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Bioactive Pigments of *Monascus purpureus*: Identification and Characterization

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Abstract: *Monascus purpureus* is a filamentous fungus that has long been recognized as one of the main producers of natural pigments. These pigments, including yellow (*ankaflavin, monascin*), orange (*rubropunctatin, monascorubrin*), and red (*rubropunctamine, monascorubramine*), are widely used as natural coloring agents. Compared to synthetic dyes, *Monascus* pigments are considered safer and possess additional biological activities, making them attractive for applications in the food, pharmaceutical, and cosmetic industries. Therefore, accurate identification of the pigments in *Monascus purpureus* is essential to ensure their safety, functionality, and potential industrial applications. The aim of this research is to determine the results of pigment identification *Monascus purpureus* using UV-Vis Spectrophotometry, Fourier Transform Infra-Red (FTIR), Thin Layer Chromatography (TLC), Preparative Thin Layer Chromatography (Preparative TLC), and Liquid Chromatography- Mass Spectrometry (LC-MS). The method used is method laboratory experiments. Samples are tested using several instruments. The extraction results show that the % yield value meets the requirements, namely >10%, then the fractionation results show that the water fraction gets more thick extract, the TLC results for the water fraction show a spot height of 4.7 cm with a value of Rf 0.78, the results of Preparative TLC on the water fraction show the presence of a single fluorescent band at wavelengths of 254 nm and 366 nm. The UV-Vis spectrophotometry results were identified at a wavelength of 477 nm with an absorbance of 0.364, then the FTIR results on Preparative TLC scrapings from the water fraction showed the presence of functional groups from the orange pigment structure and the LC-MS results showed that the sample was a compound. *Monascorubrin* (C23H28O5) with a molecular weight of 384.99 g/mol and compound *rubropunctatin* (C21H22O5) with a molecular weight of 354.74 g/mol. These results provide information that the sample is an orange pigment from *Monascus purpureus* with two compounds *monascorubrin* and *rubropunctatin*.

Keywords: *Monascus purpureus*; pigment; fractionation; identification

1. INTRODUCTION

Color is an important attribute that greatly influences consumer preference and product acceptance. Dyes are commonly added to improve visual appeal, maintain existing color, and prevent fading caused by temperature, light, and humidity [1]. As the demand for visually appealing products grows across industries, colorants have become indispensable additives in modern manufacturing.

In industries such as textiles, cosmetics, food, and household products, dyes not only enhance appearance but also increase commercial value. For the past decades, synthetic dyes have dominated the market, while natural dyes account for only about 1% of global consumption. However, the widespread use of synthetic dyes has raised increasing safety and environmental concerns, leading to growing interest in natural and eco-friendly coloring agents derived from biological sources [2].

Among these natural sources, microbial pigments have emerged as a particularly promising alternative due to their stable production and chemical diversity. In particular, *Monascus purpureus* has long been used in Asia as a natural colorant. It produces polyketide pigments—yellow, orange, and red—that are widely applied due to their attractive shades, solubility, and stability [3]. Beyond

coloring properties, *Monascus* pigments exhibit beneficial physiological effects, including antioxidant and cholesterol-lowering activities, making them not only safer but also functional bio-colorants for food, pharmaceutical, and cosmetic industries [4].

Despite their growing utilization, studies on *Monascus* pigments remain limited, particularly in comprehensive identification and structural characterization. Most previous research has emphasized pigment production or application, whereas systematic identification using multiple complementary analytical techniques is still scarce. Accurate identification is crucial to differentiate among yellow (ankaflavin, monascin), orange (monascorubrin, rubropunctatin), and red (monascorubramine, rubropunctamine) pigments and to ensure their safe industrial application [5]. To address this gap, the present study integrates chromatographic and spectroscopic methods to provide a more complete characterization of *Monascus purpureus* pigments.

In this study, we addressed this gap by applying a combination of chromatographic and spectroscopic methods. Thin Layer Chromatography (TLC) and Preparative TLC were used to separate the pigments, UV-Vis spectrophotometry and FTIR to determine their spectral and structural characteristics, and LC-MS to confirm molecular composition. The use of these complementary methods provides a more reliable and updated characterization of *Monascus purpureus* pigments, offering new insights into their chemical profiles and supporting their potential applications as natural alternatives to synthetic dyes.

2. MATERIALS AND METHODS

2.1. Tools and Materials

2.1.1. Tools

In this study, the equipment used included a Refrigerator (Samsung, Korea®), micropipette (IKA, Germany®), dry sterilizer (Ztp80a-7, Memmert GmbH, Germany®), rotary evaporator (IKA RV 10, IKA-Werke GmbH, Staufen, Germany®), water bath (Memmert, Germany®), measuring cup (Pyrex, USA®), glass jar, stir bar (Pyrex, USA®), baking pan, spatula, filter paper, analytical balance (Ohaus Pioneer, Ohaus Corp., USA®), TLC chamber, Silica gel 60 F254 TLC plates (Merck, Darmstadt, Germany®), tweezers, test tube (Iwaki Pyrex, Japan®), steam cup, capillary tube, ruler, pencil, Uv-Vis spectrophotometer (Genesys 10s UV-Vis, Thermo Fisher Scientific, USA®), Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu IRPrestige-21, Shimadzu Corp., Kyoto, Japan®) and LC-MS system (Mariner Biospectrometry, PerSeptive Biosystems, Framingham, MA, USA®).

2.1.2. Materials

In this study, the materials used included Red yeast rice Powder (ITB HDCC®, Indonesia), ethanol 96% (Emsure®), ethyl acetate, n-hexane, chloroform, methanol (Lichrosolv®), 70% ethanol (Emsure®), ethanol pro analysis (Emsure®), distilled water,water formic acid 0.1%, and CH_3CN formic acid 0.1%.

2.2. Making Extracts Monascus purpureus

2.2.1. Extraction

Powder *Monascus purpureus* got from ITB HDCC. Next, extract by cold method, namely the maceration process, using 96% ethanol solvent. This method is carried out for 3 x 24 hours in a closed condition protected from light, every 1 x 24 hours. Hour the sample in the jar is stirred then Filtering is done to separate the dregs with liquid [6;7]. After that the filtrate was evaporated using rotary evaporatorat a temperature of 50° C until a thick extract is obtained. After that, the extract was further concentrated using a water bath until a very thick extract was obtained [8].

2.2.2. Fractination (Liquid-Liquid Extraction)

Carry out fractionation with solvents that have different levels of polarity, namely polar (water), semi-polar (ethyl acetate) and non- polar (n- hexane) solvents. Weigh 10 g of the thick extract

that has been obtained, then dissolve it in a little water and add up to 100 mL of water. After that, pour the extract solution into a separating funnel, which then adds n- Hexane solvent in the same amount as water (ratio 1:1). The solution mixture was shaken evenly and left until phase separation occurred. After two different layers are formed, the layers are separated carefully. Then, put the water solution back into the separating funnel and add the same amount of ethyl acetate solvent (ratio 1:1) as before. This process is repeated to obtain the desired fractions. After the three fractions are obtained, proceed with evaporating the three fractions using waterbath and calculate the yield value [9].

2.3. Identification Pigment

2.3.1. Identification using Thin Layer Chromatography (TLC)

TLC testing was carried out using Silica gel 60 F254 TLC plates functions as a stationary phase and a mobile phase consisting of chloroform: methanol: water in the ratio (8:2:1). After that, the TLC plate was marked with a line with a distance of 1 cm (lower limit) and 0.5 cm (upper limit). The sample was spotted using a capillary tube at a distance of 1 cm from the bottom edge of the plate, then eluted with eluent, and the eluted plate was observed under ultraviolet light. Wavelengths of 254 nm and 366 nm [10]. After observing under ultraviolet light, Rf values were calculated.

2.3.2. Identification using Preparative Thin Layer Chromatography (Preparative TLC)

The test was carried out using the Preparative Thin Layer Chromatography (Preparative TLC) technique, using Silica gel 60 F254 TLC plates as a stationary phase and a mobile phase consisting of chloroform: methanol: water in the ratio (8:2:1). The extracts and fractions obtained were spotted horizontally on a Preparative TLC plate. Then, the sample was eluted and then visually viewed and detected under ultraviolet light with wavelengths of 254 nm and 366 nm.

2.3.3. Identification using UV-Vis spectrophotometry

Measurement of absorption from Preparative TLC scrapings at a wavelength of 300-800 nm. The results refer to the literature, namely the results of yellow pigment λ 330 nm – 450 nm, orange λ 460 nm – 480 nm and red λ 490 nm – 530 nm [11].

2.3.4. IdentificationFourier transform Infra-Red (FTIR)

Infrared is measured at wave numbers ranging from 4000-450 cm-1, then analyzed further [12].

2.3.5. Identification use Liquid Chromatography-Mass Spectrometry (LC- MS)

Preparative TLC scraping results *Monascus purpureus* identified by use Liquid Chromatography-Mass Spectrometry (LC-MS). An adequate sample was taken, then dissolved using H_2O, then filtered using a 0.22 μm syringe filter, and inject 5 μL into the LC-MS system.

3. RESULTS AND DISCUSSION

3.1. Material Preparation

Powder *Monascus purpureus*is the sample used in this research. The powder was obtained from the Institute of Technology Bandung (ITB), Powder *Monascus purpureus* This is produced from rice mixed with Red yeast rice which has been cultured on a slanted agar medium and placed under direct sunlight for 2-3 days until it dries and grows mycelium *Monascus* [13]. Meanwhile, other materials were obtained from the Bakti Tunas Husada Tasikmalaya University Laboratory.

3.2. Extraction Results

A total of 40 grams of powder was extracted using 400 mL of 96% ethanol. Ethanol was selected as the extraction solvent due to its universal polarity and wide availability. In addition, 96% ethanol offers several advantages, including high selectivity, non-toxic properties, optimal absorption

capacity, and the ability to extract polar, semi-polar, and non-polar compounds. Moreover, 96% ethanol is known to penetrate effectively into plant cell walls, thereby facilitating the formation of a thicker extract enriched with the desired bioactive compounds [14].

The resulting filtrate was evaporated using rotary evaporatorat a temperature of 50°C. The evaporation process is carried out using rotary so that separation occurs between the active substance obtained and the solvent, namely 96% ethanol. The concentration process is carried out using waterbath with a temperature of 50°C so that a thick extract is produced. After the thick extract is obtained, the % yield value is calculated. The calculation results of the yield values can be seen in Table 1.

Table 1. Extract yield *Monascus purpureus*

Heavy Powder (grams)	Reedmen extract obtained (grams)	Weighten (%)	Condition (%)
40	22.0768	55.19	>10

Based on Table 1, the results of calculating the % yield in the extract *Monascus purpureus* declared good, because the yield value obtained was 55.19% and met the requirements for a good yield, namely >10%.

3.3. Fractionation Results

In research, the fractionation process was carried out using a liquid-liquid extraction technique using solvents with different levels of polarity, including n-hexane, ethyl acetate and water. The results show that compounds that have different polarity properties can be separated effectively, thus enabling the isolation of compounds that have the desired potential biological activity. The results of the % yield value from fractionation can be seen in Table 2.

Table 2. Fractionation Yield of *Monascus purpureus*

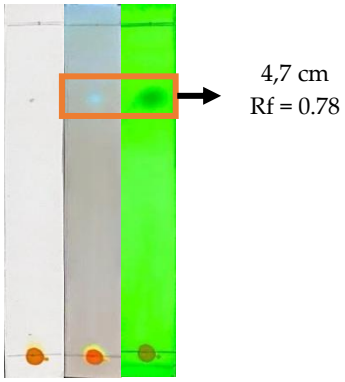
Water fraction	Red yeast rice thick extract (g)	10
	Yield value (%)	78.25
Ethyl acetate fraction	Water fraction (mL)	100
	Yield value (%)	0.18
n-Hexane fraction	Water fraction (mL)	100
	Yield value (%)	5.25

Based on Table 2, the ethanol extract fractionation process *Monascus purpureus* produces different yield values, this is due to differences in the ability of each solvent to attract compounds based on their polarity. The use of water as a solvent produces the most viscous extract compared to other fractions, because the extract *Monascus purpureus* has orange pigment contains many secondary metabolites that are polar. This research is in line with research conducted by Yuliana (2018) stating that the compounds contained in *Monascus purpureus* is polar. Therefore, because the results of the water fraction show more viscous extract results, further identification used in several instruments is water fractionation.

3.4. Pigment Identification Results

3.4.1. Identification results use TLC Thin Layer Chromatography

Identification by Thin Layer Chromatography (TLC) is a physicochemical separation technique that uses separation media such as the granules placed on a suitable substrate, which can be glass, metal, or another suitable substrate (stationary phase). This stationary phase is applied to a closed container containing the solution (mobile phase). Thin layer chromatography is a relatively simple, fast and commonly used method for identifying active compound components [15]. The results of the identification process using thin layer chromatography can be seen in Figure 1.



(a) (b) (c)

Figure 1. TLC profiles of *Monascus purpureus* fractions (n-hexane, ethyl acetate, water) on silica gel 60 F254 plates (Merck) developed with chloroform:methanol:water (8:2:1, v/v/v). Visualization: (a) visible, (b) UV 366 nm, (c) UV 254 nm. The water fraction shows a major band (migration 4.7 cm; Rf = 0.78), whereas n-hexane and ethyl acetate fractions show faint/no bands under these conditions. A ruler (cm) is included

In this test, GF_{254} silica gel plates used as the stationary phase, and the mobile phase consists of chloroform, methanol, and water in the ratio (95:25:4) [7]. After going through several tests, the best optimization results were obtained using a mobile phase consisting of chloroform: methanol: water with a ratio of (8:2:1) for the water fraction. The TLC test results in Figure 2 show that spots were detected in UV λ 254 nm and UV λ 366 at a spot distance of 4.7 cm with a value of Rf 0.78.

Based on the TLC testing literature on *Monascus purpureus* having an Rf value that meets the requirements, there is a single spot in the range of 0.2 – 0.8 [8]. Apart from that, the results of this research are also in line with research conducted by Puspita et al., (2020) which states that the results of pigment TLC analysis *Monascus purpureus* red pigment is obtained (*rubropunctamine* and *monascorubrin*) with a spot height of 3.1 cm, pigment orange (*rubropunctatin* and *monascorubrin*) with a spot height of 4.8 cm, and yellow pigment fraction (co-migrated; not resolved into monascin/ankaflavin under these TLC conditions) with a spot height of 3.5 cm. Therefore, the results of the TLC test carried out on the water fraction showed the presence of orange pigment, namely a compound *monascorubrin* and *rubropunctatin*.

3.4.2. Identification results using Preparative Thin Layer Chromatography (Preparative TLC)

Based on the analytical TLC optimization (Section 3.4.1), prep-TLC was performed on silica gel 60 F254 plates using chloroform: methanol: water (8:2:1, v/v/v) as the mobile phase. The water fraction was applied as a 1-cm band; the chamber was pre-saturated and the plate was developed to a migration distance of ~7 cm. After development, a single, intense fluorescent band was observed under UV 254 and 366 nm at Rf = 0.78 (Figure 2). The band was marked, scraped, and eluted with methanol for subsequent UV–Vis and FTIR analyses; LC–MS was then used to confirm molecular composition.

The single band does not imply a single compound: the two major orange azaphilone pigments (*monascorubrin* and *rubropunctatin*) have very similar polarity and typically co-migrate on silica under these conditions, so baseline separation was not achieved. Prep-TLC was chosen over open-column/flash chromatography because it is rapid, solvent-efficient, provides direct visual localization of the target zone on F254 plates, and is well-suited to milligram-scale isolation required for orthogonal spectroscopic/MS identification. The broader band seen in the prep plate reflects the higher loading required for collection (the analytical TLC image in Figure 1 shows the corresponding narrow band at the same Rf).

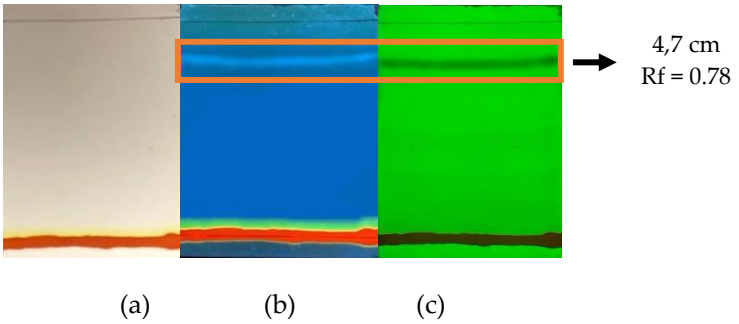


Figure 2. Preparative TLC profile of the water fraction of *Monascus purpureus* extract on silica gel 60 F254 (Merck). Mobile phase: $CHCl_3$:MeOH:H_2O (8:2:1). Visualization: (a) visible, (b) UV 366 nm, (c) UV 254 nm. A single fluorescent band (migration 4.7 cm; Rf = 0.78) was collected for UV–Vis and FTIR; LC–MS confirmed *monascorubrin* and *rubropunctatin*. The broader band reflects the higher loading required for preparative collection. Origin, solvent front, and a ruler (cm) are indicated.

Preparative TLC of the water fraction was performed on silica gel 60 F254 using chloroform:methanol:water (8:2:1). After development, a single, intense fluorescent band was observed under UV 254 and 366 nm at Rf = 0.78 (Figure 2). This does not imply a single compound: the two major orange azaphilone pigments, *monascorubrin* and *rubropunctatin*, have very similar polarity and typically co-migrate on silica under these conditions, so baseline separation was not achieved. We selected preparative TLC (rather than open-column/flash chromatography) because it is rapid, solvent-efficient, enables direct visual localization of the target zone on F254 plates, and is well-suited to milligram-scale isolation required for orthogonal identification. The band was marked, scraped, and eluted with methanol for UV–Vis and FTIR; the molecular composition was then confirmed by LC–MS as *monascorubrin* and *rubropunctatin*. Many organic compounds are colorless under visible light, hence UV visualization on F254 plates was used to detect and localize the pigment zone.

3.4.3. Identification result use UV-Vis spectrophotometry

Test analysis at maximum waves was carried out using UV-Vis Spectrophotometry with a wavelength range of 300-800nm, but for maximum wavelengths of *Monascus purpureus*is between 400-500nm, this is in accordance with research of [3]. Objectives of the process identification use UV-Vis spectrophotometry to determine the color intensity of the compounds contained in the sample. The spectrum results from UV-Vis spectrophotometric measurements from Preparative TLC scrapings can be seen in Figure 3.

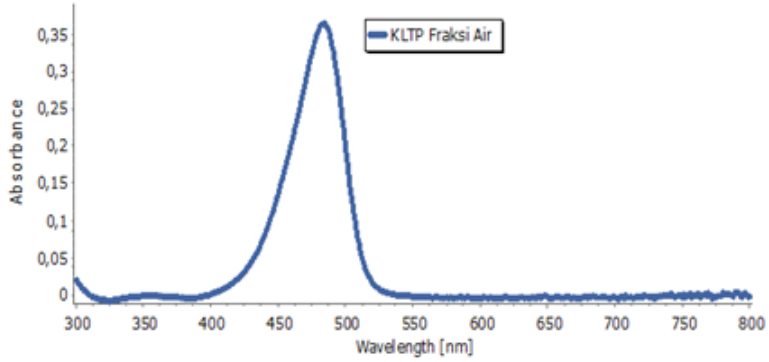


Figure 3. UV–Vis spectrum of the prep-TLC band (water fraction) of *Monascus purpureus* (λmax = 477 nm)

The Preparative TLC results were identified using UV-Vis spectrophotometry because the sample had a chromophore group. Based on Figure 1, Preparative TLC scrapings have a wavelength

of 477 nm and an absorption peak of 0.364. The results of this research are in accordance with those obtained by [11] who identified pigments *Monascus purpureus* using UV-Vis spectrophotometry which states that the yellow pigment in *Monascus purpureus* has a λ of 330-450 nm which is the identified compound *monascin* and *ankaflavin*. Orange pigment has a λ of 460-480 nm which is identified as a compound *monascorubrin* and *rubropunctatin*, and the red pigment has a λ of 490-530 nm which identified as compound *monascorubramine* and *rubropunctamine*.

Apart from that, this research is in line with research [17] who conducted the analysis with UV-Vis spectrophotometry. Their results showed that the yellow pigment was detected at 400 nm, the orange pigment at 470 nm, and the red pigment at 500 nm. Based on the wavelength obtained from the water fraction and its comparison with previous studies, the pigment identified in this research falls within the orange pigment range, corresponding to *monascorubrin* and *rubropunctatin*.

3.4.4. Identification results usingFourier Transform Infra-Red (FTIR)

The functional groups of *Monascus purpureus* pigments were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. Absorption bands at specific wavenumbers (cm^{-1}) indicate the presence of characteristic functional groups. The spectrum obtained from the material scraped from the preparative TLC band was recorded over the range of 4000–450 cm^{-1} and processed using OriginPro 2018 software (OriginLab). The resulting FTIR spectrum is presented in Figure 4.

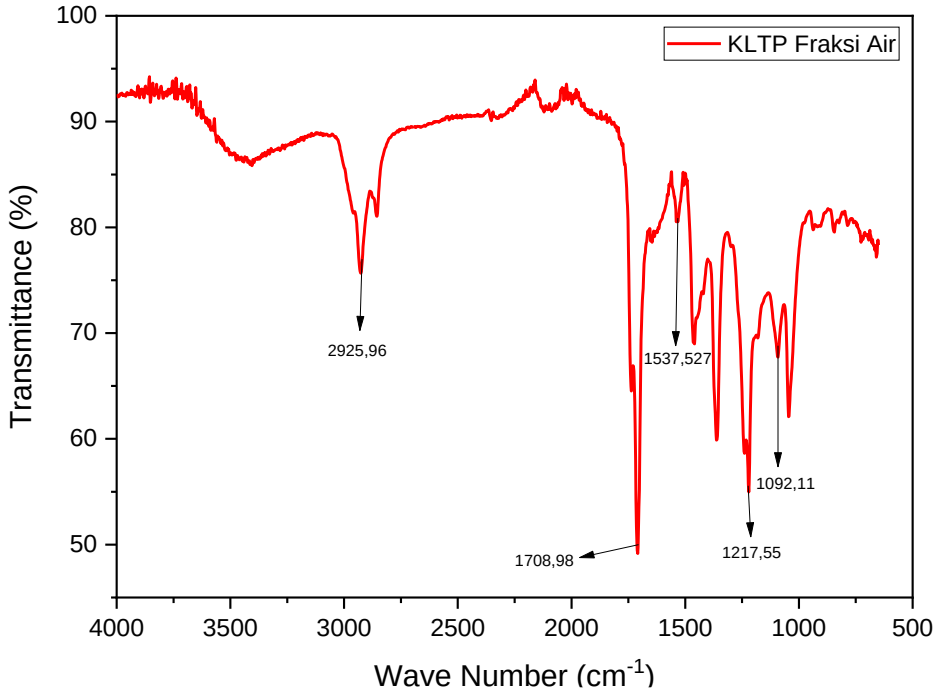


Figure 4. FTIR spectrum of the prep-TLC scraped band (water fraction).

Tabel 3. Results of FTIR identification data

Wavenumber (cm^{-1})	Functional Group
3339.695 (broad)	O-H *stretching*
1755.576	regang C=O
1634.438	regang C=C
1470.435	C-H *bending*
1101.428	C-O strong

Based on Table 3, In the FTIR spectrum a broad band at ~3339 cm^{-1} is observed, which we assign to O–H stretching (H-bonded) typical of azaphilone pigments; however, minor contribution from adsorbed moisture cannot be excluded. By contrast, aliphatic C–H stretching appears as sharp bands near 2920 and 2850 cm^{-1}; these (were observed as weak features / are not prominent) in our spectrum, supporting the assignment of the 3339 cm^{-1} band to O–H rather than C–H.

This research is in line with research [18] and Mullaiselvan et al., (2020) which uses FTIR to detect compounds *monascorubrin* and *rubropunctatin*. The results show a C=O stretch which indicates the presence of pyron in the coumarin ring. the C=C strain of the benzene ring is related to this phenomenon. There is also CH bending indicating the presence of 1,4-quinone from anthraquinone. And CH stretching is said to be present in some alkaloids.

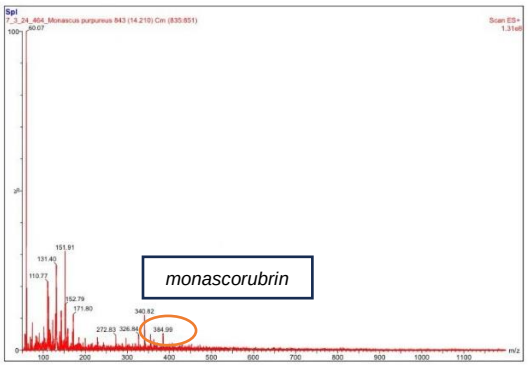
3.4.5. Identification results using Liquid Chromatography-Mass Spectrometry (LC- MS)

Guided by the TLC and UV–Vis results indicating the orange pigment class, a targeted high-resolution LC–MS analysis was performed. The selection of candidate compounds was based on previously reported dominant azaphilone pigments from *Monascus purpureus*, namely monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$), which are known to exhibit λmax values within the 460–480 nm range and characteristic orange coloration. These compounds were therefore chosen as primary targets for molecular confirmation. Extracted ion chromatograms (EICs) were generated using their theoretical $[M+H]^+$ exact masses (±5 ppm), producing two distinct peaks with accurate-mass and isotopic distributions consistent with the predicted formulas.

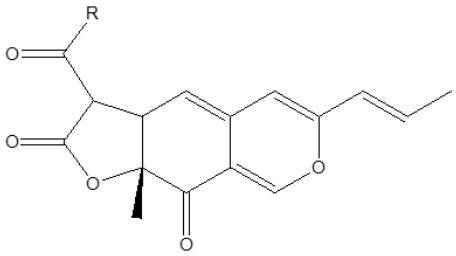
The obtained MS/MS fragmentation spectra were compared with those available in literature and spectral libraries (e.g., MassBank and published LC–MS data of Monascus pigments) to verify diagnostic fragment ions corresponding to neutral losses of H_2O (−18 u), CO (−28 u), and side-chain cleavage typical of azaphilone structures. Although authentic reference standards were not analyzed in parallel, the strong agreement between experimental and literature fragmentation patterns supports the identification at Schymanski Level 2 (probable structure based on diagnostic MS/MS).

These results confirm that the water fraction predominantly contains orange azaphilone pigments—monascorubrin and rubropunctatin—consistent with the spectral evidence obtained from TLC (Rf = 0.78) and UV–Vis (λmax = 477 nm) [20].

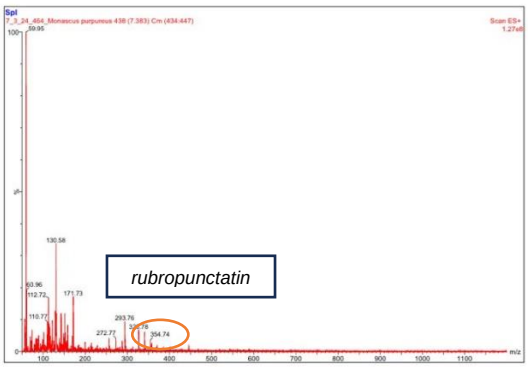
LC–MS analysis (water fraction). LC–MS measurements were performed at LPPT Universitas Gadjah Mada (Yogyakarta, Indonesia). The analyte was the water fraction of the *Monascus purpureus* extract: an aliquot was diluted with water, filtered through a 0.22 μm syringe filter (Millex), and 5 μL was injected. Chromatographic separation used a BEH C18 column (1.7 μm, 2.1 × 50 mm) under gradient elution with mobile phase A = water + 0.1% formic acid and mobile phase B = acetonitrile (ACN) + 0.1% formic acid. The mass spectrometer operated in ESI positive mode with capillary voltage 0.5 kV, cone voltage 21 V, scan range m/z 50–1200, and source/desolvation temperature 500 °C. Guided by the TLC/UV–Vis results (orange pigment class), we generated extracted-ion chromatograms (EICs) at the theoretical $[M+H]^+$ exact masses of *monascorubrin* and *rubropunctatin* (±5 ppm) and acquired data-dependent MS/MS. Accurate mass, isotopic pattern, and diagnostic fragments (e.g., neutral losses of H_2O and CO) were matched against library/literature spectra to support the assignments. Figure 5 shows the LC–MS chromatograms/EICs and corresponding MS/MS spectra.



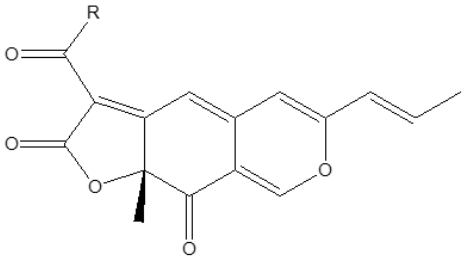
(a)



(c)



(b)



(d)

Figure 5. LC-MS chromatogram *Monascus purpureus:* (a) *Monascorubrin;* (b) *Rubropunctatin;*(c) Structure *monascorubrin*and; (d) structure *rubropunctatin.*

Based on the results of LC-MS analysis of pigments *Monascus purpureus* in Figure 5 the main pigments identified by the detector are obtained Mass-Spectrometry (MS). The main pigment obtained is orange pigment with compounds *rubropunctatin* and *monascorubrin*, because there is a molecular weight that corresponds to the chemical formula contained in the orange pigment compound, namely compound *monascorubrin* and *rubropunctatin*. Compound *monascorubrin* has the chemical formula C23H26O5with a molecular weight of 382 g/mol, but there is a slight difference with the molecular weight obtained in Figure 5 part (a), which produces a molecular weight of 384 g/mol, this occurs due to the addition of protons, because in Electrospray Ionization (ESI) molecules of a molecule can capture protons from solution which causes an increase in its molecular weight. Whereas *rubropunctatin* has the chemical formula C21H22O5with a molecular weight of 354 g/mol, the same as the molecular weight obtained in Figure 5 part (b), which produces a molecular weight of 354 g/mol.

This research is in line with research of [21] which shows that the LC-MS test results provide molecular weight results for compounds *monascin* of 358.46 g/mol, molecular weight *ankaflavin*is 385.48 g/mol, molecular weight *monascorubrin* is 382.48 g/ mol, molecular weight *rubropunctatin* is 354.42 g/mol, and the molecular weight monascorubramine is 381.4 g/mol.

In the water fraction, we targeted the two dominant orange azaphilone pigments reported for *Monascus—monascorubrin* and *rubropunctatin*. The TLC profile on silica gel 60 F254 ($CHCl_3$:MeOH:H_2O 8:2:1) showed a single band at Rf = 0.78, indicating the orange pigment zone (co-migration expected for these isomers). The UV–Vis spectrum of the scraped band displayed λmax = 477 nm, which lies in the orange region (460–480 nm). The FTIR spectrum (Table 3) showed a strong C=O (lactone/ester) at (1755.6 cm^{-1}), conjugated C=C at (1634.4 cm^{-1}), C–O 1260–1030 cm^{-1}, and a

broad O–H (3339.6 cm^{-1}) a pattern consistent with an azaphilone (pyranone) chromophore rather than coumarin/anthraquinone.

Guided by these results, LC–MS (ESI^+) was interrogated using EIC windows at the theoretical $[M+H]^+$ exact masses of *monascorubrin* and *rubropunctatin* (±5 ppm). Two retention-separated peaks were observed with accurate masses and isotopic fits within tolerance; MS/MS produced diagnostic fragments (neutral losses H_2O [−18 u], CO [−28 u], and side-chain cleavage) that match library/literature spectra. Together, the concordant evidence from TLC (Rf = 0.78) → UV–Vis (λmax 477 nm) → FTIR (lactone/C=C/C–O) → LC–MS (exact mass + MS/MS) supports the presence of monascorubrin and rubropunctatin in the water fraction. Yellow pigments (*monascin/ankaflavin*) were not detected under our EIC targets and conditions.

4. CONCLUSION

Ethanol extraction of *Monascus purpureus* powder yielded >10%. During fractionation, the water fraction produced the highest amount of concentrated extract and was therefore selected for analysis. Analytical TLC (silica gel 60 F254; $CHCl_3$:MeOH:H_2O 8:2:1) showed a major band at Rf = 0.78; preparative TLC enabled its isolation. The UV–Vis spectrum of the scraped band exhibited λmax = 477 nm; FTIR showed a strong C=O (lactone/ester) ~1755 cm^{-1}, C=C ~1634 cm^{-1}, C–O 1260–1030 cm^{-1}, and a broad O–H ~3339 cm^{-1}, consistent with an azaphilone (pyranone) chromophore. High-resolution LC–MS (ESI^+) revealed two components whose exact masses/isotopic patterns and diagnostic MS/MS fragments (−18 u, −28 u) match monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$). Taken together, the multi-technique evidence demonstrates that the water fraction is dominated by orange azaphilone pigments *monascorubrin* and *rubropunctatin*—supporting their potential as natural colorants.

Limitations and outlook. *Monascorubrin* and *rubropunctatin* were not baseline-separated on TLC (co-migration); quantitative analysis was not performed. Future work should apply HPLC with authentic standards for quantitation/purity, assess stability and safety, and optimize production/extraction to increase orange pigment yield.

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