Biopigments from Monascus sp. as Potential Peroxisome Proliferator-Activated Receptor Alpha (PPAR-*α*) Modulators: A Computational Approach

Dyahati Wahyurini[1], Evrick Yonantiko[2]*, Immanuelle Kezia[3]

[1]Faculty of Medicine and Health, Institut Teknologi Sepuluh Nopember, Surabaya, East Java, Indonesia

[2]Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia

[3]Kariadi Hospital, Semarang, Central Java, Indonesia

Corresponding Author

Evrick Yonantiko

Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia

Rungkut Madya Street, Gunung Anyar, Gunung Anyar District, Surabaya, East Java 60294

Email: evrick_yonantiko.fk@upnjatim.ac.id

ABSTRACT

Monascus sp. produces biopigments (ankaflavin, monascin, and rubropunctatin) that have various biological activities, including a role in lipid metabolism through interaction with peroxisome proliferator-activated receptor alpha (PPAR-α). It plays an important role in regulating lipid metabolism and is a therapeutic target. Fenofibrate is one of the antihyperlipidemic drugs that targets PPAR-α. Therefore, this study aimed to evaluate the interaction of ankaflavin, monascin, and rubropunctatin with PPAR-α. The biopigments were analyzed using SwissADME and molecular docking with PPAR-α (PDB ID: 5HYK). The results showed that ankaflavin, monascin, and rubropunctatin complied with Lipinski's Rule. Molecular docking revealed that all biopigments analyzed had good interactions with PPARα, with binding affinity values of ankaflavin -10.03 kcal/mol, monascin -10.38 kcal/mol, and rubropunctatin -10.62 kcal/mol. Rubropunctatin had the most negative binding affinity and the lowest predicted Ki (16.45 μM) among the tested biopigments and was most similar to the positive control, fenofibrate (−10.78 kcal/mol and 12.59 μM). In conclusion, these findings suggest that biopigments from *Monascus* sp. are potential candidates for PPAR-α modulators, particularly rubropunctatin. Further in vivo and in vitro investigations are necessary to validate these findings.

Keyword: Biopigments; lipid metabolism; monascus; ppar-α; rubropunctatin

Introduction

Hyperlipidemia is a metabolic condition in which lipid levels in the blood are elevated, especially cholesterol and triglycerides.[1] This condition is a major health concern because it is closely linked to an increased risk of atherosclerosis and cardiovascular disease and can contribute to various metabolic disorders. Therefore, regulating lipid metabolism and maintaining lipid homeostasis are crucial aspects of efforts to reduce the risk of metabolic and cardiovascular complications.[2,3]

Lipid homeostasis in the body is regulated through various metabolic pathways involving several transcription factors, including PPAR-α. PPAR-α is a nuclear receptor that acts as a transcription factor and is activated by ligands such as fatty acids and their derivatives.[4] PPAR-α activation plays a key role in regulating lipid metabolism, particularly by increasing fatty acid oxidation and regulating lipid homeostasis.[5] Activation of this receptor can increase the expression of various genes involved in the *β*-oxidation of fatty acids, particularly in the liver and other metabolic tissues.[6] Increased activity of this pathway can support the utilization of fatty acids as an energy source and help reduce lipid accumulation.[7] Additionally, PPAR-α plays a role in regulating lipoprotein metabolism and is known to contribute to a reduction in plasma triglyceride levels. Therefore, PPAR-α is a relevant molecular target in the development of strategies to manage lipid metabolism disorders.

In recent years, natural products have been increasingly explored as potential sources of therapeutic agents due to their diverse chemical structures and broad biological activities. One potential source is *Monascus* sp., a group of filamentous fungi that has long been utilized in food fermentation processes and various biotechnology applications. *Monascus* sp. produces a variety of bioactive compounds, including monascin, ankaflavin, and rubropunctatin.[8,9] Numerous studies have reported that *Monascus* pigments possess various biological activities, including antioxidant and anti-inflammatory activities, as well as the potential to modulate lipid metabolism.[10-12] Several compounds produced by Monascus have also been reported to influence cholesterol metabolism and lipid parameters.[13,14] However, information regarding the molecular interaction mechanisms between bioactive Monascus pigments and PPAR-α remains relatively limited. This situation highlights the need for further exploration of potential interactions between these compounds and PPAR-α, a key regulator of lipid metabolism.

Advances in computational technology have enabled the use of molecular modeling approaches to explore interactions between bioactive compounds and target proteins more efficiently.[15] One such approach is molecular docking, which allows prediction of ligand orientation and interactions at protein binding sites, as well as estimation of binding affinity. The results of this analysis can provide an initial insight into the potential interactions between a compound and its molecular target, as well as the characteristics of the interactions formed at the binding site. However, molecular docking results are predictive in nature and do not directly prove a compound's biological activity.

Therefore, this approach can serve as an initial step to screen and prioritize bioactive compounds that have the potential to interact with PPAR-α before validation through experimental research.

The objectives of this study were to evaluate the potential of biopigments produced by *Monascus* sp., specifically monascin, ankaflavin, and rubropunctatin, as candidates for antihyperlipidemic compounds through analysis of their interactions with PPAR-α using a molecular docking approach. In addition, drug-likeness characteristics based on Lipinski's Five Rules and were analyzed to obtain an initial overview of the pharmacological feasibility and safety of these compounds as candidates for antihyperlipidemic agents.

Material and Methods

This research is an in silico study with several stages. Bioactive compounds from *Monascus* sp. were obtained from phytochemical studies in previous research. Of the several active compounds in *Monascus* sp. with biological potential, three were selected for further analysis: ankaflavin, monascin, and rubropunctatin. Ankaflavin and monascin are known to possess antioxidant and anti-inflammatory activity, while rubropunctatin, in addition to its antioxidant role, also has antimicrobial potential.

PASS Online Prediction, Druglikeness and ADME Analysis

These three compounds were then analyzed using Prediction of Activity Spectra for Substances (PASS) Online to predict their biological activity. PASS Online can be accessed through http://www.pharmaexpert.ru/passonline. The next stage is the analysis of the three bioactive compounds using the SwissADME platform (http://www.swissadme.ch) to determine their pharmacokinetic and drug-likeness properties. SMILES (Table 1) of the three compounds were entered into the system to obtain physicochemical results, ADME (absorption, distribution, metabolism, excretion), and drug-likeness characteristics of each compound. These results were then analyzed and evaluated to assess compliance with Lipinski's Rule of Five, consisting of molecular weight, lipophilicity (LogP), number of hydrogen bond donors, number of hydrogen bond acceptors, and the number of violations of these rules. In addition, the bioavailability values generated by SwissADME were also recorded and analyzed.

Table 1. SMILES of *Monascus* pigments

Bioactive Pigments	**SMILES**
Ankaflavin	CCCCCCCC(=O)C1C2CC3=C(COC(=C3)/C=C/C)C(=O)C2(OC1=O)C
Monascin	CCCCCC(=O)[C@@H]1[C@H]2CC3=C(COC(=C3)/C=C/C)C(=O)[C@@]2(OC1=O)C
Rubropunctatin	CCCCCC(=O)C1=C2C=C3C=C(OC=C3C(=O)[C@@]2(OC1=O)C)/C=C/C

Molecular Docking

The final stage is molecular docking analysis to evaluate the interaction between bioactive compounds and target proteins, namely Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α). The target protein structure and identification information were retrieved from the RCSB Protein Data Bank, and PPAR-α has PDB ID: 5HYK. The protein structure is then cleaned by removing water molecules, bonds between the protein and its native ligand, and non-essential components that could affect the protein structure. Before molecular docking, hydrogen atoms are added, and the structure is optimized.

The three-dimensional structures of ankaflavin, monascin, and rubropunctatin, which were obtained from the database, were prepared. In addition, the three-dimensional structure of fenofibrate (one of the drugs that plays a role in antihyperlipidemia) was analyzed. Molecular docking was performed using AutoDock Vina version 1.5.7 to evaluate the binding affinity between bioactive compounds and the PPAR-α protein. Molecular docking analysis was determined from the location of the protein's active site and was performed in a grid box with center coordinates at: 8.748 Å; 33.867 Å; 19.279 Å (X; Y; Z). The coordinate range (min-max) was 1.248 Å -16.248 Å; 26.367 Å - 41.367 Å; 11.779 Å - 26.779 Å (X; Y; Z). The docking results were expressed as binding affinity (kcal/mol). A more negative binding energy value indicates a stronger binding affinity between the ligand and the target protein.

Results

Drug-Likeness and ADME Analysis

Analysis using the SwissADME platform provided information on the 3D structure, SMILES, and bioavailability of ankaflavin, monascin, and rubropuncatatin (Table 2). The bioavailability radar plot is in the pink area, which indicates that the physicochemical range is potentially similar to that of a drug. All biopigments are in the middle of the pink area, with a position almost similar to the positive control compound (antihyperlipidemic drug). The ADME analysis results are shown in Table 3. Each compound was also analyzed to fulfill Lipinski's rule criteria: namely a molecular weight of less than 500 Da, hydrogen bond acceptors < 10, hydrogen bond donors < 5, lipophilicity (Log P) < 5, and molar refractivity between 40-130.[16] The results confirmed that biopigments complied with Lipinski's Rule of Five (Table 3).

Table 2. Basic molecular characteristics

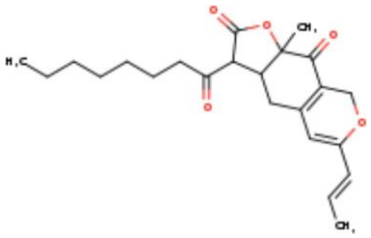
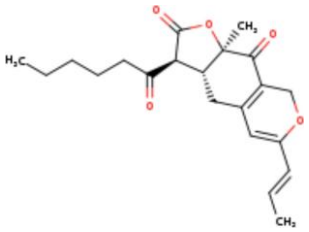
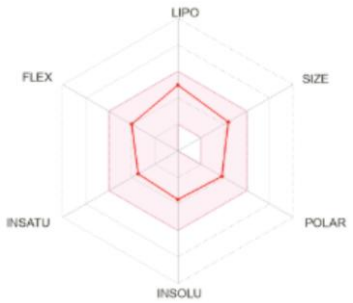
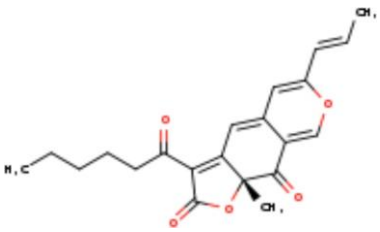
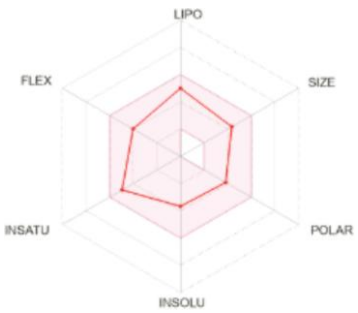
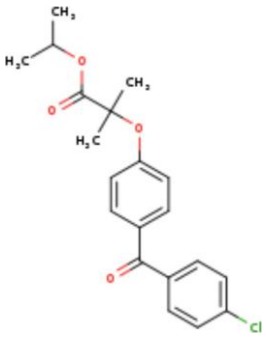
Bioactive Pigments	Structures	Bioavailability Radar
Ankaflavin		
Monascin		
Rubropunctatin		
Positive Control (Fenofibrate)		

Table 3. Physicochemical properties and drug-likeness of bioactive pigments

Bioactive Pigments	Molecular Weight (g/mol)	H-Bond Acceptors	H-Bond Donors	Rotatable Bonds	TPSA* (A^2)	LogP	Log S (ESOL)	BS**	Lipinski
Ankaflavin	386.48	5	0	8	69.67	4.22	-4.40	0.55	Yes
Monascin	358.43	5	0	6	69.67	3.44	-3.68	0.55	Yes
Rubropunctatin	354.4	5	0	6	69.67	3.63	-3.68	0.85	Yes
Positive Control (Fenofibrate)	360.83	4	0	7	52.6	4.68	-5.23	0.55	Yes

*TPSA: Topological Polar Surface Area. **BS: Bioavailability score.

Molecular Docking

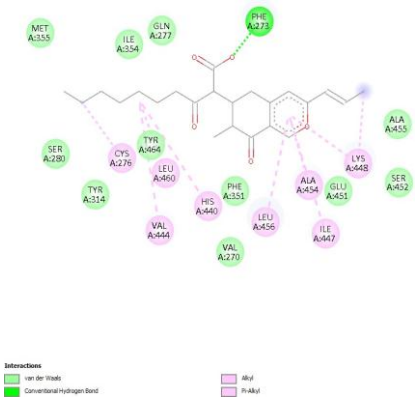
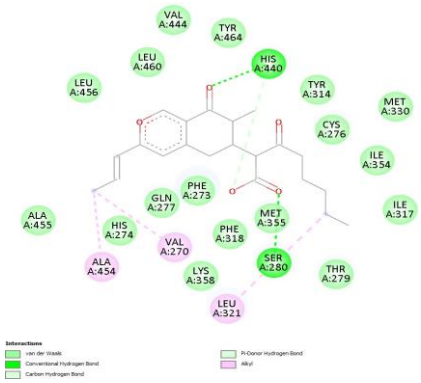
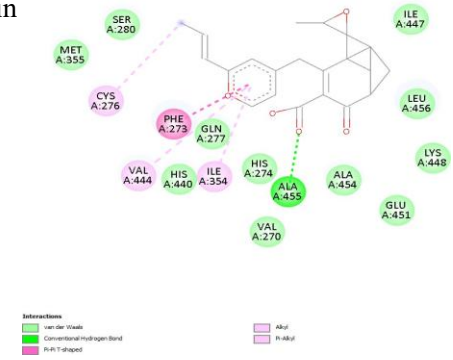
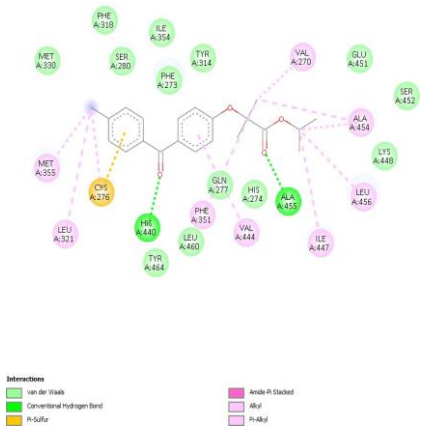
This analysis was conducted to evaluate the interaction between bioactive compounds from *Monascus* biopigments and the PPAR-α protein based on binding affinity, inhibition constant, and hydrogen bonds (Table 4). Table 4 displays a docking visualization illustrating the interaction between ankaflavin, monascin, and rubropunctatin with PPAR-α, as well as the interaction between fenofibrate and PPAR-α for comparison. The results show that each compound has similar hydrogen-bond and binding-affinity values. The binding affinity of rubropunctatin (-10.62) is the most negative among ankaflavin (-10.03) and monascin (-10.38). The more negative the binding affinity, the stronger the interaction is predicted to be. The binding affinity of rubropunctatin is also the most similar to that of the positive control, fenofibrate (-10.78). Similarly, rubropunctatin has the lowest inhibition constant (Ki) value (Ki=16.45) among other bioactives and is closest to fenofibrate (Ki=12.59). Each compound exhibits hydrogen bonds, although none are identical to fenofibrate. Based on these analysis results, rubropunctatin is the compound with the greatest potential to interact by modulating PPAR-α and has potential as an antihyperlipidemic.

Discussion

Previous research has shown that *Monascus* sp. has biological activity and can act as an antioxidant and anti-inflammatory agent, and also plays a role in lipid metabolism and other metabolite effects.[10] The analysis shows that the biopigment compounds monascin and ankaflavin have the potential to interact with PPAR-α, which plays a key role in regulating lipid metabolism by increasing fatty acid oxidation and *β*-oxidation. These findings are consistent with the role of the PPAR-α/AMPK pathway in maintaining energy and lipid homeostasis. AMPK activation can promote fatty acid oxidation and inhibit lipogenesis. At the same time, PPAR-α increases the expression of genes involved in *β*-oxidation.[6] Thus, the potential interaction of monascin and ankaflavin with PPAR-α supports the hypothesis that these two compounds may contribute to improved lipid metabolism.

Analysis of the interaction between ankaflavin and PPAR-α suggests that ankaflavin has the potential to modulate PPAR-α, which is a key regulator of lipid metabolism. These findings are in line with reported research showing that ankaflavin can increase PPAR-α activity and support fatty acid oxidation.[17] Activation of the PPAR-α pathway can increase the expression of genes involved in *β*-oxidation, thereby helping to reduce lipid accumulation and maintain lipid homeostasis. Thus, the interaction of ankaflavin with PPAR-α supports its potential as a candidate bioactive compound for regulating lipid metabolism. However, this potential for PPAR-α activation still requires experimental validation.

Table 4. Interaction affinity of the tested bioactive compounds with PPAR-α

Bioactive Pigments	Docking Visualization	Energy (Kcal/Mol)	Inhibition Constanta (μM)	Hydrogen Bond
Ankaflavin		-10.03	44.33	PHE A: 273
Monascin		-10.38	24.74	HIS A: 440 SER A: 280
Rubropunctatin		-10.62	16.45	ALA A: 455
Positif Control (Fenofibrate)		-10.78	12.59	HIS A: 440 ALA A: 455

The results of the analysis of monascin and ankaflavin also suggest that these compounds may influence lipid profiles, including LDL-C, HDL, LDL assembly, and apoA1. These findings indicate that both compounds not only have the potential to affect triglyceride metabolism but may also play a role in regulating lipoprotein metabolism. The effects on LDL-C and the LDL assembly process may support the control of atherogenic lipoprotein levels. In contrast, the association with apoA1 may support the maintenance of HDL and cholesterol transport function. Thus, the activity of monascin and ankaflavin on various components of lipoprotein metabolism demonstrates their potential as bioactive compound candidates for modulating the lipid profile.

Ankaflavin, monascin, and rubropunctatin are the primary biopigments produced by *Monascus* sp. and exhibit a variety of biological activities.[10] These biological activities highlight the potential of Monascus biopigments as a valuable source of bioactive compounds with broad biological effects, particularly anti-inflammatory and antioxidant activities, as well as modulation of lipid metabolism. These findings strengthen the rationale for selecting these three compounds in this study as bioactive candidates with the potential to play a role in managing lipid metabolism disorders. However, the differences in the biological activities and mechanisms of each pigment highlight the need for further evaluation of specific molecular targets to determine their therapeutic potential. Fenofibrate was used as a reference ligand because it is a PPAR-α agonist known to regulate lipid metabolism. Activation of PPAR-α by fenofibrate can increase fatty acid oxidation, help lower triglyceride levels, and improve the lipoprotein profile. Therefore, using fenofibrate as a comparator in molecular docking analysis provides a relevant basis for evaluating the potential interaction of Monascus pigment compounds with PPAR-α. Similarities or close matches in interaction patterns and affinity values with fenofibrate can serve as an initial indication of these compounds' potential as PPAR-α modulators.

Molecular docking results also showed that rubropunctatin and fenofibrate both have hydrogen bonds with the ALA455 residue in PPAR-α. A study by Kamata *et al*. (2020) showed that interaction patterns in the ligand-binding domain contribute to the potency and selectivity of ligands, including PPAR-α ligands, and their role in lipid metabolism.[18] The number of rubropunctatin studies remains limited, with fewer studies reported than for ankaflavin and monascin.[10] Therefore, this research can be a pilot study and a basic foundation for continuing research on rubropunctatin, which can be a candidate for antihyperlipidemic effects through interaction with PPAR-α. However, it should be noted that this research is the result of a molecular docking analysis and still has many limitations, especially confounding factors that were not included in the analysis. It is hoped that this research can be continued with in vivo testing to confirm the biological potential of Monascus pigments, especially rubropunctatin, which is still limited, and continued with further studies. In vitro studies are needed to evaluate the effect on target gene expression of PPAR-α.

Conclusion

In conclusion, rubropunctatin, monascin, and ankaflavin meet Lipinski's Rule of Five criteria, demonstrating a potential bioavailability. The three compounds analyzed can bind stably to the active site. PPAR-α has the potential to play a role in regulating lipid metabolism as an antihyperlipidemic. Rubropunctatin shows the strongest binding affinity and has the highest binding energy value, which is close to the positive control, namely fenofibrate. However, this study is still limited to in silico analysis and prediction; therefore, further experimental studies (in vivo and in vitro investigations) are needed to confirm the potential of *Monasucs* sp. biopigment as a PPAR-α modulator that regulates lipid metabolism and has potential as an antihyperlipidemic.

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