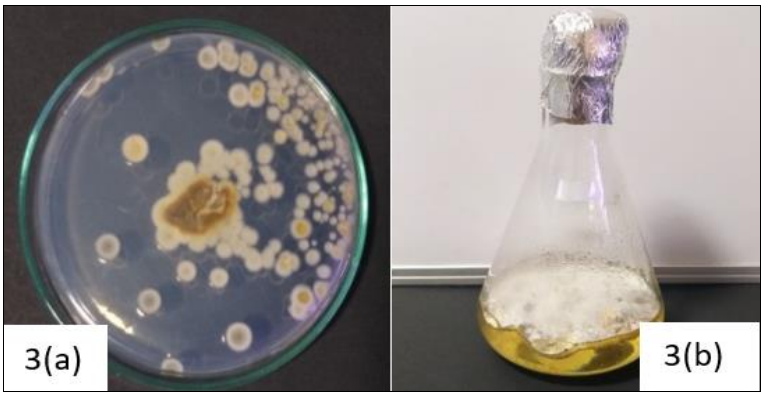

Fig 3 (a, b): Yellow pigment produced by *Penicillium sp.- 3*

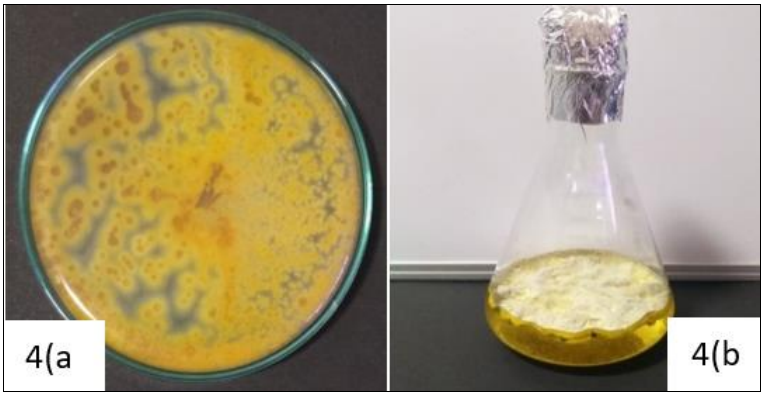

Fig 4 (a, b): Yellow pigment produced by *Penicillium sp-4*

Table 1: Effect of partially purified DMSO and ethanol-dissolved crude extracts using polar to nonpolar solvents against 2nos. of bacteria (Gram -ve Bacterial strain -1 and Gram -ve Bacterial strain -2) and 2nos. of fungi *Aspergillus sp.* and *Penicillium sp.*

Antibacterial activity					Antifungal activity				
Sr. No	**Test Organisms**		**Gram -ve Bacterial strain-I**	**Gram -ve Bacterial strain-II**	**Sr. No**	**Test Organisms**		***Aspergillus sp.***	***Penicillium sp.***
1	*Penicillium Sp.*-1 /Dmso	A	-	-	1	*Penicillium Sp.*-1 /Dmso	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	-
		D	+	+			D	-	-
	Penicillium Sp.-1 /Ethanol	A	-	-		*Penicillium Sp.*-1 /Ethanol	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	+
		D	-	+			D	-	-
2	*Penicillium Sp.*-2 /Dmso	A	-	+	2	*Penicillium Sp.*-2 /Dmso	A	-	-
		B	-	-			B	-	-
		C	-	+			C	+	+
		D	-	+			D	-	-
	Penicillium Sp.-2 /Ethanol	A	-	-		*Penicillium Sp.*-2 /Ethanol	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	+
		D	-	+			D	-	-
3	*Penicillium Sp.*-3 /Dmso	A	-	-	3	*Penicillium Sp.*-3 /Dmso	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	+
		D	-	+			D	-	-
	Penicillium Sp.-3 /Ethanol	A	-	-		*Penicillium Sp.*-3 /Ethanol	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	+
		D	-	+			D	-	-
4	*Penicillium Sp.*-4 /Dmso	A	-	+	4	*Penicillium Sp.*-4 /Dmso	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	+
		D	+	+			D	-	-
	Penicillium Sp.-4 /Ethanol	A	-	+		*Penicillium Sp.*-4 /Ethanol	A	-	-
		B	-	-			B	-	-
		C	+	+			C	+	+
		D	-	+			D	-	-

A, Water; B, Acetic Acid; C, Methanol; D, Ethanol.

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Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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