Studies on red-pigment production by *Talaromyces atroroseus* TRP-NRC mutant II from wheat bran via solid-state fermentation

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Received: 22 June 2022
Revised: 19 September 2022
Accepted: 18 September 2022
Published: 31 March 2023

Egyptian Pharmaceutical Journal 2023, 22:18–29

Background
Recently, the need of finding eco-friendly and less-hazardous pigments focused on an important alternative to harmful synthetic dyes. High productivity of various pigments from microorganisms, their rapid growth throughout the year, stability, and solubility of their pigments provide them advantages more than pigments produced from other natural sources.
Objective
The objective of this study is to improve red-pigment production from local isolated fungus *Talaromyces atroroseus* TRP-NRC on an inexpensive substrate (wheat bran) under solid-state fermentation system by using different mutants. Then, comparing between pigment released from fungi after mutation by different mutants, comparing the efficiency of different solvents for the extraction of red biopigments under different conditions, and then extraction of pigment and studying its structure.
Materials and methods
A novel locally non-mycotoxin-producing fungus *T. atroroseus* TRP-NRC was treated with γ-ray radiation followed by subjecting to ultraviolet rays and grown on wheat bran as a complete medium via solid-state fermentation technique. Different solvents, including water, ethanol, methanol, and acetone, were applied to extract pigment from dried fermented wheat bran. The effect of pH, temperature, and contact time on yield of pigment extraction was studied. Stability of extracted pigment to heat, autoclaving, and ultraviolet rays was studied. Antimicrobial activity of extracted pigment was studied. The extracted sample was subjected to high-performance liquid-chromatography analysis and gas chromatography–mass spectrometry analysis. Statistical analyses were performed using SPSS program at *P* value less than 0.05.
Results and conclusion
The mutant fungus (I) by gamma radiation achieved 30% increase in red pigment compared with the wild type. The mutant fungus (I) was subjected to ultraviolet rays, mutant (II) added 22% increase in pigment production compared with mutant obtained by gamma radiation. About 70% v/v of methanol, ethanol, and acetone were more efficient for extracting pigment with an advantage of 70% v/v acetone. The yield of pigment extraction was affected by pH, temperature, and contact time, and was at pH 6.5 at 50°C after 16 h. The produced pigment appeared to be heat-stable when subjected to heat from 30 to 80°C for 6 h. The pigment was also stable when autoclaved at 121°C for 15 min. The pigment was stable when subjected to ultraviolet rays for 6 h. The extracted pigment showed antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative). Gas chromatography–mass spectrometry analysis revealed that eighteen compounds were identified in the acetone extract of pigment. In general, the prevailing two compounds of fermented wheat bran by *T. atroroseus* TRP-NRC mutant-II extract were 9, octadenoic acid (43.72) and 1,1′-bicyclopropyl-2-octanoic acid, 2′-hexyl-, methyl ester 43.72%.

Keywords:
heat stability, mutant, red pigment, solid-state fermentation, solvent, *Talaromyces atroroseus* TRP-NRC, wheat bran

Egypt Pharmaceut J 22:18–29

1687-4315

Introduction

Color of food is an important charactrestic that the first impressions are made based on it. Also, its appearance is very important in food industry. Synthetic pigments are widely used in foods, cloth, cosmetics, paintings,

plastics, and pharmaceuticals, but produce harmful effects on humans and pollute water and soil [1]. Using artificial color is associated with negative health problems for consumers [2]. This has generated interest in the production of alternative natural colors. The demand for natural pigments is repeatedly raised by the food industry due to the deficiencies of existing natural food colorants. Discovering new pigment procedures became the aim of several researches. Pigments from microorganisms have several advantages because they are more stable and soluble than those from plant or animal sources. Microorganisms can grow rapidly, which can lead to high productivity, and can produce various pigments throughout the year without limitations [3,4]. Also, some of their spores can resist harmful environmental conditions such as ultraviolet radiation. Carotenoids, melanins, flavins, quinones, monascins, and violacein or indigo are common microbial pigments that are industrially significant [5]. Microorganisms from the genus *Aspergillus* and *Penicillium* are potential producers of natural pigments [6,7]. Other microorganisms can produce pigments in high quantities, such as those belonging to the genus *Paecilomyces* [8], *Talaromyces atroroseus* [9], and *Penicillium* species [10,11]. Pigments in fungi are not essential for their growth and are classified as secondary metabolites that are derived from the products of primary metabolism and have been used as antimicrobial or antitumor agents.

This study has been performed as fungal pigments are industrially important and isolation of novel microbial strains with improved characteristics for pigment is highly desirable. This study focused on the production of red pigment from the local isolated fungus *T. atroroseus* TRP-NRC, which produced industrially relevant red pigments from the common inexpensive substrates like wheat bran under solid-state fermentation system [12,13]. The objective of this study is to improve the *T. atroroseus* red-pigment production using physical mutagenesis (γ-ray radiation and ultraviolet rays), and compare the pigments produced by fungi after mutation. Then, comparing the efficiency of different extraction solvents of the produced red biopigments from different treatments studies the pigment yield, effect of temperature, pH, pigment stability, and antimicrobial activities. Pigment was extracted from fungi and subjected to high-performance liquid-chromatography (HPLC) analysis and gas chromatography–mass spectrometry analysis (GC–MS).

Materials and methods

Substrate and microorganism

Wheat bran was purchased from a local market and *T. atroroseus* TRP-NRC used in this study was previously isolated from Egyptian compost in Microbial Chemistry Laboratory and deposited in the gene bank under accession number MW282329. This fungus is preserved in YPM medium at 4°C.

Inoculum-seed preparation

Ten milliliters of sterilized water were added to 5-day-old fungal-culture slants to prepare inoculum in the form of spores. Culture loop was used to crush fungal growth, 0.01% Tween 80 was added, vortexed, and used to inoculate production flasks at 1%.

Experimental and cultural conditions

Two hundred and fifty milliliters conical flasks containing 5 g of wheat bran were taken for pigment production, moistened with tap water. The flasks were sterilized by autoclaving at 121°C for 15 min. Then, they were inoculated after cooling and incubated under static conditions at 25°C.

Mutation induction using gamma radiation and ultraviolet rays

Fungal spores of 5-day-old culture of *T. atroroseus* TRP-NRC grown on PDA medium were exposed to different doses from γ-ray (200–800 Gry). Fungal spores obtained from each treatment cultivated in experimental conical flasks 250 ml contained 5 g of wheat bran (70% moisture) for 7 days, and then pigment was extracted by 95% ethanol (5 ml/g). The extracted pigment was diluted and measured at 500 nm. Spores exposed to 400 Gry that gave the high pigment concentration were grown on PDA medium for 5 days, crouched in 10 ml of distilled water, poured in open Petri dishes, and exposed to ultraviolet rays at a distance of 20 cm for 30 min. Fungal spores were obtained and cultivated on wheat bran (70% moisture) for 7 days, then pigment was extracted, determined, and compared with the wild fungus that was exposed to gamma radiation at 400 Gry.

Effect of incubation period and moisture content on pigment production from *Talaromyces atroroseus* TRP-NRC (mutant II)

The effect of the incubation period on pigment production was studied over the range of 5–14 days. Also, moisture-content range 1 : 1–1 : 4 w/v solid–liquid ratio was tested. Experiment flasks were incubated under static conditions for 7 days at 25°C.

Pigment extraction

The fermented matter was mixed with 90% ethanol in a conical flask (adding 5 ml of ethanol per gram of fermented wheat bran on dry-mass basis). The content was mixed on a rotary shaker at 200 rpm for 1 h, allowed to stand for 15 min, and filtered through Whatman filter paper No. 1.

Red-pigment estimation

The extracted pigments were diluted by ethanol and the absorbance was measured by spectrophotometer at 500 nm. The solvent extracts of unfermented substrates served as blank control.

Effect of solvent type on the pigment extraction efficiency

Different solvents were applied for pigment extraction from fermented wheat bran, including water, ethanol (90, 70, and 25%), methanol (90, 70, and 25%), and acetone (90, 70, and 25%) (adding 5 ml/g of fermented wheat bran on original dry wheat-bran basis). The content was mixed on a rotary shaker at 200 rpm for 1 h, allowed to stand for 15 min, and filtered through Whatman No. 1 filter paper, then estimated.

Effect of the contact time on extraction efficiency

After addition of solvent, the flasks were shaken on the rotary shaker at 150 rpm for 15 min and left for soaking for different periods from 4 to 24 h at the end of soaking contents. Time: The flasks were placed on rotary shaker at 150 rpm for 15 min. The flasks were filtered through filter paper Whatman No. 1 and the density of diluted color was measured at 500 nm.

Effect of temperature on efficiency of pigment extraction

The flask that contained fermented matter was mixed with the solvent (adding 5 ml of solvent per gram of fermented matter on dry-mass basis). The content was mixed on a rotary shaker at 200 rpm for 1 h at different temperatures (30, 40, 50, 60, and 70°C), allowed to stand for 15 min, and filtered through Whatman No. 1 filter paper, then estimated.

Effect of pH on the pigment extraction efficiency

The flask that contained fermented matter was mixed with the solvent (adding 5 ml of solvent per gram of fermented matter on dry-mass basis). The content with different pH was mixed on a rotary shaker at 200 rpm, allowed to stand for 15 min, and filtered through Whatman No. 1 filter paper, then estimated.

Red-pigment yield

About 70% acetone was used for extraction of red pigment from fermented wheat bran as mentioned above. A rotary vacuum evaporator is used for removing solvent. To calculate the pigment yield per gram of wheat bran, the obtained dry weight was divided by an original substrate [10].

Red-pigment stability

The aqueous phase of the extract solution was stored and used to investigate red-pigment stability. Briefly, glass tubes containing 50 ml of the samples were incubated in a water bath at 40, 60, 80, and 100°C, the sample was withdrawn at intervals of 1 h for 6 h for measuring the color density. The samples were also tested for pigment stability after sterilization at 15 psi, at 121°C for 15 min. The samples were cooled to room temperature and then the color density was estimated. Another set of plastic plates containing 20 ml of the samples were exposed to sunlight at different times, UV light, for 4 h. The OD values of the heated samples, sterilized samples, sunlight-exposed samples, and UV-exposed samples were measured at 500 nm against water as a blank. The results were normalized by dividing the obtained absorbance.

Antimicrobial activity of extracted pigment

A 24-h-grown bacterial culture was spread on agar plates. About 20 µl of dissolved extract was loaded on a 5-mm sterilized disc of Whatman filter paper 1 kept over agar surface. The plates were then incubated at 30°C for 24 h. Control for solvent and for each test organism was also taken simultaneously to nullify the inhibition due to solvent. The zone of inhibition was calculated after incubation as described in Jain and Pandey [14].

Extraction and analysis of secondary metabolites

About 70% acetone was used for extraction of red pigment from fermented wheat bran as mentioned above. The content was mixed on a rotary shaker at 200 rpm for 1 h, allowed to stand for 15 min, and filtered through Whatman filter paper No. 1. A rotary vacuum evaporator was used for removing solvent. To calculate the pigment yield per gram of wheat bran, the obtained dry weight was divided by the original substrate. The extracted sample was subjected to HPLC analysis and GC–MS.

High-performance liquid-chromatography analysis

HPLC is an analytical technique used to separate, identify, or quantify each component in a mixture. HPLC separates compounds dissolved in a liquid sample and allows qualitative and quantitative analysis of what components and how much of each component are contained in the sample. The sample is injected into the mobile phase. The individual

components of the sample migrate through the column at different rates because they are retained to a varying degree by interactions with the stationary phase. After leaving the column, the individual substances are detected by a suitable detector and passed on as a signal to the HPLC software on the computer. In the end, a chromatogram in the HPLC software on the computer is obtained. The chromatogram allows the identification and quantification of the different substances. The resulting chromatogram has a peak for every component in the sample.

High-performance liquid-chromatography conditions

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm×250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A), 0–5 min (80% A), 5–8 min (60% A), 8–12 min (60% A), 12–15 min (82% A), 15–16 min (82% A), and 16–20 (82% A). The multiwavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40°C [15].

Gas chromatography–mass spectrometry analysis

Mass sample derivatization

The sample was extracted, dried, and resuspended in 50 µl of bis(trimethylsilyl)trifluoroacetamide (BSTFA) +trimethylchloro-silane (TMCS) 99:1 silylation reagent and 50 µl pyridine for derivatization-sample functional groups to trimethylsilyl groups (abbreviated TMS) before GC analysis.

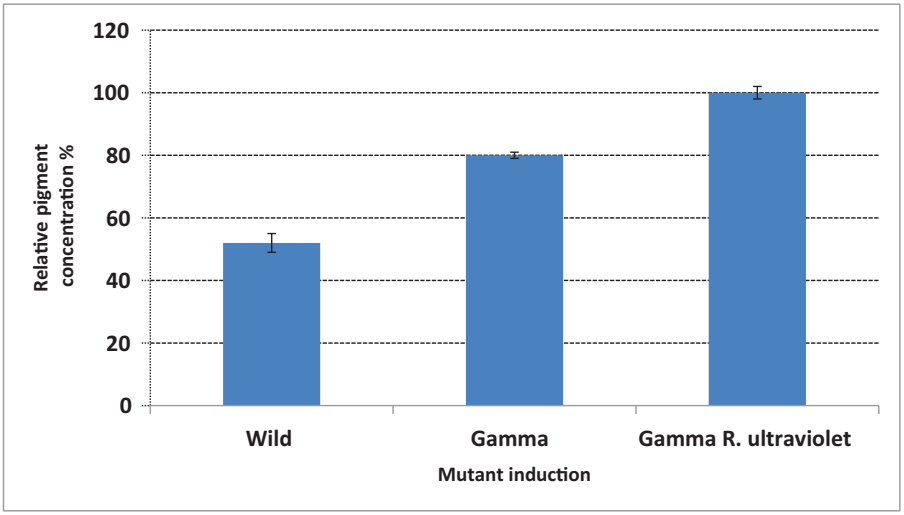
Gas chromatography–mass spectrometry analysis

he GC–MS system (Agilent Technologies, 5301 Stevens Creek Blvd. Santa Clara, CA 95051, United States) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977 A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m×0.25-mm internal diameter and 0.25-µm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 2.0 ml/min at a split-less injection volume of 2 µl and the following temperature program: 50°C for 5 min, rising at 5°C/min to 100°C and held for 0 min, and rising at 10°C/min to 320°C and held for 10 min. The injector and detector were held at 280 and 320°C. Mass spectra were obtained by electron ionization (EI) using a spectral range of *m/z* 25–700 and solvent delay 6 min. The mass temperature was 230°C and Quad 150°C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data [16].

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software and Mstat-c Program. Descriptive statistics and analysis of variance (one-way analysis) for the parametric variables were tested followed by LSD. All measurements were repeated three times (n=3) [17].

Figure 1



Production of red pigment from *Talaromyces atroroseus* TRP-NRC (wild type, γ-ray radiation and radiation with ultraviolet rays) cultivated on wheat bran under solid-state fermentation after 7 days at 25°C. Values are represented as means±SD of at least three experiments (n=3).

Results

Production of pigment

Effect of mutation

Data in Fig. 1 clearly show that exposure of *T. atroroseus* TRP-NRC wild fungus to gamma rays 400 Gry (mutant I) increased the red-pigment concentration released in growth medium by about 30% compared with the wild fungus. Exposure of the mutant obtained from gamma rays' treatment at 400 Gry to ultraviolet rays (mutant II) resulted in another increase in pigment production by 22% compared with gamma mutant and 50% compared with wild type.

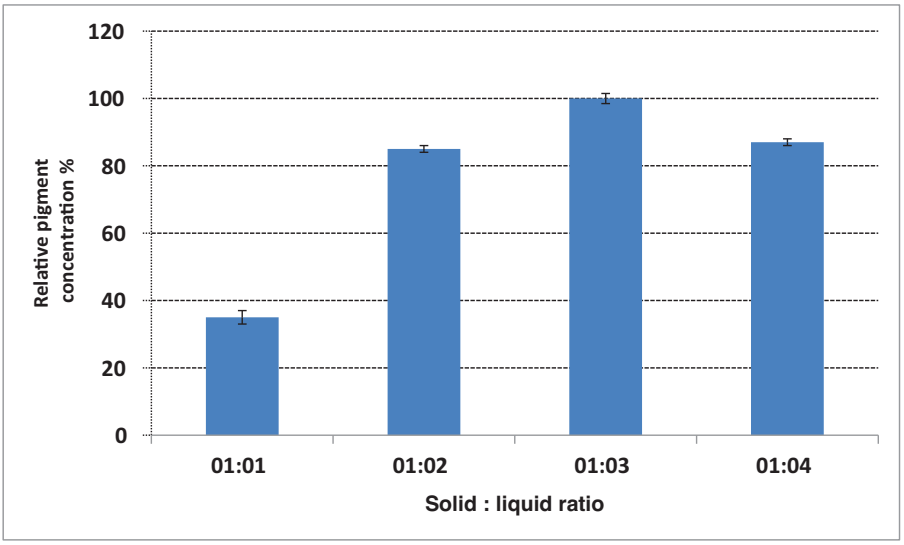
Effect of moisture content

Figure 2 illustrates that when the solid:liquid ratio was 1 : 3, the extraction of pigment/g substrate was more than that other ratio applied.

Effect of incubation periods

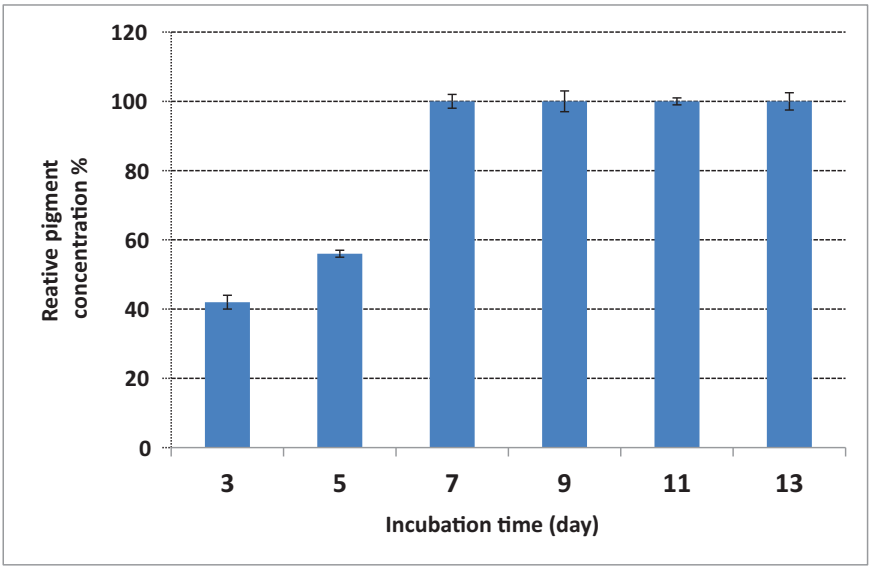
Figure 3 illustrates that after 10 days, the production of the pigment was at maximum concentration and stable in the formation medium without decaying till 13 days.

Figure 2



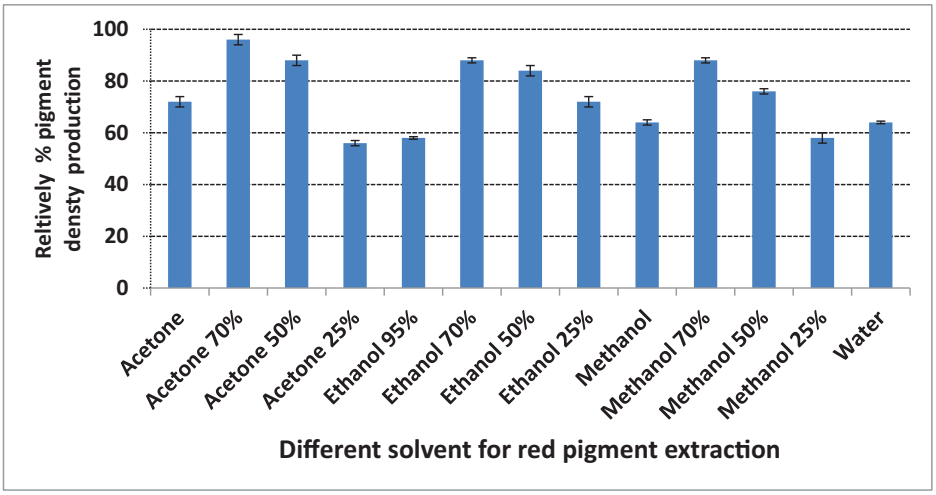
Effect of solid : liquid ratio (1 : 1,1 : 2,1 : 3,1 : 4) on red-pigment production by *Talaromyces atroroseus* TRP-NRC mutant II cultivated on wheat bran under solid-state fermentation after 7 days at 25°C. Values are represented as means±SD of at least three experiments (n=3).

Figure 3



Effect of incubation time on efficiency of red-pigment density yield from fermented wheat bran by *Talaromyces atroroseus* TRP-NRC mutant II incubated at 25°C under solid-state fermentation. Values are represented as means±SD of at least three experiments (n=3).

Figure 4



Effect of different solvents with different concentration on the efficiency of red-pigment density yield from fermented wheat bran by *Talaromyces atroroseus* TRP-NRC mutant II incubated for 10 days at 25°C under solid-state fermentation. Values are represented as means±SD of at least three experiments (n=3).

Effect of solvent type on the pigment extraction efficiency

Data presented in Fig. 4 showed that extracted red pigment from fermented dried wheat bran for 10 days by *T. atroroseus* TRP-NRC mutant II was varied according to solvent applied. However, the application of nonpolar solvents, that is, ethanol, acetone, and methanol, as well as too high polar solvent water as polar, did not result in best extraction of pigment from fermented wheat bran. The mixture of ethanol, acetone, and methanol with water 30% v/v to become moderately polar enhanced too much efficiency of pigment extraction. Applied acetone 70% v/v has an advantage in extracting more red-pigment yield from fermented wheat bran followed by 70% v/v ethanol and 70% v/v methanol, respectively.

Effect of contact time on the pigment extraction efficiency

Table 1 shows that contact time has an important factor in efficiency in releasing the pigment from fermented wheat bran by *T. atroroseus* TRP-NRC (mutant II). The relative density of pigment was increased by increasing the contact time to 16 h as pigment density was doubled compared with 1 h of contact time.

Effect of temperature on the pigment extraction efficiency

Figure 5 illustrates that the density of the eluted pigment was increased by the increase of temperature applied for elution from 30 to 50°C and decreased slightly at 60°C.

Effect of pH on the pigment extraction efficiency

Table 2 reveals that at pH 6 and 6.5, the obtained concentration of red pigment was more than the other tested pH values, giving 2.4-fold from fermented wheat bran by *T. atroroseus* TRP-NRC mutant II.

Table 1 Effect of contact time between fermented substrate and solvent on efficiency of red-pigment density yield from fermented wheat bran by *Talaromyces atroroseus* TRP-NRC mutant II incubated for 10 days at 25°C under solid-state fermentation

Contact time between fermented substrate and solvent (h)	Relative% pigment-density production
1	112 d±2
4	124 c±1
8	136 b±1.5
16	206 a±1
24	206 a±0.5
LSD at α 0.05	3.372

Values are represented as means±SD of at least three experiments (n=3). Means with different letters within each column are significant at α=0.05 level and means without letters are not significant.

Pigment stability

The produced pigments by *T. atroroseus* TRP-NRC mutant II were stable under high temperatures and long time. The produced pigment retained the full density color when subjected to heating under 40–100°C for 6 h, or after sterilization for 15-min autoclave at 121°C. Also, the produced pigments were stable after exposure to UV for 4 h or after direct sunlight exposure for 8 hrs, whereas they were degraded after long-time UV exposure.

Antimicrobial activity of extracted pigment

With concerning to antimicrobial of extracted pigment, the filter-paper method was used to test the effects on bacteria based on the diameter of

inhibition zones and Fig. 6 shows the results obtained. It was observed that extracted pigment showed antibacterial activity against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative). It was apparent that the fungus pigment extract has good antibacterial effect against Gram-positive bacteria, *B. subtilis* ATCC 6633 and *E. coli* ATCC 25955, Gram-negative bacteria.

High-performance liquid-chromatography

After extraction, the pigments are purified via chromatographic techniques. HPLC – separates based on retention time. HPLC is also used to characterize pigments apart from purification, such as, in this case, the red pigment from *T. atroroseus* TRP-NRC mutant II, which is characterized to be a mixture of 12 components. Table 3 and Fig. 7 summarize the results, area, and concentration of each component.

Table 2 Effect of pH value on efficiency of red-pigment density yield from fermented wheat bran by *Talaromyces atroroseus* TRP-NRC mutant II incubated for 10 days at 25°C under solid-state fermentation

pH value	Relative% pigment-density production
4.5	105 e±1
5	105 e±2
5.5	206 d±0.5
6	220 b±0.4
6.5	236 a±0.5
7	217 c±1
LSD at α 0.05	1.874

Values are represented as means±SD of at least three experiments (n=3). Means with different letters within each column are significant at α=0.05 level and means without letters are not significant.

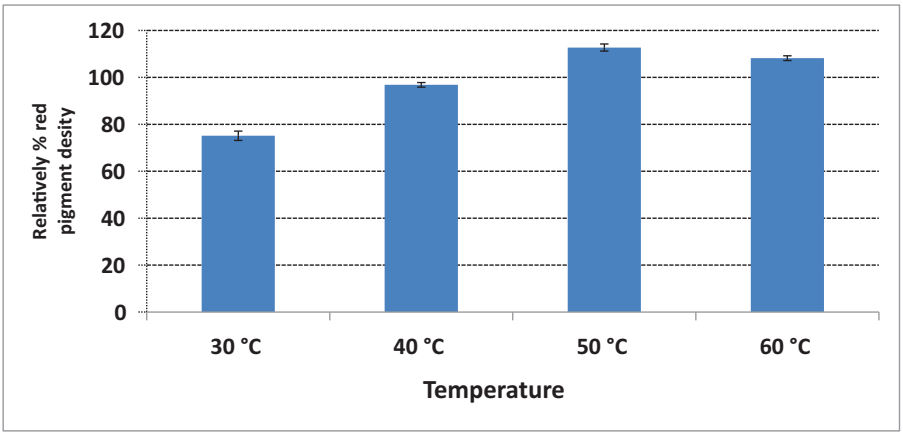
Gas chromatography–mass spectrometry analysis

The components present in the acetone extract of fermented wheatbran by *T. atroroseus* TRP-NRC mutant II were identified by GC–MS. The active components with their retention time, molecular formula, molecular weight, and concentration (%) in the acetone extract of fermented wheat bran by *T. atroroseus* TRP-NRC mutant II are presented in Table 4. Eighteen compounds were identified in the acetone extract of pigment. In general, the prevailing two compounds of fermented wheat bran by *T. atroroseus* TRP-NRC mutant-II extract were 9-octadenoic acid (43.72) and [1,1′-bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester (23.15%).

Discussion

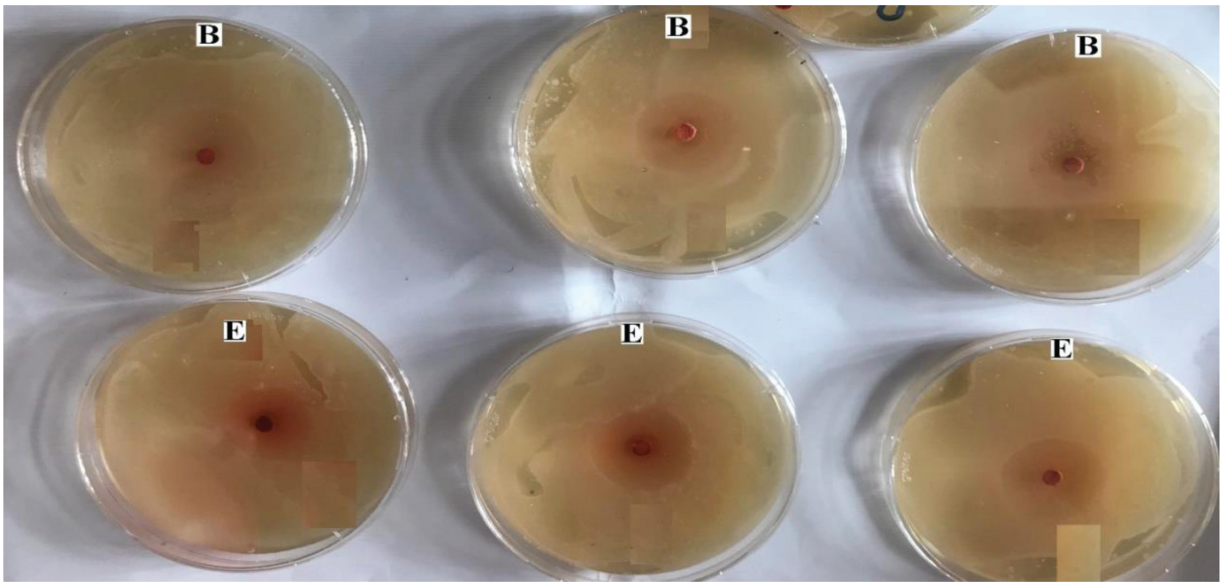
The present study reported an increase in pigment production by 50% from *T. atroroseus* TRP-NRC (mutant II) compared with wild type. This is supported by a study that explained the mechanism of mutation and increasing pigment concentration may be due to increasing fungal-membrane permeability and gene copy number or upregulation of gene expression involved in pigment production [18]. Other studies concluded that the effect of different doses of gamma radiation for enhanced secondary metabolites from fungi, which affects the performance of pigment-production pathway, resulted from the formation of free radicals and reactive oxygen species due to gamma irradiation on fungi. While ultraviolet rays can cause rearrangement in amino acids in the protein as well as alter genes' position on the chromosome [19]. Moisture is a key factor for the successes of solid-state fermentation system for two main factors, the first is the solubility of the nutrients in the substrate to be utilizable by the

Figure 5



Effect of temperature on efficiency of red-pigment density yield from fermented wheat bran by *Talaromyces atroroseus* TRP-NRC mutant II (incubated for 10 days at 25°C under solid-state fermentation). Values are represented as means±SD of at least three experiments (n=3).

Figure 6



Antimicrobial activity of extracted pigment against [B] *Bacillus subtilis* ATCC6633 (Gram-positive bacteria) and [E] *Escherichia coli* ATCC 25955 (Gram-negative bacteria).

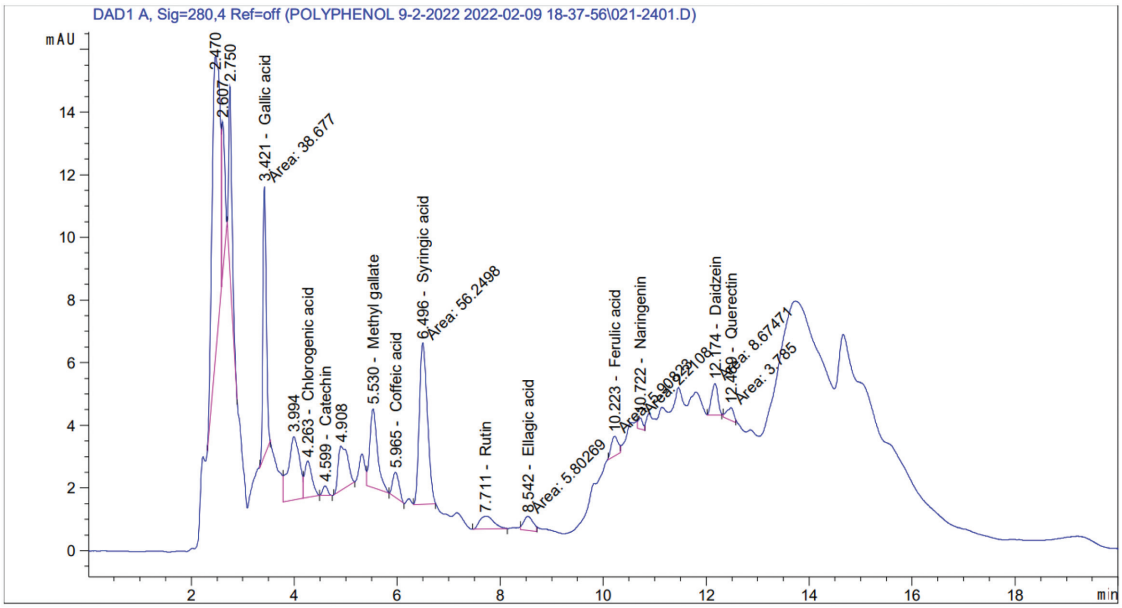
Table 3 Components of pigment extract

Peak	*RetTime	Width (min)	*Area (mAU*s)	Area %	Conc. (μg/ml=μg/207 mg)	Conc. (μg/g)	Name
1	3.421	0.0749	38.68	10.4951	3.35	16.17	Gallic acid
2	4.263	0.1498	11.59	3.1453	1.58	7.64	Chlorogenic acid
3	4.599	0.1088	1.97	0.5358	0.51	2.48	Catechin
4	5.530	0.1533	25.73	6.9815	1.78	8.59	Methyl gallate
5	5.965	0.1440	6.79	1.8435	0.55	2.64	Caffeic acid
6	6.496	0.1818	56.25	15.2635	4.05	19.59	Syringic acid
7	7.711	0.2702	7.73	2.0984	0.92	4.43	Rutin
8	8.542	0.2140	5.80	1.5746	3.98	19.25	Ellagic acid
9	10.223	0.1562	5.91	1.6032	0.37	1.77	Ferulic acid
10	10.722	0.1029	2.21	0.5999	0.25	1.19	Naringenin
11	12.174	0.1441	8.67	2.3539	0.54	2.60	Daidzein
12	12.489	0.1508	3.79	1.0271	0.47	2.27	Querectin

Retention time (Rt) is the time interval between sample injection point and the apex of the peak. Peak area is the area enclosed by the peak and baseline.

fungus, and the second is the availability of oxygen in growth medium that demanded optimum growth. The solid: liquid ratio in solid-state fermentation system is varied mainly according to the microorganisms and the substrate type and nature, configuration, as well as substrate granule size. The present result is supported by research, which found that lower moisture content led to sharp decrease in pigment yield produced by *Monascus purpureus* FRR 2190 cultivated under solid-state fermentation [20]. The achieved results for the effect of incubation periods on pigment production gave economically an advantage for the red-pigment production from cultivating *T. atroroseus* TRP-NRC mutant II on wheat bran as a complete economic medium under solid-state fermentation. The previous workers stated that different incubation periods for production of high red pigment depend on types of fermentation system, fermented substrates, and microorganisms [21–24]. A suitable extraction technique and solvent selection are very important to increase the extraction-yield efficiency as well as performance of higher quality of pigments. Some researchers reported that ethanol 60% was the best solvent for red-pigment extraction from rice fermented by *M. purpureus* FTC 5357 under solid-state fermentation [25]. Other studies found that methanol was the best solvent for extraction of red pigment from *M. purpureus* MTCC 410 cultivated on coconut residue and *Monascuss anguineus* cultivated on potato peel when applied to extract pigment among different polar and nonpolar solvents [23]. The obtained results reported that a mixture of ethanol,

Figure 7



Chromatogram of pigment extract.

acetone, and methanol with water 30% v/v to become moderately polar enhanced too much efficiency of pigment extraction. These results ensure the previous reported data that moderate polar compounds were preferable for efficient pigment extraction [22]. The result proved that the longer the exposure of solute to the solvent, the greater the pigment can be extracted from the fermented wheat bran. Similar findings were reported that at the longer soaking time, the contact time allows the phase equilibrium to be established. Hence, the reaction is complete, as a result, greater pigment yield is extracted from the fermented substrate [26–28]. The temperature applied for the pigment extraction affects the efficiency of elution of the produced red pigment from the cultivation of *T. atroroseus* TRP-NRC mutant II on wheat bran under solid-state fermentation. Temperature is identified as the efficiency to extract the red pigment produced by different extraction solvents. Several studies reported 55°C as optimal for extraction [29,30], while others stated 80°C for pigment extraction from *Planococcus maritimus* AHJ_2 isolated from distillery spent wash [31–33]. The pH value considers an important parameter role in the pigment extraction process from fermented substrates. The obtained results, which revealed that at pH 6 and 6.5, the obtained concentration of red pigment was more than the other tested pH values, agreed with that reported by many investigators [34–36]. Thermal stability of natural pigments is critical for food and textile applications because thermal treatments are applied in the food industry. The present study revealed that produced pigments by *T. atroroseus* TRP-NRC mutant II were stable under high temperatures and long time. The results agreed with other studies, which reported that the thermal stability of pigments produced by Talaromyces covered the temperature range between 25 and 100°C for different treatment times. Also, the produced pigments were stable after exposure to UV for 4 h, whereas they were degraded after long-time UV exposure. These properties make *T. atroroseus* TRP-NRC mutant II pigments that are useful for industrial processes and can applied for industrial scale [37]. The antimicrobial properties of fungal pigments were important biological properties. The present study reported the antibacterial activity of extracted pigment against *B. subtilis* and *E. coli*. Similarly, other studies have demonstrated the antibacterial activity of pigments produced by *T*alaromyces *purpureogenus* CFRM02 against foodborne pathogens, both Gram-positive and Gram-negative bacteria [38–40]. The biological properties could be attributed to other bioactive secondary metabolites present in the pigmented extract. According to this result, applications of pigmented extract in food processing would be valuable and used to extend food shelf life and other preservation strategies. HPLC is an analytical technique used to separate compounds dissolved in a liquid sample and allows qualitative and quantitative analysis of what components and how much of each component is contained in the sample. HPLC can separate and detect each compound by the difference of each

Table 4 Gas chromatography–mass spectrometry analysis

Peak	RT	Name	Formula	Area	Area sum %
1	16.309	Glycerol, 3TMS derivative	C12H32O3Si3	825348.56	0.73
2	22.289	1,1,1-Trifluoroheptadecen-2-one	C17H31F3O	747220.19	0.66
3	22.949	Xylitol 5TMS	C20H52O5Si5	3297403.3	2.92
4	24.171	Oleic acid	C18H34O2	6906062.1	6.11
5	24.74	D-lyxose, 4TMS derivative	C17H42O5Si4	3309371.8	2.93
6	24.786	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]m	C27H52O4Si2	1133712.6	1
7	24.93	Ribitol, 5TMS derivative	C20H52O5Si5	639302.83	0.57
8	25.127	Mannitol TMS		4066802.7	3.6
9	25.666	D-lyxose, 4TMS derivative	C17H42O5Si4	2007238.8	1.78
10	25.939	Hexadecanoic acid, trimethylsilyl ester	C19H40O2Si	2525084.5	2.24
11	26.288	Oleanitrile	C18H33N	3365275.9	2.98
12	26.471	[1,1′-Bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester	C21H38O2	26150910	23.15
13	26.622	Octanal, 7-methoxy-3,7-dimethyl	C11H22O2	1736096.7	1.54
14	27.123	1, E-11, Z-13-octadecatriene	C18H32	551553.27	0.49
15	27.594	9-Octadecanoic acid, (E)-, TMS derivative	C21H42O2Si	49386199	43.72
16	27.632	11-Octadecanoic acid, (E)-, TMS derivative	C21H42O2Si	4681540.7	4.14
17	27.723	Stearic acid, TMS derivative	C21H44O2Si	798042.03	0.71
18	28.724	9-Octadecanamide, (Z)-	C18H35NO	837352.03	0.74

compound's speed through the column. In the column, the stronger the affinity (e.g. van der Waals force) between the component and the mobile phase, the faster the component moves through the column along with the mobile phase. On the other hand, the stronger the affinity with the stationary phase, the slower it moves through the column. The elution speed in the column depends on the affinity between the compound and the stationary phase. At the end, the resulting chromatogram has a peak for every component in the sample [41]. Eighteen compounds were identified in the acetone extract of pigment. In general, the prevailing two compounds of fermented wheat bran by *T. atroroseus* TRP-NRC mutant II extract were 9-octadecanoic acid and [1,1′-bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester. 9-octadenoic acid is a natural product found in *Eleutherococcus sessiliflorus*, *Dipteryx lacunifera*, and other organisms with data available. An unsaturated fatty acid is the most widely distributed and abundant fatty acid in nature. It is used commercially in the preparation of oleates and lotions, and as a pharmaceutical solvent [42]. It was found that [1,1′-bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester has anti-inflammatory, antitumor, antiviral, antibacterial, and antifungal properties. Hydroxytyrosol also improves endothelial dysfunction, decreases oxidative stress, and is neuroprotective and cardioprotective. The obtained results of all these biological futures revealed that the obtained red pigments are currently the most actively investigated natural phenol. The evidence presented in suggests that fungal red pigment obtained from wheat bran fermented by *T. purpureogenus* CFRM02 under solid-state fermentation system has great pharmacological potential [14,42,43]. The obtained red pigment by *T. purpureogenus* CFRM02 is considered an excellent food supplement by the nutraceutical and food industries due to its bioavailability, chemical properties, and easy formulation along with its lack of toxicity [44,45].

Conclusion

Searching for isolation microorganisms of high-producing biopigment from inexpensive familiar substrates is the primary step to achieving successes on road for economically and commercially pigment yield. The applied scientific techniques for mutant of new isolate organisms for improving the efficiency for performance of pigment release make the production more economical and encourage to be useful in industrial application. The applied suitable extraction techniques like selecting of proper solvent, suitable pH, and temperature as well as contact time, help to increase the extraction yield and stimulate higher quality of pigments. Compounds of fermented wheat bran by *T. atroroseus* TRP-NRC mutant II extract were 9-octadenoic acid and [1,1′-bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester. 9-Octadecanoic acid is a natural product fungal red pigment obtained from wheat bran fermented by *T. purpureogenus* CFRM02 under solid-state fermentation system with data available. It is an unsaturated fatty acid used commercially in the preparation of oleates and lotions, and as a pharmaceutical solvent. It was found that [1,1′-bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester has anti-inflammatory,

antitumor, antiviral, antibacterial, and antifungal properties. Also, it improves endothelial dysfunction and decreases oxidative stress, as well as is neuroprotective and cardioprotective. The obtained red pigment by *T. purpureogenus* CFRM02 is considered an excellent food supplement by the nutraceutical and food industries due to its bioavailability, chemical properties, and easy formulation along with its lack of toxicity.

Acknowledgments

The authors are grateful to National Research Centre for providing facilities of instruments and needed materials.

Fadel M. and Yomna A.M. Elkhateeb conceptualized, analyzed the data for this work, and necessary inputs were given toward the design of the paper. All authors discussed the methodology and conclusion and contributed to the final paper. Fadel M. sharing in practical section 30%. Yomna A.M. Elkhateeb sharing in practical section 70%. All authors have read the paper and approved it.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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