



Article

Monascus-Derived Biologically Active Compounds Fermented by Khao Klong Broken Rice (Brown Rice) as Bio-Ingredient for Anti-Aging Potential: Chemical Profiles, Antioxidant, Anti-Collagenase, and Anti-Tyrosinase Activities

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Abstract

This study aimed to optimize pigment production from *Monascus purpureus* TISTR3090 using Khao Klong broken rice (brown rice) as a solid substrate and to evaluate the antioxidant and anti-aging activities of the resulting pigment extracts. Solid-state fermentation showed a progressive increase in red pigment production, reaching a maximum on day 8 ($OD_{680} = 0.10$). The water extract (WAE) exhibited a higher total phenolic content (148.86 mg GAE/g) than the ethanol extract (ETE) and demonstrated stronger antioxidant activities, including DPPH radical inhibition (74.66%) and ABTS scavenging activity (65.53%). In addition, WAE showed superior anti-aging potential, with greater inhibition of tyrosinase (75.29%) and collagenase (32.88%) compared to ETE. The MP extract exhibited the presence of R-NH₂ functional groups, identified by FTIR, indicating the primary criterion for fungal red pigment. Gas chromatography–mass spectrometry (GC-MS) analysis identified 25 bioactive compounds, mainly fatty acid methyl esters and aromatic derivatives, which may contribute to the observed bioactivities. Liquid chromatography–mass spectrometry (LC-MS) further confirmed higher levels of gallic acid, chlorogenic acid, epigallocatechin gallate, and oroxylin A in WAE. Thus, *Monascus* pigments derived from broken brown rice-based fermentation represent a promising natural colorant and functional bioingredient with potential applications in nutraceutical and cosmeceutical industries, warranting further studies on formulation, stability, and in vivo validation.

Keywords: *Monascus purpureus*; pigment; anti-aging; solid-state fermentation; bioingredients



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1. Introduction

Microbial pigments, especially those generated by *Monascus* fungi, have drawn considerable interest due to their vivid colors and potential health advantages [1]. Historically, *Monascus* species have been employed in Asian nations for ages, chiefly in the manufacture of red yeast rice, a fermented product recognized for its use as a natural food dye and its therapeutic attributes [2,3]. Normally, the agroindustry and food industry byproducts

have been used as a carbon source for microbial pigment production such as broken rice hydrolysate [4], corn residues [5], orange processing waste [6], corncob hydrolysate [7], and sugarcane bagasse hydrolysate [8], which are widely used as cost-effective and nutrient-rich substrates for microbial pigment production, primarily through fermentation processes.

The pigments derived from *Monascus* fermentation are not only valued for their coloring properties but also for their biological activities [9]. Notably, these pigments exhibit significant antioxidant activities, which is crucial in preventing cellular damage. Additionally, the total phenolic content of these pigments correlates with their antioxidant capacity, as phenolic compounds are known for their free radical scavenging abilities. The relationship between total phenolic content and antioxidant activity is pivotal, as higher phenolic content often translates to enhanced antioxidant capacity [9,10]. This increased antioxidant activity can improve cell viability by protecting cells from oxidative stress-induced damage, thereby reducing the risk of chronic diseases and supporting overall cellular health [1]. In addition to their antioxidant properties, *Monascus* pigments (MPs) have been investigated for their cytotoxic effects against various cancer and normal cell lines to evaluate their potential biological activities and safety profiles. Previous studies have reported differential cytotoxic effects of certain MPs among cell lines, with greater inhibitory effects observed in some cancer cell lines than in normal cells. These findings suggest that MPs may warrant further investigation as potential candidates for anticancer drug development [11,12]. MPs are aphilone derivatives, including substances like monascin, ankaflavin, rubropunctatin, and monascorubrin [13]. Although these chemicals provide advantageous qualities, safety issues have been highlighted, especially with the synthesis of citrinin, a mycotoxin that may arise during fermentation [14]. Citrinin poses nephrotoxic risks, and its presence necessitates stringent control measures in the production process to ensure the safety of *Monascus*-derived products. *Monascus* species can synthesize monacolin K, a secondary metabolite structurally similar to lovastatin, via the polyketide biosynthetic pathway during submerged or solid-state fermentation. Monacolin K has garnered significant attention in the health sector for its cholesterol-lowering properties through the inhibition of HMG-CoA reductase; however, excessive intake or uncontrolled fermentation conditions may result in detrimental effects, such as hepatotoxicity and myopathy, especially when produced alongside citrinin, a nephrotoxic mycotoxin. Consequently, optimization and safety surveillance are crucial for the industrial use of *Monascus*-derived products [15,16].

Despite the extensive research on MPs, there remains a gap in understanding how different fermentation substrates, such as various rice varieties, influence the production of novel compounds and their associated biological activities [17,18]. The utilization of broken brown rice as a substrate for fungal fermentation has garnered significant interest as a novel and sustainable method for producing natural colors and high-value bioproducts for the food, nutraceutical, and cosmetic sectors. Although its economic value is diminished by physical damage incurred during milling, broken brown rice maintains substantial concentrations of carbs, proteins, lipids, vitamins, minerals, and bioactive phytochemicals, rendering it an exceptional nutrient-dense substrate for microbial bioprocesses [19]. Filamentous fungi, specifically *Monascus purpureus*, *Aspergillus* spp., and *Talaromyces* spp., exhibit significant potential for pigment biosynthesis through solid-state fermentation on rice-based substrates, yielding bioactive natural colorants such as red, orange, and yellow pigments with antioxidant and antimicrobial attributes [20,21]. Exploring the fermentation of Khao Klong broken rice (brown rice) with *Monascus* fungi could lead to the discovery of unique pigments with specific bioactivities, paving the way for their application in health care and cosmetic products. Hence, the objective of this study was to optimize the MPs production from *M. purpureus* TISTR3090, which was fermented by Khao Klong broken rice (brown rice) as solid substrate and antioxidant activity of MPs.

2. Materials and Methods

2.1. Fungal Strain and Culture Condition

Test fungal-free citrinin strain, *Monascus purpureus* TISTR3090, was obtained from Thailand Institute of Scientific and Technology Research (Bangkok, Thailand), Thailand. The fungal was cultured on Potato dextrose agar (PDA) and incubated at 30 °C for 30 days; additionally, there was maintenance of fungal culture at 4 °C before use.

2.2. Fungal Pigment Production by Solid-State Fermentation (SSF)

Khao Klong broken rice (brown rice) was carefully cleaned with distilled water. The solid rice medium was added to a 250 mL Erlenmeyer flask by immersing 20 g of rice in 47 mL of distilled water to set a beginning moisture level of 70%. The pH was adjusted to pH 6 before sterilization at 121 °C and 15 psi for 15 min. After cooling spontaneously to the ambient temperature, the flask was moving around evenly. The fungal spore suspension (4.5×10^6 spores) was injected onto the solid medium and incubated for 30 days at 30 °C.

2.3. Extraction of MPs

The fermented rice solids were oven-dried at 60–65 °C overnight to remove moisture and blended. The dried material was then subjected to extraction using the following two different solvents for comparison: distilled water (as the control) called WAE and 95% ethanol called ETE. Each extraction process utilized 1 g of the dried fermented rice solids per 160 mL of the respective solvent, following the methodology described by Shaleha et al. [22]. The percent yield of MP extracts was calculated using following formula:

$$\text{Yield (\%)} = [\text{Weights of solvent free extract (g)}/\text{Dried fermented rice}] \times 100 \quad (1)$$

2.4. Total Phenolic Content (TPC)

The TPC was determined using the Folin–Ciocalteu reagent (FC). Basically, 0.5 mL of the extract was mixed with 0.5 mL of FC. The solution was maintained at 25 °C for exactly 5 min before adding 2 mL of 7.5% sodium carbonate mixture and mixing it with 8 mL of water. The absorbance at 725 nm was measured two hours later. The calibration curve used gallic acid as its standard. The TPC was determined using UV spectrometer (Tecan Sunrise Basic, Groedig, Austria) in milligrams of gallic acid equivalent per gram of sample [23]. The TPC result was expressed as mg gallic acid equivalent per g of extract (mg GAE/g sample).

2.5. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Assay

The radical scavenging activity was measured using the DPPH assay, which had minor changes as opposed to Lee et al.'s approach [24]. To summarize, 40 µL of MPs (10 mg/mL in DMSO) was combined with 2.96 mL of DPPH (0.1 mM) solution. The combination of reactions was rapidly agitated before the incubation process for 30 min in a dark place at an ambient temperature. The absorbance at 517 nm was measured using microplate reader (Tecan Sunrise Basic, Groedig, Austria). DPPH was used as a control. Ascorbic acid was employed as a means of control. The percentage of inhibitory radical scavenging activity was determined by applying a particular formula:

$$\% \text{ Inhibition} = [(\text{Abs Control} - \text{Abs Sample})/\text{Abs Control}] \times 100 \quad (2)$$

2.6. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out in accordance with Wu et al. [25], with a few modifications. The stock solutions included 300 mM acetate buffer at pH 3.6, 10 mM TPTZ (2-, 4-, and 6-tripyridyl-s-triazine), and 20 mM FeCl₃ solution. MPs (50 µL, 1 mg/mL) were mixed with 1.5 mL of the FRAP solution and incubated for 5 min in dark circumstances. The

colorful product was then measured using UV spectrometer (Tecan Sunrise Basic, Groedig, Austria) at 593 nm and reported as milligram equivalents of FeSO₄ per milligram of dry weight. The calibration line was created employing the following subsequent values of FeSO₄: 0.0025, 0.005, 0.01, and 0.02 mg/mL.

2.7. 2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Assay

The ABTS stock solution was prepared by mixing 7 mM ABTS in water with 2.45 mM potassium persulfate in an equal volume. After diluting the initial solution with water, the working solution had an absorbance of 0.706 ± 0.02 at 734 nm. Each experiment used a freshly generated ABTS⁺ solution. In darkness, 20 µL of the substance was mixed with 180 µL of ABTS solution for 30 min. The wavelength of the absorbance was measured using microplate reader (Tecan Sunrise Basic, Groedig, Austria) at 734 nm with a microplate spectrophotometer reader. The formula for calculating ABTS radical scavenging was as follows:

$$\text{ABTS radical scavenging} = [(AB - AA)/AB] \times 100 \quad (3)$$

When

AB = absorbance of ABTS radical + distilled water;

AA = absorbance of ABTS radical + sample extract [25].

2.8. Anti-Tyrosinase Determination

Jiang et al. [26] demonstrated that this approach was used under specific conditions to block tyrosinase. The mixture included 50 µL of WAE and ETE (2.0 mg/mL), 50 µL of 0.2 M sodium phosphate buffer (pH 6.6), and 50 µL of tyrosinase (50 units/mL). After 10 min of incubation at 37 °C, 50 µL of a 2.5 mM L-DOPA solution was added. The reaction was finally observed using microplate reader (Tecan Sunrise Basic, Groedig, Austria) at 492 nm. The proportion of tyrosinase inhibition was approximated using the following equation:

$$\text{Inhibition is calculated as } (A - B)/A \times 100 \quad (4)$$

When

A = serving as the control slope at 492 nm;

B = representing the test sample slope.

2.9. Anti-Collagenase Determination

The reaction mixture contained 20 µL of each sample and 20 µL of an enzyme mixture (0.8 units/mL in 50 mM Tricine buffer, pH 7.5, and incubated at 37 °C for 10 min before use). After applying 200 µL of N-[3-(2-furyl)acryloyl]-L-leucyl-glycyl-L-prolyl-L-alanine (FALGPA) substrate (0.5 mM in buffer), the mixture was incubated for 10 min and measured using microplate reader (Tecan Sunrise Basic, Groedig, Austria) at 340 nm for 1 min intervals by a spectrophotometer. The enzyme activity was determined by lowering kinetic absorbance, and the percentage of collagenase inhibition was calculated using the following equation [27]:

$$\text{Inhibition (\%)} = [\text{Activity (con)} - \text{Activity (In)}]/[\text{Activity (con)}] \times 100. \quad (5)$$

2.10. Flavonoid and Phenolic Compound Determination

Flavonoid compounds, including oroxin B (purity ≥ 98%, HPLC), oroxylin A (purity ≥ 98%, HPLC), baicalin (purity ≥ 95%, HPLC), and baicalein (purity ≥ 98%), purchased from Sigma-Aldrich (St. Louis, MO, USA), were quantified using high-performance liquid chromatography with photodiode array detection (HPLC-PDA). The analysis was performed using an Accela HPLC system (Thermo Fisher Scientific, San Jose, CA, USA)

equipped with an Accela 600 Pump, an Accela Autosampler (AS), and an Accela Photodiode Array (PDA) Detector. The PDA Detector Conditions (Accela PDA) were executed for 35 min at a discrete wavelength of 280 nm. The Autosampler Conditions (Accela AS) were configured with a volume injection of 2 μ L, a tray temperature of 25 °C, and a column oven temperature of 35 °C. Chromatographic separation was performed on a Luna[®] C8 column (150 mm $\times$ 2.0 mm, 5 μ m particle size; Phenomenex (Torrance, CA, USA)). The evaporated dried extract was scraped and accurately weighed (1 mg) and then dissolved in acetonitrile (ACN) to a final volume of 1 mL.

The solution was sonicated at 30 °C for 5 min and subsequently filtered through a 0.45 μ m syringe filter prior to chromatographic analysis. All reference standards of phenolic compounds, namely gallic acid (GA) (purity $\geq$ 97.5%), catechin (CAT) (purity $\geq$ 98%), epicatechin (EPI) (purity $\geq$ 99%), vanillic acid (VA) (purity $\geq$ 97%), *p*-coumaric acid (PCA) (purity $\geq$ 98%), quercetin (QUE) (purity $\geq$ 95%), kaempferol (KAE) (purity $\geq$ 97%), chlorogenic acid (CLA) (purity $\geq$ 97%), and epigallocatechin gallate (EGCG) (purity $\geq$ 95%), were purchased from Sigma-Aldrich (St. Louis, MO, USA) and analyzed using liquid chromatography–mass spectrometry (LC-MS). The mobile phase consisted of 0.1% (*v/v*) acetic acid in water (Solvent A) and 0.1% (*v/v*) acetic acid in acetonitrile (Solvent B). Chromatographic separation was performed on a Luna[®] C8 column (150 mm $\times$ 2.0 mm, 5 μ m particle size; Phenomenex). The injection volume was 2.5 μ L, with the autosampler tray maintained at 25 °C and the column oven set at 35 °C. Mass spectrometric detection was carried out using a Surveyor MSQ Plus system (Thermo Scientific Surveyor MSQ Plus) equipped with an electrospray ionization (ESI) source operating in negative ion mode. The probe temperature was set at 450 °C, the needle voltage at 4.5 kV, and nitrogen was used as the nebulizing gas at a pressure of 5 bar. Data acquisition was performed over a scan time of 0–19 min [28].

2.11. Chemical Characterization

2.11.1. GC–MS Analysis

The Red pigment was conducted using the same conditions and protocol as previously reported by Girija et al. [29] with some modifications. Briefly, the pigment (ETE) was dissolved in hexane (1:20 *v/v*) and was injected into the capillary column (30 m $\times$ 0.25 mm diameter $\times$ 0.25 μ m) of an Agilent 7890 gas chromatograph. The injection temperature was 280 °C, the column oven temperature was 45 °C, and the helium flow rate was 1.40 mL/min. Also, 50 min is the total holding time. Conditions for the mass spectra were electron impact (40 eV, 200 °C ion source temperature, and 240 °C interface temperature). The MPs' chemical profiles were analyzed using a GC–MS (Agilent, Santa Clara, CA, USA). Mass-spectrum interpretation was measured using the National Institute of Standards and Technology (NIST) database, which had over 62,000 patterns. The spectra of unknown components were compared to those of known components contained in the NIST library. The names, molecular formulas, weights, and chemical structures of the substances were determined.

2.11.2. LC–MS Analysis

The chemical constituents of the *Monascus purpureus* TISTR 3090 pigment extract were analyzed using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6545 Q-TOF mass spectrometer (Agilent, CA, USA) equipped with a Dual Agilent Jet Stream electrospray ionization (Dual AJS-ESI) source operated in positive ion mode. Chromatographic separation was performed on a reversed-phase C18 column at 25 °C using 0.1% formic acid in water (A) and acetonitrile (B) as the mobile phases. The gradient was 5% B (0–5 min), increased linearly to 100% B (35–40 min), maintained at 100% B (40–45 min), and returned to 5% B for re-equilibration until 50 min. The flow rate and

injection volume were 0.20 mL min⁻¹ and 10 µL, respectively. Mass spectra were acquired using Auto MS/MS over m/z 100–1200 for full-scan MS and m/z 50–1200 for MS/MS. MS and MS/MS acquisition rates were 1.0 and 2.0 spectra s⁻¹, respectively. Data-dependent MS/MS was performed for up to three precursor ions per cycle using an approximately 4 amu isolation width and collision energies of 10, 20, and 40 eV. The source parameters were: capillary voltage, 4000 V; nozzle voltage, 2000 V; fragmentor voltage, 175 V; skimmer voltage, 65 V; octopole RF peak, 750 V; drying gas, 325 °C at 13 L min⁻¹; sheath gas, 275 °C at 12 L min⁻¹; and nebulizer pressure, 35 psig. Continuous mass calibration was performed using reference ions at m/z 121.0509 and 922.0098. Putative compound identification was based on accurate mass, isotope distribution, and MS/MS fragmentation patterns, with comparison against available spectral databases and published data on *Monascus azaphilone* pigments. In the absence of authentic reference standards, compound assignments were considered tentative.

2.11.3. FTIR-ATR Analysis

The functional groups and chemical characteristics of the *Monascus purpureus* TISTR 3090 pigment extract were characterized using a Thermo Scientific Nicolet Summit X FTIR spectrometer equipped (Thermo Scientific, Madison, WI, USA) with a DTGS KBr detector and an Everest attenuated total reflectance (ATR) accessory (Smart Accessory ID: B124649). The instrument was equipped with a KBr beamsplitter and an IR source. The pigment extract was directly placed onto the ATR accessory and analyzed over the wavenumber range of 4000–450 cm⁻¹. Spectral acquisition was performed using the instrument's standard optical configuration, with an aperture setting of 100 and 24-bit digitization. The resulting spectra were evaluated based on characteristic absorption bands to identify the major chemical bonds and functional groups present in the pigment extract [22].

2.12. Statistical Analysis

All data were collected in triplicate and shown as means ± standard deviation. The statistical analysis was performed using GraphPad Prism Version 5.01 (San Diego, CA, USA) to determine significant variations between extracts at $p < 0.05$.

3. Results and Discussion

3.1. Production of Fungal Red Pigment on Solid-State Fermentation

Figures 1 and 2 displayed the MP production by *M. purpureus* TISTR3090 under solid-state fermentation conditions using brown rice as a carbon source. The pigment product was measured by UV-spectrophotometer (680 nm) as an indicator of pigment concentration. The initial day recorded a minimal average value of the percentage of red pigment extract yield around 0.26 ± 0.03 , which progressively increased until day 30, reaching a maximum output value of 1.41 ± 0.09 ($p < 0.001$). The results demonstrate that the development and production of secondary metabolites in *M. purpureus* TISTR3090 correlate with fermentation duration, as pigment accumulation escalates over time, aligning with the fungi's advancement into the stationary phase typically linked to secondary metabolite synthesis. Contrary to comparable studies, research by Ren et al. [23] demonstrated that mannitol supplementation combined with cellulase pretreatment of a substrate can promote *M. purpureus* growth and improve MP biosynthesis with rice bran as the substrate. Optimization of *Monascus purpureus* culture conditions in rice bran for enhanced *Monascus* pigment biosynthesis was conducted with rice bran as the substrate. The concentration stabilizes or reduces prior to any pigment degradation or modifications. The pH of the system, aligned with the study's

results indicating maximum output on day 30, confirmed that the fermentation conditions were appropriate and markedly improved pigment production from *Monascus* fungi.

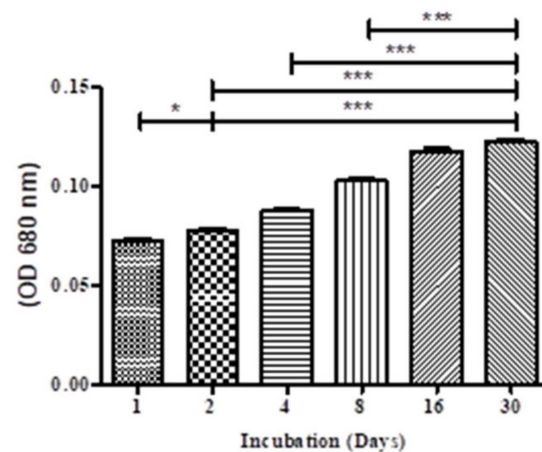


Figure 1. The red pigment production of *M. purpureus* TISTR3090 under solid-state fermentation. * $p < 0.05$, *** $p < 0.001$.

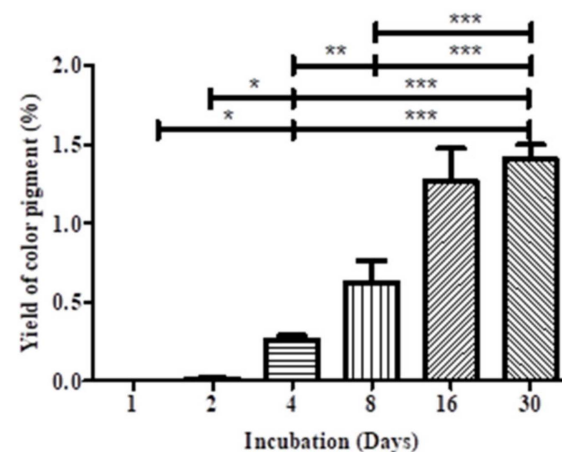


Figure 2. The percentage of red pigment produced from *M. purpureus* TISTR3090 under solid-state fermentation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. Antioxidant Activity and Total Phenolic Content

According to the results obtained, the rice extracts exhibited potent antioxidant activities. Among them, the WAE demonstrated the most potent antioxidant capacity and exhibited the highest TPC, which was calculated at 148.86 ± 0.40 mg GAE/g sample. The TPC of ETE was calculated at 71.68 ± 0.24 mg GAE/g sample. The statistical analysis revealed significant differences ($p < 0.001$) between the WAE and ETE. The DPPH radical scavenging assay—WAE exhibited the highest DPPH inhibition, which was calculated at $74.66 \pm 1.47\%$. The DPPH inhibition of ETE was calculated at $56.79 \pm 0.82\%$. The statistical analysis revealed significant differences ($p < 0.01$) between the WAE and ETE. The FRAP value—WAE exhibited the highest FRAP value, which was calculated at 0.36 ± 0.00 mM. The FRAP value of ETE was calculated at 0.17 ± 0.01 mM. The statistical analysis revealed significant differences ($p < 0.001$) between the WAE and ETE. The ABTS—WAE exhibited the highest ABTS, which was calculated at $65.53 \pm 1.03\%$. The ABTS of ETE was calculated at $21.61 \pm 1.05\%$. The statistical analysis revealed significant differences ($p < 0.001$) between the WAE and ETE (Table 1). The findings indicated that the aqueous extract of *Monascus* exhibited a superior DPPH scavenging action compared to the ethanolic extract, consistent with the observations of Abd Raza et al. [30] about the influence of polarity on the

compound. The red pigment extracted by *Rhizopus oligosporus* and *M. purpureus* utilizing solid-state fermentation exhibited a phenolic content and antioxidant activity, which was consistent with the results reported in Abd Razak et al. [30]. Moreover, the red pigments of *Monascus* species, notably monascorubramine and rubropunctamine, have acknowledged antioxidant effects, as well as other health-enhancing attributes [31].

Table 1. TPC and antioxidant activities of MPs extract.

Extract (10 mg/mL)	Total Phenolic Content (mg GAE/g)	DPPH (% Inhibition)	FRAP Value ($\mu\text{M Fe}^{2+}/\text{g Sample}$)	ABTS (% Scavenging Activity)
WAE	148.86 $\pm$ 0.40	74.66 $\pm$ 1.47	0.36 $\pm$ 0.00	65.53 $\pm$ 1.03
ETE	71.68 $\pm$ 0.24	56.79 $\pm$ 0.82	0.17 $\pm$ 0.01	21.61 $\pm$ 1.05
Vit. C	-	93.54 $\pm$ 2.16	1.14 $\pm$ 0.00	96.70 $\pm$ 0.01

3.3. Anti-Tyrosinase and Anti-Collagenase Activity

The anti-aging properties of MP extracts were assessed in vitro. The findings demonstrated a favorable efficacy of fungal extract at a dosage of 2.0 mg/mL against the enzymes tyrosinase and collagenase. The extracts of WAE and ETE showed inhibitory action against two enzymes (Table 2). The WAE demonstrated significant anti-tyrosinase and anti-collagenase activity, with inhibition percentages of 75.29 $\pm$ 0.09 and 32.88 $\pm$ 0.54, respectively. In contrast, ETE had a lower inhibition of tyrosinase and collagenase, measuring 56.57 $\pm$ 0.55 and 19.27 $\pm$ 0.54, respectively. Previous research has demonstrated the solid and liquid fermentation of chestnut inner shell utilizing *M. kaoliang*. The ethanol extracts from submerged fermentation exhibited superior levels of phenolic compounds, antioxidant activity, tyrosinase activity, and elastase activity compared to solid-state fermentation on day 3 [18]. It indicates that fungal species significantly contribute to the generation of bioactive chemicals, even within the same genus, together with the fermentation techniques and substrates employed in the fermentation process [12,24]. However, this study conducted a single concentration as a preliminary assessment of the potency of MP extract, necessitating further in-depth investigations with several concentrations for confirmation.

Table 2. Anti-aging potential of MP extract.

Extract	Anti-Aging Potential (% Inhibition)	
	Anti-Tyrosinase	Anti-Collagenase
WAE (2.0 mg/mL)	75.29 $\pm$ 0.09	32.88 $\pm$ 0.54
ETE (2.0 mg/mL)	56.57 $\pm$ 0.55	19.27 $\pm$ 0.54

3.4. HPLC Phenolic Compounds of MPs

The MPs demonstrated the presence of flavonoid and phenolic compounds in the extract when compared with phenolic compound standard (Figure 3). Among the flavonoids, Oroxylin A was detected at a concentration of 0.24 mg/kg. Regarding phenolic compounds, gallic acid (GA) was present at 0.38 mg/kg, while chlorogenic acid (CLA) was the predominant phenolic compound, with a concentration of 9.69 mg/kg. In addition, epigallocatechin gallate (EGCG) was quantified at 3.34 mg/kg. Our study was comparable to that by Husakova and Patakova [12], which revealed that antioxidants in substrate—such as phenolic and flavonoids in rice—enhance *monascus* pigments in fermented food. Duarte et al. [32] also found that brown seaweed fermented with *M. purpureus* contained phenolic substances.

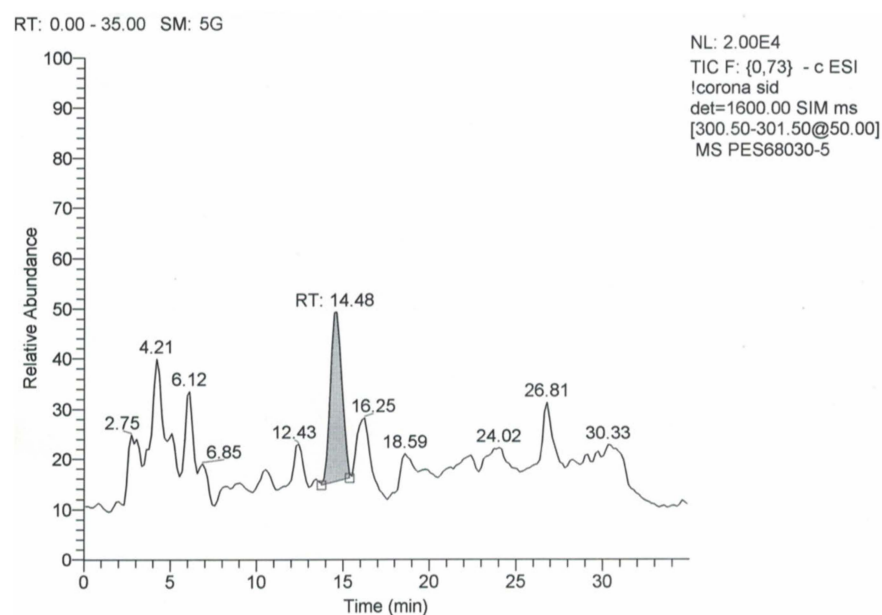


Figure 3. HPLC chromatogram of phenolic compounds of MPs.

3.5. Chemical Analysis of Ethanolic Extract of MPs

GC–MS Analysis of Red Pigment

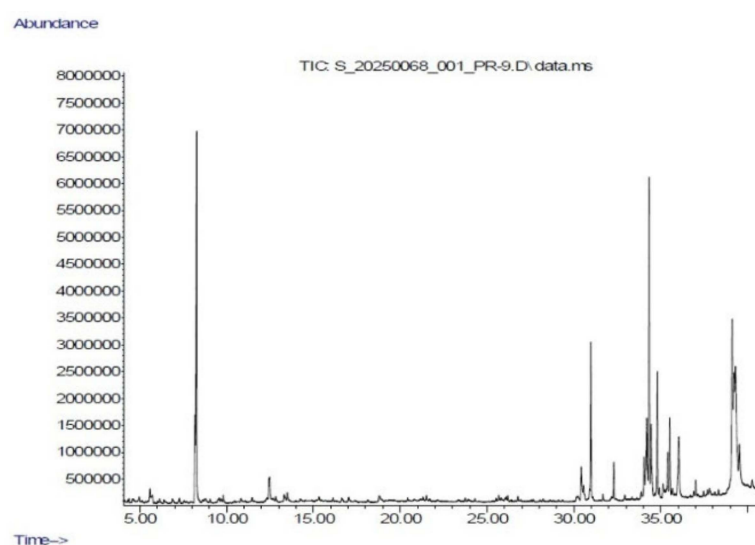
The chemical analysis of the MP extract was conducted utilizing the GC–MS technique. The outcome of the chromatogram is distinctly depicted in Figure 4 and Table 3. Twenty-five molecules were discovered in the MP extract. The main components included cis-13-octadecenoic acid, methyl ester (14.50%), methyl palmitate (8.55%), p-xylene (8.18%), 11-octadecenoic acid, methyl ester (7.56%), ethylbenzene (5.56%), methyl 9-cis,11-trans-octadecadienoate (4.05%), methyl stearate (3.87%), ethyl oleate (3.38%), 1,1'-biphenyl, 2-methyl- (3.44%), and benzene, 1-ethyl-2-methyl- (3.43%), respectively. Previous investigations have indicated that cis-13-octadecenoic acid methyl ester possesses antioxidant properties [25,33] and functions as an emollient in cosmetics [34]. Moreover, additional chemical compounds, such as methyl palmitate [35] and methyl 9-cis,11-trans-octadecadienoate, exhibited effectiveness in antioxidant activity. In addition, certain scientific data revealed that the ethanol extraction of *M. purpureus* BCRC 38110 produced the following eight chemical compounds: 5S,6S-monaspurpyridine A, 5R,6R-monaspurpyridine A, monasxanthones A and B, monasnaphthalenone, monapurpurin, and two identified compounds, exhibiting anti-melanogenic potential in mice [29]. Consequently, *M. purpureus* demonstrated potential for development as a cosmeceutical application.

Table 3. Chemical components from ethanolic extract detected with GC–MS of *Monascus* extract by NIST11.

No.	Components	RT	Area %	m/z
1	Ethylbenzene	4.942	5.56	51.1, 65.1, 77.1, 91.1 , 106.1, 124.2, 137.0, 206.9
2	p-Xylene	5.574	8.18	51.1, 58.0, 65.1, 77.1, 84.0, 91.1 , 99.2, 106.1, 128.1
3	Benzene, propyl-	6.918	2.25	51.0, 65.0, 78.1, 91.1 , 105.0, 113.1, 120.1, 136.2
4	Benzene,1-ethyl-2-methyl-	7.556	3.43	51.2, 57.9, 65.1, 71.1, 77.2, 85.1, 91.1 97.8, 105.1 , 120.1
5	Benzene,1-ethyl-3-methyl-	7.984	2.64	57.1, 65.0, 77.0, 91.1, 105.1 , 117.1, 127.9, 142.0
6	D-Limonene	8.805	0.76	55.1, 68.1 , 79.0, 93.1, 107.1, 121.1, 136.2
7	Benzene, 1-propynyl-	9.402	0.82	54.9, 62.9, 70.0, 77.2, 89.0, 97.0, 105.1, 115.1 , 133.9
8	Benzene, (1-methylpropyl)-	9.857	1.14	51.1, 65.0, 79.0, 91.1, 105.1, 115.0 , 127.1, 134.1

Table 3. Cont.

No.	Components	RT	Area %	m/z
9	Naphthalene	13.530	2.53	57.2, 71.1, 81.2, 91.0, 102.0 115.0, 128.1 , 148.1, 170.3
10	Benzene, hexyl-	15.336	0.12	51.0, 65.2, 77.1, 91.1 , 105.2, 115.1, 129.1, 144.2, 162.2
11	Naphthalene, 2-methyl-	17.013	2.25	55.0, 63.1, 74.0, 89.1, 98.0, 107.1, 115.0, 127.9, 142.1
12	Biphenyl	18.879	2.44	55.0, 64.9, 76.0, 91.0, 102.1, 114.9, 128.1, 141.0, 154.1
13	1,1'-Biphenyl, 2-methyl-	19.165	3.44	50.9, 63.1, 73.1, 83.0, 97.9, 115.1, 128.1, 141.0, 154.1, 168.1
14	Methyl palmitate	30.984	8.85	55.1, 74.1 , 97.1, 115.0, 143.1, 171.1, 199.1, 227.2, 270.3, 295.0
15	Ethyl palmitate	32.307	1.43	55.1, 88.0 , 115.1, 157.1, 199.2, 241.2, 284.2, 310.1 362.3
16	Methyl linoleate	34.188	1.43	55.1 , 81.1, 109.1, 136.1, 164.1, 182.0, 202.1, 220.1, 263.1, 294.2
17	Cis-13-Octadecenoic acid, methyl ester	34.317	14.50	55.1 , 83.1, 110.1, 137.1, 157.1, 180.2, 200.1, 222.2, 264.2, 296.3, 327.2
18	11-Octadecenoic acid, methyl ester	34.439	7.56	55.1 , 74.1, 96.1, 123.1, 152.1, 180.2, 203.0, 222.2, 241.2, 264.2, 296.2
19	Methyl stearate	34.806	3.87	55.1, 74.1 , 97.1, 115.1, 143.1, 171.1, 199.1, 227.0, 255.2, 298.3
20	9-Methyl-Z,Z-10,12-hexadecadien-1-ol acetate	35.145	1.48	55.1, 81.1, 105.1 , 133.1, 161.0, 183.1, 201.0, 220.1, 239.4, 258.1, 290.1
21	Ethyl 9-cis,11-trans-octadecadienoate	35.410	1.40	67.1 , 95.1, 135.0, 163.1, 185.1, 207.1, 238.9, 262.2, 284.4, 308.1
22	Ethyl oleate	35.525	3.38	55.1 , 83.1, 110.1, 137.1, 157.1, 180.2, 199.1, 222.2, 241.1, 265.2, 284.1, 310.2
23	Methyl 9-cis,11-tran-octadecadienoate	36.000	4.05	55.1 , 88.1, 109.0, 129.0, 157.1, 185.2, 213.2, 243.0, 269.1, 290.2, 312.2
24	Cyclooctadecane, ethyl-	36.998	1.18	55.1 , 83.1, 111.1, 151.0, 179.0, 207.0, 235.1, 263.2, 295.2, 329.2, 356.1
25	Oleic acid amide	39.157	1.58	59.1 , 95.1, 126.2, 154.1, 180.0, 207.1, 238.3, 264.2, 313.9, 354.9, 394.2

Figure 4. GC–MS chromatogram of *Monascus purpureus*-derived red pigment extract.

3.6. FTIR Determination of Red Pigment

FTIR spectra of MP extract from *Monascus purpureus* TISTR3090 was represented in Figure 5, indicating comparable chemical structures with variations in functional group intensities. The spectra exhibited broad absorption bands in the region of 3269–3296 cm^{-1} , corresponding to O–H stretching vibrations, suggesting the presence of hydroxyl groups [36–38], and hydrogen bonding. Peaks around 2932–2936 cm^{-1} were attributed to aliphatic C–H stretching, confirming the presence of alkyl side chains characteristic of polyketide compounds [36]. A prominent absorption band at approximately 1629 cm^{-1} was observed in both samples, corresponding to conjugated C=O or C=C stretching vibrations, which are essential components of the chromophore system responsible for pigment coloration [39]. Notably, this sample exhibited absorption bands in the region of 1530–1550 cm^{-1} , indicative of N–H bending or C–N stretching vibrations, suggesting

a higher concentration of nitrogen-containing compounds. The presence of a R-NH₂ stretching at 1558 cm⁻¹ indicates that the MP pigment was red pigment [40]. Typically, the red pigments generated by *Monascus purpureus* are monascorubramine (C₂₃H₂₇NO₄) and rubropunctamine (C₂₁H₂₃NO₄) [41]. Additional bands observed at 1227–1233 cm⁻¹ and 1038–1082 cm⁻¹ correspond to C–O stretching vibrations, confirming the presence of ester or lactone groups [42], which are typical structural features of *Monascus* pigments.

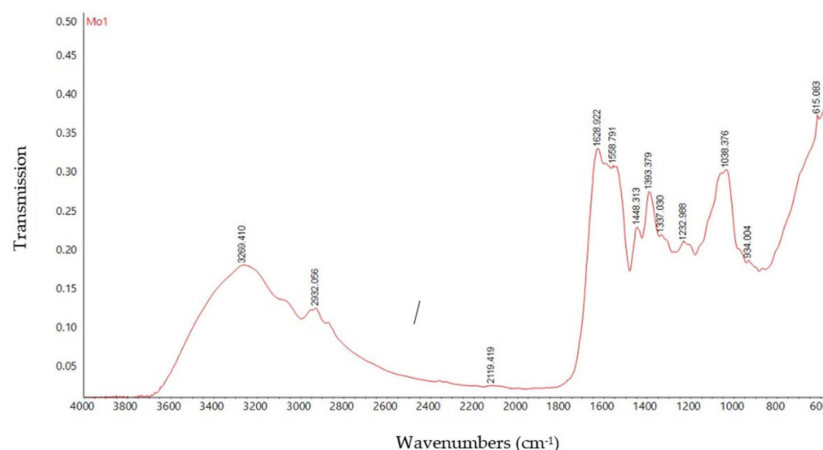


Figure 5. FTIR spectrum of MPs extracted by ethanol of *Monascus purpureus* TISTR3090. The spectrum shows characteristic absorption bands including O–H stretching (~3296 cm⁻¹), aliphatic C–H (~2932 cm⁻¹), conjugated C=O/C=C (~1629 cm⁻¹), and C–O stretching (~1233 and 1038 cm⁻¹). The presence of a band near 1537 cm⁻¹ suggests nitrogen-containing functional groups, indicating the presence of red pigment derivatives.

3.7. LC–MS Profiling of Compounds Detected in Negative and Positive ESI Mode

LC–MS analysis of the *Monascus purpureus* TISTR 3090 sample was performed using positive electrospray ionization (ESI) mode to characterize the detected metabolites and pigment-related compounds. Several molecular features were detected across the chromatographic profile and were tentatively assigned by comparison with the mass spectral library based on their retention times and observed *m/z* values.

In negative ESI mode (Figures 6 and 7), the detected 38 compounds and positive ESI mode (Figures 8 and 9), the detected 21 compounds (Table 4), the compounds detected at the later retention times, particularly those observed at RT 11.814, 17.175, 23.865, and 24.265 min, represent relatively higher-molecular-weight components of the sample. However, their library-based assignments should be considered tentative because library matching alone does not provide definitive structural confirmation. In particular, the compounds tentatively assigned as piperpenone, bisindolylmaleimide I, desoxycorticosterone acetate, and 3-hydroxyquinidine cannot be conclusively classified as *Monascus* pigments based solely on the available LC–MS data.

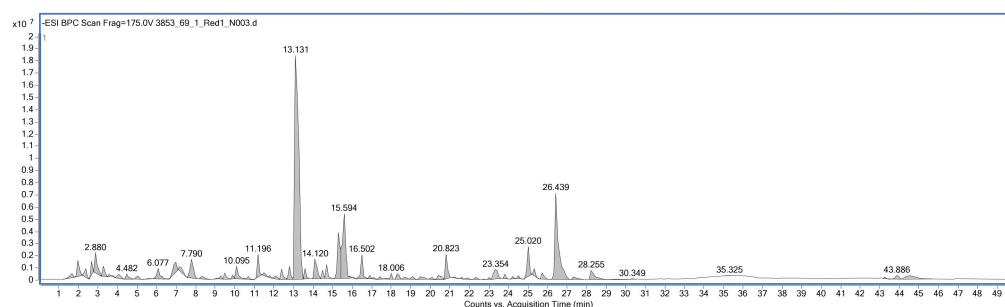


Figure 6. Negative electrospray ionization (ESI) BPC scan chromatogram: chromatogram created by plotting the base peak ion on current in a series of MS scan records as a function of retention time.

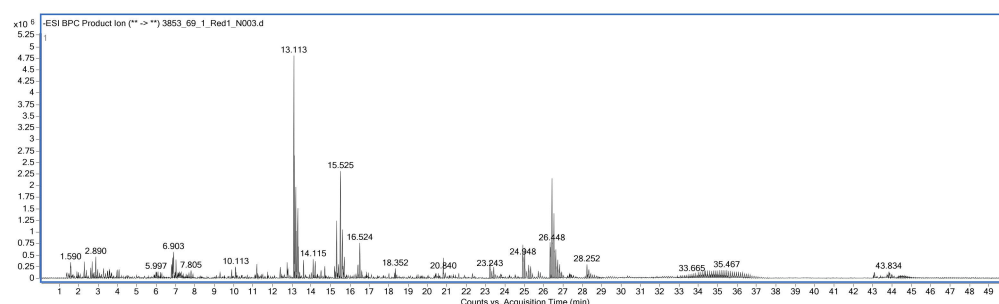


Figure 7. Negative electrospray ionization (ESI) BPC product ion chromatogram: chromatogram created by plotting the base peak ion in a series of product ions records as a function of retention time.

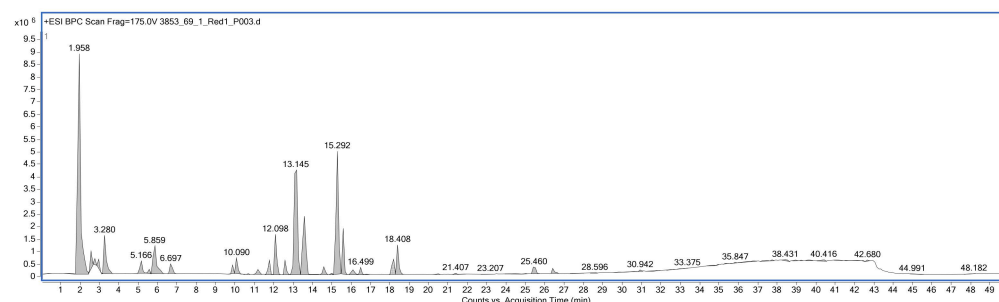


Figure 8. Positive electrospray ionization (ESI) BPC scan chromatogram: chromatogram created by plotting the base peak ion on current in a series of MS scan records as a function of retention time.

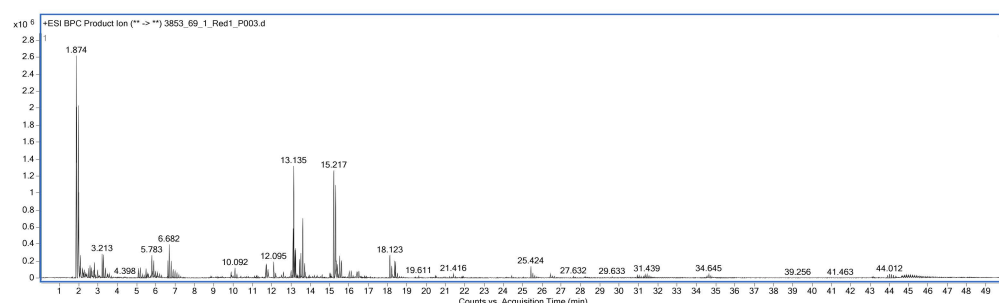


Figure 9. Positive electrospray ionization (ESI) BPC product ion chromatogram: chromatogram created by plotting the base peak ion in a series of product ions records as a function of retention time.

Additional production measurements of the positive ESI chromatographic profile showed prominent features at RT 13.191 min with an observed m/z of 431.2399 (neutral mass 430.2327) and RT 15.208 min with an observed m/z of 251.0918 (neutral mass 250.0846). These molecular features were not directly assigned to specific *Monascus* pigments from the available library-based identification and should therefore be considered additional pigment-associated or metabolite-associated features requiring further structural characterization.

Overall, positive ESI LC–MS analysis demonstrated a complex chemical profile in the *M. purpureus* TISTR 3090 sample, comprising amino acids, vitamins, nucleobases, organic acids, and higher-molecular-weight metabolites. The detected molecular features provide evidence of a chemically diverse metabolite profile; however, definitive identification of *Monascus* pigment compounds requires confirmation using MS/MS fragmentation patterns, accurate molecular formulas, authentic reference standards, and/or complementary spectroscopic techniques.

Table 4. Liquid chromatography–positive and negative electrospray ionization mass spectrometry of MPs.

Negative Polarity					Positive Polarity				
RT	<i>m/z</i>	Score (Lib)	Compound Identification	Formula	RT	<i>m/z</i>	Score (Lib)	Compound Identification	Formula
2.007	149.0092	92.12	Tartaric acid	C ₄ H ₆ O ₆	1.992	118.0867	96.05	Betaine	C ₅ H ₁₂ NO ₂
2.144	105.0191	98.13	Glyceric acid	C ₃ H ₆ O ₄	2.092	104.1072	95.30	Choline	C ₅ H ₁₄ NO
2.482	187.1086	90.40	Nε-Acetyl-L-lysine	C ₈ H ₁₆ N ₂ O ₃	2.518	124.0399	98.69	Niacin (nicotinic acid)	C ₆ H ₅ NO ₂
2.946	151.0260	89.02	Xanthine	C ₅ H ₄ N ₄ O ₂	2.618	113.0349	97.44	Uracil	C ₄ H ₄ N ₂ O ₂
3.447	147.0298	88.49	(S)-Citramalic acid	C ₅ H ₈ O ₅	2.694	152.071	84.98	N-Methylanthranilic acid	C ₈ H ₉ NO ₂
3.547	130.0870	53.73	L-Isoleucine	C ₆ H ₁₃ NO ₂	2.743	170.0815	92.21	Pyridoxine (vitamin B6)	C ₈ H ₁₁ NO ₃
4.124	121.0292	89.28	4-Hydroxybenzaldehyde	C ₇ H ₆ O ₂	2.856	153.041	96.63	Xanthine	C ₅ H ₄ N ₄ O ₂
6.053	164.0714	96.93	L-Phenylalanine	C ₉ H ₁₁ NO ₂	3.119	132.1022	89.74	L-Leucine	C ₆ H ₁₃ NO ₂
6.354	122.0244	99.74	Isonicotinic acid	C ₆ H ₅ NO ₂	3.244	146.0601	84.98	Oxyquinoline	C ₉ H ₇ NO
9.161	137.0244	97.98	p-Salicylic acid	C ₇ H ₆ O ₃	3.482	127.0502	97.77	Thymine	C ₅ H ₆ N ₂ O ₂
10.839	181.0504	86.21	Hydroxyphenyllactic acid	C ₉ H ₁₀ O ₄	3.608	140.0346	85.74	6-Hydroxynicotinic acid	C ₆ H ₅ NO ₃
11.115	470.1891	82.81	MK 886	C ₂₇ H ₃₄ ClNO ₂ S	6.050	166.086	98.57	L-Phenylalanine	C ₉ H ₁₁ NO ₂
11.265	144.0455	100	4-formyl Indole	C ₉ H ₇ NO	7.504	220.1183	89.17	Pantothenic acid	C ₉ H ₁₇ NO ₅
11.566	113.0607	99.87	3-Methyl-4-pentenoic acid	C ₆ H ₁₀ O ₂	8.130	132.102	90.19	L-Isoleucine	C ₆ H ₁₃ NO ₂
12.969	113.0606	95.78	3-Methyl-4-pentenoic acid	C ₆ H ₁₀ O ₂	10.861	146.06	88.16	Oxyquinoline	C ₉ H ₇ NO
13.245	130.0874	86.56	Aminocaproic acid	C ₆ H ₁₃ NO ₂	11.814	394.1865	89.78	Pirenperone	C ₂₃ H ₂₄ FN ₃ O ₂
13.445	130.0873	51.62	L-Isoleucine	C ₆ H ₁₃ NO ₂	12.941	169.0763	90.36	Norharman	C ₁₁ H ₈ N ₂
13.846	498.2927	32.34	Tauroursodeoxycholic acid	C ₂₆ H ₄₅ NO ₆ S	14.770	128.1434	80.17	Coniine	C ₈ H ₁₇ N
13.971	129.0554	100	3-Methyl-2-oxovaleric acid	C ₆ H ₁₀ O ₃	17.175	413.1939	87.39	Bisindolylmaleimide I	C ₂₅ H ₂₄ N ₄ O ₂
13.984	263.1401	93.65	Val Phe	C ₁₄ H ₂₀ N ₂ O ₃	23.865	373.2354	87.01	Desoxycorticosterone acetate	C ₂₃ H ₃₂ O ₄
14.134	107.0500	92.47	p-Cresol	C ₇ H ₈ O	24.265	341.1865	47.48	3-Hydroxyquinidine	C ₂₀ H ₂₄ N ₂ O ₃

Table 4. Cont.

Negative Polarity					Positive Polarity				
RT	<i>m/z</i>	Score (Lib)	Compound Identification	Formula	RT	<i>m/z</i>	Score (Lib)	Compound Identification	Formula
14.648	302.1509	89.52	Val Trp	C ₁₆ H ₂₁ N ₃ O ₃					
14.861	165.0558	91.49	D-(+)-3-Phenyllactic acid	C ₉ H ₁₀ O ₃					
14.861	147.0453	95.99	trans-Cinnamic acid	C ₉ H ₈ O ₂					
16.176	187.0974	94.40	Methyl N-(α -methylbutyryl)glycine	C ₉ H ₁₆ O ₄					
16.414	144.0454	87.12	4-formyl Indole	C ₉ H ₇ NO					
16.891	181.0507	82.68	3-Methylorsellinic acid	C ₉ H ₁₀ O ₄					
17.454	205.0505	100	Eugenitol	C ₁₁ H ₁₀ O ₄					
18.457	299.1652	88.30	2-Methoxyestrone	C ₁₉ H ₂₄ O ₃					
18.733	193.0869	97.46	Orthothymotinic acid	C ₁₁ H ₁₄ O ₃					
18.757	149.0971	90.47	Thymol	C ₁₀ H ₁₄ O					
20.699	207.0661	86.95	DL-Benzylsuccinic acid	C ₁₁ H ₁₂ O ₄					
23.293	187.1337	88.26	10-hydroxy capric acid	C ₁₀ H ₂₀ O ₃					
24.721	299.1649	84.64	4-Androsten-3,17-dione 19-aldehyde	C ₁₉ H ₂₄ O ₃					
27.189	297.1532	87.30	Iso-Olomoucine	C ₁₅ H ₁₈ N ₆ O					
28.380	277.1445	96.59	Phthalic acid Mono-2-ethylhexyl Ester	C ₁₆ H ₂₂ O ₄					
31.913	311.2013	61.71	Tibolone	C ₂₁ H ₂₈ O ₂					
35.821	339.1995	95.12	Norethindrone Acetate	C ₂₂ H ₂₈ O ₃					

Remarks: RT; retention time, *m/z*; mass to charge ratio, Lib; library.

4. Conclusions

This study reveals that *M. purpureus* TISTR3090 can take advantage of Khao Klong broken rice (brown rice) as a substrate, yielding maximal secondary metabolites at 30 days. MP extract possesses potential as a natural source of antioxidants and anti-aging properties. The aqueous extract (WAE) exhibited greater antioxidant, anti-tyrosinase, and anti-collagenase activities compared to the ethanol extract (ETE). The MP extract exhibited the presence of R–NH₂ functional groups, identified by FTIR, indicating the primary criterion for fungal red pigment. The GC–MS analysis found 25 bioactive molecules, primarily fatty acid methyl esters and aromatic substances. The WAE exhibited elevated levels of phenolic compounds (gallic acid, chlorogenic acid, and epigallocatechin gallate) and flavonoids (oroxylin A). Subsequent research will concentrate on elucidating the structure of the red pigment utilizing nuclear magnetic resonance (NMR) and examining the stability of the compounds. This study illustrates the enhanced utilization of agricultural by-products using microbial biotechnology and emphasizes their potential as future raw materials for cosmetics and health applications.

Author Contributions: S.T.: conceptualization; methodology; formal analysis; production of the original draft. T.D., W.N. and K.J.: antioxidant, antityrosinase, and anti-collagenase analysis. W.K.: statistics; data curation and review; editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

<i>M. purpureus</i>	<i>Monascus purpureus</i>
MPs	Monascus pigments
GC-MS	Gas chromatography–mass spectrometry
PDA	Potato dextrose agar
SSF	Solid-state fermentation
DPPH	1,1-diphenyl-2-picrylhydrazyl
TPTZ	2, 4, 6-tripyridyl-s-triazine
GA	Gallic acid
CLA	Chlorogenic acid
EGCG	Epigallocatechin gallate

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