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Research Article

Biopigments Produced by *Monascus purpureus* Through Solid State Fermentation: HPTLC Finger Printing Analysis

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Abstract

The current study aims to conduct HPTLC finger printing analysis of various extracts of *Monascus purpureus* biopigments (water extract, acetone extract, chloroform extract, acetonitrile extract, petroleum ether extract, methanol extract, ethanol extract, hexane extract). Natural and synthetic pigments both play an important roles as biocolourants in the food and pharmaceutical industries. The most recent HPTLC technique helps useful fingerprint analysis to determine the identity and composition of natural phytomolecules. Solid state fermentation was carried out with the help of a production medium contains long grain, unripe basmati rice. Water, acetone, chloroform, petroleum ether, acetonitrile, ethanol, methanol, and hexane were used to extract fermented rice (5g), which was then analysed at 485 nm. For scanning at different wavelengths of 370 nm (yellow pigment), 420 nm (red pigment), 470 nm (orange pigment), 500 nm, and 530 nm (red pigment), various mobile phases have been developed. When chromatographed with mobile phase 5 (ethyl acetate: isopropanol: water- 8.0:1.0:1.0 v/v), an acetone extract of *Monascus* biopigment showed good resolution.

***Keywords:** HPTLC, Monascus purpureus, Biopigments, Solid state fermentation, Finger printing analysis*

1. Introduction

The majority of natural dyes are plant extracts or microorganism-produced dyes, which have certain advantages in terms of production. Pigments, whether natural or synthetic, play an important role in the food and pharmaceutical industries as colourants. The number of permitted synthetic colourants has decreased due to undesirable toxic effects (mutagenicity and potential carcinogenicity). The emphasis is on the production of natural food pigments. Synthetic red pigments such as azorubin or tartrazine [1,2,3] cause allergic reactions, and C-red is carcinogenic and teratogenic [4]. Researchers all over the world are looking for naturally occurring red pigments in a variety of natural sources. While many natural colourants are available today, microbial colourants play an important role as food colouring agents due to their versatility in production, simple and quick down-streaming process and reduced cost. Among the pigment-producing microorganisms, *Monascus* has been shown to produce non-toxic pigments that can be used as a food colourant [5,6,7]. *Monascus* biopigments are a beneficial secondary metabolite produced by the *Monascus* species. They are widely used in the food industry as food additives, colour intensifiers, and nitrite substitutes in meat products. They have therapeutic potential and are widely used as dyes in the textile and cosmetic industries [8]. Several fungi have been reported for biopigment production, including *Monascus purpureus* [9,10], *M. ruber* [11], *M. paxi* [12], and *M. anka* [9].

Species of *Monascus* are well known for producing pigments like monas-corubrine, rubropunctatine (orange pigment) [13,14]. Monascorubramine and rubropunctamine (red pigments), monascin and ankaflavin (yellow pigments) have also been identified [15,16,17]. More recently monascusones from *Monascus mutant* [18] have also been recognized. Until 2011, thirty-nine new pigment compounds have been identified [15].

Long grain, unripe rice is substarte of choice for solid-state fermentation in *Monascus* pigments production. Non-rice substrates (corn, adlay, sorghum, dioscorea, jackfruit seed, durian seed) have also been reported for the production of *Monascus* pigment [19,20,21,22, 23]. Production and yield of *Monascus* pigment is higher in solid state fermentation as compared to submerged femenattion because pigments are directly released into the grains [24].

From the previous research it has been shown that *Monascus purpureus* produced red, orange and yellow pigments. But investigation of *Monascus* yellow pigments is still limited. Due to antioxidant, antidiabetes and anticancer properties, *Monascus* yellow pigment is employed in various functional food as well as medicines [21,25]. Their potential use as a natural colourant for biscuit, mayonnaise and wheat noodle has also been reported [16,25]. It is reported that *Monascus* yellow pigment have encouraging potential for application in food and pharmaceutical industries based on their bioactivities. Anti-atherosclerosis effect, antiobesity activity [26,27], antitumor activity [28,29], memory and learning ability [30] have also been reported for monascin and ankaflavin.

The HPTLC (high-performance thin layer chromatography) is considered as an advanced form of TLC which gives high resolution with accurate data. It is well recognized as one of the most potent analytical techniques for phytochemical and biological analysis. It is the most extensively used method for estimating chemical components present in test samples since it is inexpensive, simple, and quick. The recent HPTLC method enables the achievement of useful fingerprints analysis to establish the identity and composition of the natural phytomolecules. The HPTLC method is used as a valuable method for evaluating the emerging types of natural products [31]. The *Monascus* pigments were identified in HPTLC plate as visible bands of red, orange and yellow colour when scanned at different wavelengths [32]. The most recent HPTLC method enables useful fingerprint analysis to determine the identity and composition of natural phytomolecules. The HPTLC method is a valuable tool for evaluating new types of natural products [33,34].

The present study was performed for the fingerprint profiling of different extracts of *Monascus purpureus* pigments by the HPTLC technique.

2. Methodology

2.1 Microorganism

Monascus purpureus MTCC 369 culture was obtained from the Institute of Microbial Technology (IMTECH) in Chandigarh, India. It was grown on Potato-Dextrose Agar (PDA) slants at 4^{o}C and subcultured every 30 days.

2.2 Preparation of Seed Culture

Monascus purpureus MTCC 369 spore suspension was prepared from actively growing slants in sterile water and diluted to a concentration of 5.7×10^{3}. A hemocytometer was used to count the spores. The basal medium was inoculated with 15% spore suspension in conical flasks (100g dextrose, 10g peptone, 2g KNO_3, 2g $NH_4H_2PO_4$, 0.5g $MgSO_4.7H_2O$, 0.1g $CaCl_2$ in 1000 ml distilled water; adjusted to pH 6.0). These cultures were incubated in a shaker incubator at 110 RPM for 48 hours at 30 ^{o}C [35,36].

2.3 Solid State Fermentation

Experiments was conducted in a 250 ml Erlenmeyer flask with 40 ml of production medium (dextrose 29.59 g, ammonium chloride 3.86 g, potassium-dihydrogen phosphate 1.73 g, magnesium sulphate 0.86 g, manganese sulphate 0.19 g, distilled water 1l). 20 g of long grain unripe basmati rice was added to this. 0.1N HCl or 0.1N NaOH were used to adjust the pH of the broth to 6. Each flask was autoclaved for 15 minutes at 15 psi at 121^{o}C before being inoculated with 10% of the seed culture and incubated at 30 ºC at 70% R.H. for 14 days on a shaker incubator [36].

2.4 Extraction of *Monascus purpureus* biopigment

Fermented rice (5g) was dissolved in 10 ml of different solvents in a 250 ml Erlenmeyer flask. Water, acetone, chloroform, petroleum ether, acetonitrile, ethanol, methanol, and hexane were used as solvents. These flasks were shaken in an orbital shaker at 100 rpm for 15 minutes. The solvents were then filtered through whattman filter paper. For 10 minutes, the filtrate was centrifuged at 2400 rpm. The supernatants produced were collected for UV spectrophotometery analysis [37].

2.5 Estimation of *Monascus purpureus* biopigment

The maximum absorbance of the *Monascus purpureus* biopigments in tap water was observed at 485 nm [38,39].

2.6 High performance thin layer chromatography (HPTLC)

HPTLC finger printing of the biopigment was performed in different organic solvent mixture extracts using HPTLC plate (Aluminum Sheet 20x50 cm. TLC Silica gel 60 F_{254}, Merck.) and HPTLC instrument (Camag) with Scanner (Camag TLC Scanner 3), for spotting (Camag Linomat 5) and for photographing (Camag Reprostar 3). The plates size was determined to be 20x10 cm. Water, acetone, chloroform, petroleum ether, acetonitrile, ethanol, methanol, and hexane were used to extract the biopigment (Table 3). The spot volume for each organic solvent extract was 3µl. HPTLC instrument performed finger printing analysis using software Wincats Version 2.1:0.

3. Result and Discussion

3.1 Extraction of *Monascus purpureus* biopigment

The biopigments were extracted in various organic solvents using a fixed amount of fermented mass (5g) in a fixed volume (10 ml) of various organic solvent. Throughout the extraction process, the time (15 minutes) and temperature (25° C) were kept constant. The fermented broth and solvent were placed in a 100 mL conical flask and shaken at 150 rpm for 15 minutes in an orbital shaker. After filtering, the mixture was centrifuged at 2400 rpm. At 485 nm, the resultant was analysed in a U.V Spectrophotometer (SHIMADZU, Japan). The biopigments concentration (Table 1) in different solvents were, water (22.917 mg/ml), acetone (196.140 mg/ml), choloroform (34.367 mg/ml), Pet. Ether (0.073 mg/ml), acetonitrile (185.152 mg/ml), ethanol (174.995 mg/ml), methanol (192.354 mg/ml), and hexane (0.000 mg/ml). The outcomes of the extraction in the various solvent indicated that in acetone have maximum concentration of biopigments. Furthermore, 5 ml of acetone were added in thirteen intervals to the 10 ml acetone extract, bringing the total volume of the sample to 75 ml. The concentration of red pigment in each level was examined; initially, the concentration in 10 ml was 196.140 mg/ml, and in 75 ml, the concentration was 0.005 mg/ml (Table 2).

Table 1. Concentration of biopigments in fermented mass.

Sr. No.	Name of Solvent	Volume (ml)	Fermented broth (g)	Time (minute)	Temperature (°C)	Concentration (mg/ml)
1	Water	10	5	15	25	22.917
2	Acetone	10	5	15	25	196.140
3	Chloroform	10	5	15	25	34.367
4	Pet. Ether	10	5	15	25	0.073
5	Acetonitrile	10	5	15	25	185.152
6	Ethanol	10	5	15	25	174.995
7	Methanol	10	5	15	25	192.354
8	Hexane	10	5	15	25	0.000

Table 2. Concentration of red pigment in acetone.

Sr. No.	Solvent	Volume (ml)	Fermented broth (g)	Time (minute)	Temperature (°C)	Concentration (mg/ml)
1	Acetone	10	5	15	25	196.140
2	Acetone	5	5	15	25	67.793
3	Acetone	5	5	15	25	44.986
4	Acetone	5	5	15	25	25.595
5	Acetone	5	5	15	25	13.868
6	Acetone	5	5	15	25	7.682
7	Acetone	5	5	15	25	4.450
8	Acetone	5	5	15	25	4.081
9	Acetone	5	5	15	25	2.142
10	Acetone	5	5	15	25	1.68
11	Acetone	5	5	15	25	1.680
12	Acetone	5	5	15	25	0.572
13	Acetone	5	5	15	25	0.295
14	Acetone	5	5	15	25	0.005

Table 3. Different mobile phases for HPTLC.

Mobile Phase	Solvents	Ratio (v/v)	Scanning Wavelength (nm)	Colour of biopigment
1	Ethyl acetate Glacial acetic acid Formic acid Water	6.75 0.74 0.74 1.75	370 420 470 500 530	Yellow Red Orange Red Red
2	Water Glacial acetic acid Water	5.71 1.42 2.85	370 420 470 500 530	Yellow Red Orange Red Red
3	Ethyl acetate Isopropanol Water	6.5 2.5 1.0	370 420 470 500 530	Yellow Red Orange Red Red
4	Ethyl acetate Isopropanol Water	5.0 4.0 1.0	370 420 470 500 530	Yellow Red Orange Red Red
5	Ethyl acetate Isopropanol Water	8.0 1.0 1.0	370 420 470 500 530	Yellow Red Orange Red Red

Table 4. R_f values and A % of fractions in different extract.

Extract	Wave-length (nm)	Mobile phase 1			Mobile phase 2			Mobile phase 3			Mobile phase 4			Mobile phase 5		
		No. of peaks	R_f	A %	No. of peaks	R_f	A %	No. of peaks	R_f	A %	No. of peaks	R_f	A %	No. of peaks	R_f	A %
Acetone	530	1	0.94	100	1	0.73	100	1	0.88	100	1	0.84	100	1 2 3 4 5 6	0.01 0.17 0.54 0.77 0.90 0.93	4.83 9.31 12.10 48.38 10.62 14.77
Water	370	1 2 3 4	0.02 0.25 0.63 0.94	7.05 29.61 24.09 39.32	1 2	0.76 0.88	38.79 61.20	1 2 3	0.79 0.87 0.90	10.09 42.63 47.27	1	0.82	100	NIL	-	-
Chloro-form	530	1	0.95	100	1	0.75	100	NIL			1	0.86	100	1 2 3	0.77 0.90 0.92	13.27 42.08 44.66
Ether	500	1	0.95	100	NIL	-	-	1 2	0.84 0.87	36.29 63.71	1	0.85	100	NIL	-	-

Table continued...

Acetonitrile	530	1	0.94	100	1	0.74	100	1	0.86	100	1	0.85	100	1 2 3 4 5	0.07 0.18 0.55 0.78 0.93	3.91 22.08 13.69 42.87 17.45
Ethanol	370	1 2 3 4 5	0.07 0.13 0.48 0.58 0.94	3.51 10.19 16.82 14.57 48.91	1 2	0.25 0.73	2.5 5 97. 45	1	0.90	100	1	0.85	100	1	0.94	100
	530	1	0.94	100	1	0.73	100	1	0.87	100	1	0.84	100	1 2 3 4	0.02 0.07 0.17 0.79	27.86 7.51 36.47 28.16
Methanol	370	1 2 3 4 5	0.08 0.13 0.48 0.58 0.94	3.22 13.75 14.61 15.95 52.48	1 2	0.25 0.75	2.3 7 97. 63	1 2	0.85 0.90	33. 26 66. 74	1 2	0.85 0.89	57. 15 42. 85	1	0.95	100
	530 nm	1	0.94	100	1	0.75	100	NIL	-	-	NIL	-	-	1 2 3 4	0.02 0.07 0.17 0.79	56.85 6.33 32.72 4.10
Hexane	420 nm	1 2 3	0.94	100	1 2	0.75 0.76	60. 15 39. 35	1	0.91	100	1	0.86	100	1	0.95	100

3.2. High performance thin layer chromatography (HPTLC) analysis of *Monascus purpureus* biopigments

Water extract

Mobile phase 1: **-** Mobile phase 1 shows 4 peaks of R_f value 0.02, 0.25, 0.63 and 0.94 with A % 7.05, 29.61, 24.09 and 39.32 respectively when scan at different wavelengths. Water extract of pigments are well resolved by mobile phase 1 (Table 4).

Acetone extract

Mobile phase 5: - Eight peaks of R_f values 0.01, 0.17, 0.54, 0.77, 0.89, 0.90, 0.93 and 0.94 are found when scan at different wavelengths. The peak of R_f 0.93 (98.62 A %) are well resolved at 420 nm. The peak of R_f 0.89 (23.80 A %) and 0.94 (63.85 A %) are well resolved at 470 nm. The peak of R_f 0.01 (24.97 A %) and 0.90 (23.74 A %) are well resolved at 500 nm while the peak of R_f 0.17 (9.31 A %), 0.54 (12.10 A %) and 0.77 (48.38 A %) are very well resolved at 530 nm (Table 4).

Chloroform extract

Mobile phase 5: – Four peaks are appeared with R_f values of 0.77, 0.90, 0.92 and 0.93 when scan at different wavelengths. The peak of R_f value 0.77 (13.27 (A %) is well resolved at 530 nm, the peak of R_f value 0.90 (45.45 A %) and 0.92 (54.55) are well resolved at 500 nm and the peak of R_f 0.93 are well separated only at 470 nm (Table 4).

Ether extract

Mobile phase 3: - 3 peaks having R_f value of 0.84, 0.87 and 0.88 are appeared. Only the peak of R_f value 0.84 (36.74 A %) are well resolved at 500 nm (Table 4).

Acetonitrile extract

Mobile phase 5: - Six peaks of R_f values 0.07 (3.91 A %)), 0.18 (22.08 A %), 0.55 (13.69 A %), 0.78 (42.87 A %), 0.93 (17.45 A %) and 0.94 (100 A %) are found when scan at different wavelengths. All these are well resolved only at 530 nm (Table 4).

Ethanol extract

Mobile phase 1: - Five peaks are found at R_f value of 0.07, 0.13, 0.48, 0, 58 and 0.94 having A % of 3.51, 10.19, 16.82, 14.57 and 48.91 respectively. All these peaks are well resolved properly at 370 nm except that of peak R_f 0.07 (3.51 A % only) (Table 4).

Mobile phase 5: Seven peak of R_f values 0.01, 0.07, 0.17, 0.79, 0.93, 0.94 and 0.95 are found at different wavelengths. The peak of R_f 0.94 (97.04 A %) are well resolved at 420 nm. while peak of R_f 0.01 (70.63 A %) are well resolved at 500 nm. The peak of R_f 0.02 (27.86 A %), 0.07 (7.51 A %), 0.17 (36.47 A %) and 0.79 (28.16 A %) are well resolved at 530 nm. The peak of R_f 0.95 (82.10 A %) are well resolved at 470 nm (Table 4).

Methanol extract

Mobile phase 1: Six peaks are found at R_f values of 0.08, 0.13, 0.47, 0.48, 0.58 and 0.94. The peak of R_f 0.08 is not resolved at any wavelengths except at 370 nm (with only 3.22 A %) but peak of R_f 0.13 (13.75 A %) and 0.58 (15.95 A %) are well resolved at 370 nm. On the other hand peak of R_f 0.47 (14.61 A %) & 0.48 (14.84 A %) are close to each other and resolved slightly more at 420 nm. The pigments are absorbed at 420 nm having R_f 0.94 (74.07 A %) (Table 4).

Mobile phase 5: Seven peaks of R_f values 0.01, 0.02, 0.07, 0.17, 0.79, 0.92 and 0.95 are found when scan at different wavelength. The peak of R_f value 0.01 (37.22 A %), 0.92 (21.95 A %) and 0.95 (40.83 A %) are well resolved at 470 nm while remaining peaks of R_f value 0.02 (56.85 A %), 0.07 (6.33 A %), 0.17(32.72 A %) and 0.79 (A % 4.10 area %) are well absorbed at 530 nm (Table 4).

3.3. Extraction of biopigment

The red pigment was extracted in different organic solvent. From present study, it was found that acetone gives maximum (196.140 mg/ml) concentration of biopigment while hexane gives poor concentration of biopigment. It was found that red pigment extraction was depending on polarity of the solvent. Polar solvents like water, acetone, ethanol, methanol and acetonitrile the pigment concentration was maximum while in nonpolar solvents like hexane and petroleum ether poor concentration was found. This suggests that the red pigments may be polar in nature. Although water is a highly polar solvent, the concentration of red pigment in water was found less than acetone, methanol, ethanol, and acetonitrile (medium polar solvent) implies that red pigments are well extracted in medium polar solvents. But the concentration of red pigments in chloroform and petroleum ether (least polar solvent) was found more than hexane (non polar solvent) implies that the red pigments are not well extracted in non polar solvents. From the present study it was concluded that *Monascus purpureus* pigmnets were well extracted in medium polar solvent like acetone, acetonitrile, ethanol and methanol.

3.4. HPTLC Finger printing of *Monascus purpureus* red pigments

Water Extract

Mobile phase 1 (ethyl acetate: glacial acetic acid: formic acid: water- 6.75:0.74:0.74:1.75 v/v) with 4 peaks of R_f values 0.02, 0.25, 0.63 and 0.94 with A % 7.05, 29.61, 24.09 and 39.32 respectively [Figure 1a]. The R_f values 0.02 and 0.94 were well resolved when scanned at 420 nm implies that it may contain red pigments, while R_f values of 0.25 and 0.63 were well resolved at 370 nm implies it may be the yellow pigment.

Mobile phase 2: As the mobile phase 2 contain polar solvents like glacial acetic acid and water, unable to separate the *Monascus purpureus* pigments. Only 2 peaks of R_f values 0.76 with 38.79 A % and 0.88 with 91.10 A % were absorbed chromatograms. The pigment appeared at 0.76 shows maximum absorbance at 370 nm and pigment which is appeared at 0.88 R_f value showed maximum absorbance at 530 nm. Therefore pigment at 0.88 R_f value may be a red pigment while pigment at 0.76 R_f value may be a yellow pigment.

Mobile phase 3: It showed total of 4 peaks scan at different wavelength having R_f values of 0.79, 0.81, 0.87 and 0.90. The R_f values 0.79 (10.09 A %) and R_f value of 0.90 (47.27 A %) was well absorbed at 370 nm. While the peak of R_f 0.87 (74.91 A %) & R_f 0.81 (25.09) were well resolved at 420 nm. Therefore the R_f 0.79 and 0.90 may be yellow pigment while R_f of 0.87 & 0.81 may be red pigments.

Mobile phase 4: Two peaks was found at R_f values of 0.82 (100 A %) and 0.83 (100 A %). As both the R_f values was very close to each other, mobile phase 4 (ethyl acetate: isopropanol: water- 50:40:10) was unable to separate the pigments properly.

Mobile phase 5: Single peak of R_f value 0.01 with 100 A% was found at 470 and 500 nm showed orange and red pigment respectively but were unable to separate properly.

Acetone Extract

Mobile phase 1: Mobile phase 1 (ethyl acetate: glacial acetic acid: formic acid: water- 6.75:0.74:0.74:1.75 v/v) showed total of 5 peaks having R_f values 0.11, 0.51, 0.61, 0.74 and 0.94 scan at different wavelengths. The peaks of R_f value 0.94 (82.03 A %) was well resolved at 420 nm while remaining pigments were well absorbed at 370 nm. Although peaks of R_f 0.94 was appeared at 470 nm, 500 nm and 530 nm but were not resolved properly at these wavelengths. Therefore peak showing R_f 0.94 may be red pigment while remaining peaks may contain yellow pigments.

Mobile phase 2: Two peaks of R_f values 0.73 & 0.74 were appeared at different wavelength. As the R_f value was very close to each other mobile phase 2 (n-butanol: glacial acetic acid: water- 40:10:20) was unable to separate the pigments properly.

Mobile phase 3: Mobile phase 3 (ethyl acetate: isopropanol: water- 6.5:2.5:1.0 v/v) showed 5 peaks of R_f 0.05, 0.83, 0.84, 0.85, 0.87 and 0.88 were found when scanned at different wavelengths. The peak of R_f value 0.83 (30.32 A %) was resolved at 470 nm implies that it may be orange pigments. The peak of R_f value 0.88 (70.48 A %) was well resolved at 420 nm implies that it may a red pigments. The remaining peaks of R_f values 0.05 (15.86 A %), 0.84 (36.32 A %) and 0.87 (47.81 A %) were well resolved at 500 nm implies that it may contain red pigments.

Mobile phase 4: Mobile phase 4 (ethyl acetate: isopropanol: water- 5.0:4.0:1.0 v/v) showed 3 peaks of R_f values 0.61, 0.83 and 0.84 were found when scanned at different wavelengths. The peak of R_f 0.61 (13.27 A %) and 0.83 (86.73 A %) were well resolved at 470 nm which implies that it may be a orange pigments while peak of R_f 0.84 was appeared at 420 nm and 530 nm with same A % of 100 but are not well resolved at this wavelengths. Therefore it may be a red pigments.

Mobile phase 5: Mobile phase 5 (ethyl acetate: isopropanol: water- 8.0:1.0:1.0 v/v) showed eight peaks of R_f values 0.01, 0.17, 0.54, 0.77, 0.89, 0.90, 0.93 and 0.94 when scanned at different wavelengths. The peak of R_f 0.93 (98.62 A %) was well resolved at 420 nm implies that it may be a red pigments. The peak of R_f 0.89 (23.80 A %) and 0.94 (63.85 A %) were well resolved at 470 nm implies that it may be a orange pigments. The peak of R_f 0.01 (24.97 A %) and 0.90 (23.74 A %) were well resolved at 500 nm implies that it may be a red pigments while the peak of Rf 0.17 (9.31 A %), 0.54 (12.10 A %) and 0.77 (48.38 A %) were very well resolved at 530 nm. Therefore this pigment may be a red pigments (Figure 1b).

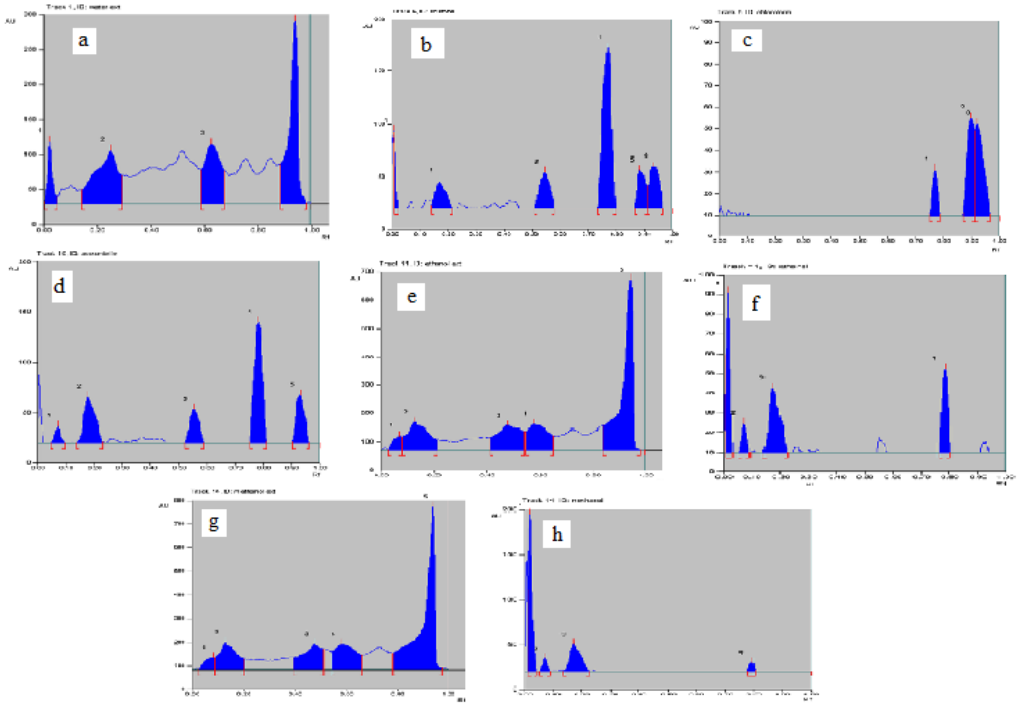

Figure 1: *Graph showing different peaks in water extract (a), acetone extract (b), chloroform extract (c), acetonitrile extract (d), ethanoel extract (e,f) and metnanol extract (g, h).*

Chloroform Extract

Mobile phase 1: Single peak was found having R_f value of 0.95 with same A % of 100 when scanned at different wavelengths. Therefore mobile phase 1 was unable to separate the pigments properly.

Mobile phase 2: Three peaks were found when scanned at different wavelengths with R_f values of 0.73, 0.74 and 0.75. All R_f values were close to each other implies that mobile phase 2 is not able to separate the *Monascus purpureus* pigments.

Mobile phase 3: Two peaks were obtained when scanned at different wavelengths having R_f values of 0.05 and 0.87 with A % of 14.56 and 85.44 respectively. The peak of R_f value 0.87 was very well resolved only at 500 nm while the peak of R_f value 0.05 at 500 nm with A % of 14.56. Therefore both the pigment may be red.

Mobile phase 4: Two peak of R_f values 0.85 and 0.86 were appeared at different wavelengths but ware not well separated. Therefore mobile phase 4 was unable to separate the pigments properly.

Mobile phase 5: Four peaks were appeared with R_f values of 0.77, 0.90, 0.92 and 0.93 when scanned at different wavelengths. The peak of R_f value 0.77 (13.27 A %) was well resolved at 530 nm (Figure 1c), the peak of R_f values 0.90 (45.45 A %) and 0.92 (54.55) were well resolved at 500 nm and the peak of R_f 0.93 were well separated only at 470 nm. Therefore R_f values of 0.77, 0.90 & 0.92 and 0.93 were red, red and orange pigment respectively.

Ether Extract

Mobile phase 1: The R_f values were same when scanned at different wavelengths i.e. 0.95 with A % 100. This implies that mobile phase 1 is unable to separate the pigments properly.

Mobile phase 2: Two peak of R_f values 0.74 and 0.75 were found when scanned at different wavelength which were very close to each other. Therefore mobile phase 2 is unable to separate the pigments properly.

Mobile phase 3: Three peaks having R_f values of 0.84, 0.87 and 0.88 were appeared. Only the peak of R_f value 0.84 (36.74 A %) was well resolved at 500 nm implies that it may contain red pigments.

Mobile phase 4: Single peak of R_f value 0.85 (100 A %) was appeared at different wavelengths. Therefore this mobile phase was unable to separate the pigments properly.

Mobile phase 5: Single peak of R_f 0.93 with 100 A % was appeared implies that mobile phase 5 is not able to separate the pigment.

Acetonitrile Extract

Mobile phase 1: Three peaks of R_f values 0.56, 0.94 and 0.95 were appeared at different wavelengths. The peak of R_f value 0.94 (88.39 A %) was well absorbed at 370 nm implies that it may be a yellow pigments. The remaining two peaks were not absorbed properly at any wavelengths.

Mobile phase 2: Two peak of R_f values 0.74 and 0.94 were appeared at different wavelengths. The peaks were not resolved properly having same A % of 100. Therefore mobile phase 2 was unable to separate the pigments.

Mobile phase 3: Four peaks were found at R_f values of 0.86, 0.88, 0.89 and 0.91, out of which only peaks of R_f values 0.88 (63.53 A %) and 0.91 (36.47 A %) were well resolved at 370 nm implies that it may be a yellow pigments. The remaining peaks were not resolved properly.

Mobile phase 4: Three peaks of R_f values 0.84, 0.85 and 0.86 with same A % of 100 were appeared when scanned at different wavelengths. Therefore mobile phase 4 was unable to separate any pigments properly.

Mobile phase 5: Six peaks of R_f values 0.07 (3.91 A %), 0.18 (22.08 A %), 0.55 (13.69 A %), 0.78 (42.87 A %), 0.93 (17.45 A %) and 0.94 (100 A %) were found when scanned at different wavelengths. All peaks were well resolved only at 530 nm implies that they all may contain red pigments (Figure 1d).

Ethanol Extract

Mobile phase 1: Five peaks were found at R_f values of 0.07, 0.13, 0.48, 0, 58 and 0.94 having A % of 3.51, 10.19, 16.82, 14.57 and 48.91 respectively. All these peaks were well resolved properly at 370 nm except that of peak having R_f 0.07 (3.51 A % only). Therefore all these pigments may red (Figure 1e).

Mobile phase 2: Mobile phase 2 showed two peaks of R_f values 0.25 and 0.73 were found when scan at different wavelengths. The peak of R_f 0.25 (2.55 A %) was not resolved properly at 370 nm but the peak of 0.73 (97.45 A %) was well resolved at 370 nm which implies that mobile phase 2 is able to separate only yellow pigment.

Mobile phase 3: Five peaks of R_f values 0.83, 0.87, 0.88, 0.89 and 0.90 were found at different wavelengths. The peak of R_f 0.83 (26.24 A % and 0.88 (75.76 A %) were well separated at 370 nm implies presence of red pigments. The remaining peaks were not absorbed properly.

Mobile phase 4: Two peak of R_f values 0.84 and 0.85 were found when scanned at different wavelengths. Both values were very close to each other. Therefore mobile phase 4 is not able to separate the pigments.

Mobile phase 5: Mobile phase 5 showed seven peak of R_f values 0.01, 0.07, 0.17, 0.79, 0.93, 0.94 and 0.95 scanned different wavelengths. The peak of R_f 0.94 (97.04 A %) was well resolved at 420 nm implies that of red pigments while peak of R_f 0.01 (70.63 A %) was well resolved at 500 nm implies that it may red pigments. The peak of R_f 0.02 (27.86 A %), 0.07 (7.51 A %), 0.17 (36.47 A %) and 0.79 (28.16 A %) were well resolved at 530 nm implies that it may contain red pigments. The peak of R_f 0.95 (82.10 A %) was well resolved at 470 nm implies that may be orange pigments (Figure 1f).

Methanol Extract

Mobile phase 1: Six peaks were found at R_f values of 0.08, 0.13, 0.47, 0.48, 0.58 and 0.94. The peak of R_f 0.08 was not resolved at any wavelengths except at 370 nm (with only 3.22 A %) but peak of R_f 0.13 (13.75 A %) and 0.58 (15.95 A %) were well resolved at 370 nm (Figure 1g) . On the other hand peak of R_f 0.47 (14.61 A %) & 0.48 (14.84 A %) were close to each other and resolved slightly more at 420 nm (Figure 1h). The pigments were absorbed at 420 nm having R_f 0.94 (74.07 A %). Therefore peaks of R_f values 0.13 and 0.58 may be a yellow pigment while R_f values of 0.48 and 0.94 may be a red pigments.

Mobile phase 2: Four peaks of R_f values 0.25, 0.73, 0.74 and 0.75 were found when scanned at different wavelengths. The peak of R_f 0.75 (97.63 A %) was well resolved at 370 nm. The remaining peaks were not separated properly. Therefore the peak of R_f 0.75 may be yellow pigments.

Mobile phase 3: Three peaks of R_f values 0.85, 0.89 and 0.90 were found at different wavelengths. The peak of R_f 0.85 (57.19 A %) was well separated at 500 nm, peak of R_f 0.89 (51.76 A %) was well separated at 470 nm and the peak of R_f 0.90 (66.74 A %) was well separated at 370 nm. Therefore peaks of R_f values 0.85, 0.89 and 0.90 may contain red, orange and yellow pigment respectively.

Mobile phase 4: It showed three peaks of R_f values 0.82, 0.85 and 0.89. The peak of R_f values 0.82 (39.55 A %), 0.85 (58.79 A %) and 0.89 (71.06 A %) were resolved at 500 nm, 420 nm and 470 nm respectively. Therefore peaks of R_f values 0.82, 0.85 and 0.89 may be red, red and yellow pigments respectively.

Mobile phase 5: Seven peaks of R_f values 0.01, 0.02, 0.07, 0.17, 0.79, 0.92 and 0.95 were found when scanned at different wavelength. The peak of R_f values 0.01 (37.22 A %), 0.92 (21.95 A %) and 0.95 (40.83 A %) were well resolved at 470 nm while remaining peaks of R_f values 0.02 (56.85 A %), 0.07 (6.33), 0.17(32.72 A %) and 0.79 (4.10 A %) were well absorbed at 530 nm. Therefore the peak of R_f values 0.01, 0.92 and 0.95 may be of orange pigment and the R_f values of 0.02, 0.07, 0.17 and 0.79 may be of red pigments.

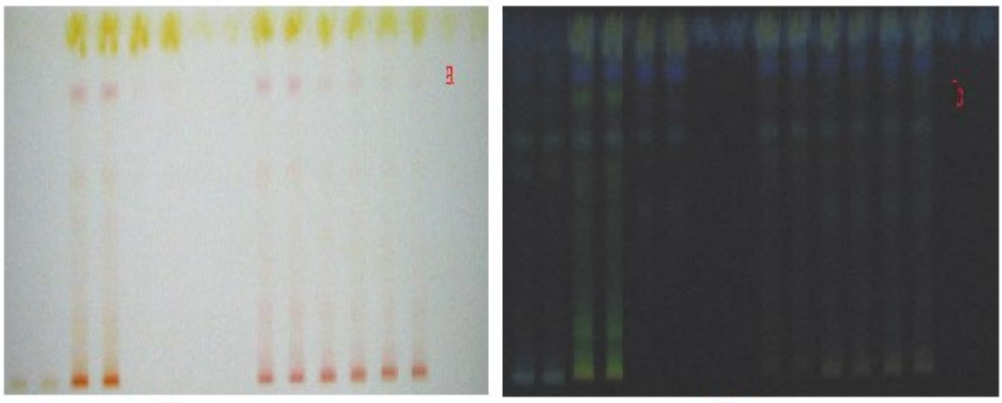

Figure 2: *Different bands in visible light (a) and in 365 nm (b) for mobile phase 5 (Ethyl acetate: Isopropanol: Water- 8.0:1.0:1.0 v/v).*

Hexane Extract

Mobile phase 1: Single peak of 0.94 R_f value with 100 A % was found. Therefore this mobile phase was unable to separate *the Monascus purpureus* pigments.

Mobile phase 2: Three peaks of R_f 0.75, 0.76 and 0.77 were found. The peak of R_f 0.76 with 60.58 A % was well resolved at 370 nm, peak of R_f 0.75 with 60.15 A % was well resolved at 420 nm and peak of R_f 0.77 with 49.82 A % was well resolved at 500 nm. Therefore R_f 0.75 & Rf 0.77 may be of red pigment while R_f 0.76 may be of yellow pigments.

Mobile phase 3: Two peak of R_f values 0.90 and 0.91 were found in mobile phase 3. The R_f values were very close to each other with same A % of 100. Therefore mobile phase 3 was unable to separate the pigments properly.

Mobile phase 4: Two peak of R_f values 0.86 and 0.87 were found scan at different wavelengths. These values are very close to each other with same A % of 100. Therefore mobile phase 4 was unable to separate the pigments.

Mobile phase 5: Single peak of 0.94 R_f value with 100 A % scanned at different wavelengths was found. Therefore this mobile phase was unable to separate the *Monascus purpureus* pigments.

4. Conclusion

In the present study HPTLC finger printing of extract of *Monascus purpureus* biopigment (water extract, acetone extract, chloroform extract, acetonitrile extract, petroleum ether extract, methanol extract, ethanol extract, hexane extract) were carried out using different mobile phases and scanned at different wavelength of 370 nm (for yellow pigment), 420 nm (for red pigment), 470 nm (for orange pigments), 500 nm (for red pigments) and 530 nm (for red pigments). The acetone extract of *Monascus purpureus* biopigments was found to showed good resolution when chromatographed with mobile phase 5 (ethyl acetate: isopropanol: water- 8.0:1.0:1.0 v/v).

Conflict of Interest

The authors do not have any conflict of interest.

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