ANTIBACTERIAL CANDIDATE OF *Monascus* PIGMENTS

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ABSTRACT

Microbial resistance problems have become a serious threat in the medical science. Governments around the world have made serious efforts to prevent and develop effective treatments against inflectional diseases which increasing. Microbes as bio active material could become an alternative for an antimicrobial such as Monascus. Monascus pigments as natural food colorants have been widely utilized in food industries in the world, especially in China and Japan. Moreover, Monascus pigments possess a range of biological activities, such as anti mutagen and anticancer properties, antimicrobial, and potential anti obesity. The potential function of Monascus pigments as antimicrobial compounds using computational methods which are docking and molecular dynamics was carry out by software MarvinSketch, Molegro Molecul Viewer (MMV), ArgustLab, Ligplot, Gromacs . The result indicates that from six main and fifty seven derivatives pigments from Monascus, Monaspyridine C met all the Drug Scan, ADME, and Toxicity test requirements. Data result show MW is 357.4434 g/mol, proton donor 1, proton acceptor 7, lipophilia 3,90 and MR 99.88 which all comply with Lipinski's Rule of Five rules. For the ADME test and toxicity test obtained Caco-2 values of 22.7729 nm/sec, HIA 96.648798%, PPB 89.685299 and for toxicity test showed negative results both for mutagenic properties or carcinogenic. Monaspyridine C also has activity in the 1PW1 receptor with peptidoglycan formation inhibition mechanism. Based on Docking result, Monaspyridine C has binding affinity energy -9,16515 kkal/mol, interacts with amino acid fit with the active site of 1PW1 receptor with Hydrogen bound of Asn 161 and hydrophobic contact of Tyr 159, Trp 233, Gln 303 and Phe 120. Molecular dynamic shows Monaspyridine C stable in 300 K, 310 K, and 312 K, although a longer simulation time still needed for 310 K and 312 K temperature, to achieve system stability so that can exhibit the interaction between ligand bond test receptor. Overall, Monaspyridine C is The most potential compound for antimicrobial candidate.

Keywords : *Monascus* pigments, antimicrobial, *docking*, molecular dynamics

INTRODUCTION

The Indonesian Ministry of Health (2016) states that the death rate due to antimicrobial resistance until 2014 amounted to 700,000 per year. With the rapid development and spread of bacterial infections, it is estimated that by 2050, deaths from antimicrobial resistance are greater than those caused by cancer, which reaches 10 million people.

An increasing number of microbial resistance cases have triggered a search for new sources of antimicrobial compounds. In order to obtain new antimicrobials, researchers have carried out various methods such as biotransformation of certain compounds with microbial assistance or making semisynthetic antimicrobial derivatives, mutations of antimicrobial producing strains or searching for new antimicrobial compounds from microbes present in nature.

Microbial diversity in Indonesia and its potential as a producer of bioactive ingredients have not been widely disclosed. One of the widely spread microorganisms in nature is mold or fungi. Monascus is one of the homotalic molds which belongs to the Ascomycetes group. Monascus is used traditionally as a food coloring, preservative, food supplement and traditional medicines. During fermentation, Monascus sp produces at least 6 pigments which are categorized into 3 colors, namely yellow, orange and red (Blanc et al., 1994). Yuliana et al. (2017) stated that currently there are 57 compounds of dyes that were successfully isolated from Monascus fungi. The orange pigment from Monascus has antibacterial activity against Escherichia coli with the mechanism of interaction

between the orange pigment and phospholipid so that there is interference and permeation of bacterial membranes (Zhao et al, 2015). The main active substances found in Monascus sp, monascidin are antibacterial (Wong and Bau, 1977) so Monascus sp can treat various diseases including infections (Permana et al., 2004).

The labarotorium test of one Monascus sp pigment has been shown to have antimicrobial activity and has been successfully carried out by color pigment isolation from Monascus sp so that further research is needed on other pigments from isolation from Monascus sp which can be a search solution for new drug candidate compounds that have the effect of antimicrobial.

Then,this study was designed to determine the potential antimicrobial candidate in silico potential as by identifying the target protein of the dyestuff compound from Monascus sp mold based on a series of physicochemical characterization tests, namely drugscan test, ADME test and toxicity test along with energy calculations and visualization of receptor ligand interactions through docking processes and molecular dynamics simulations.

RESEARCH METHODOLOGY

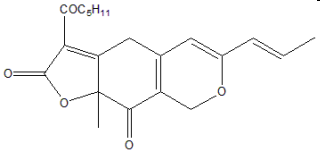
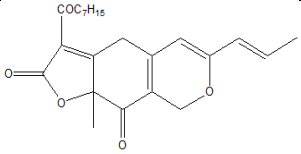
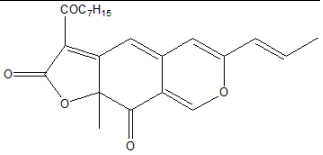
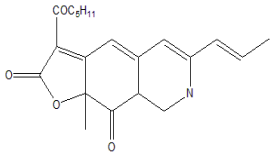
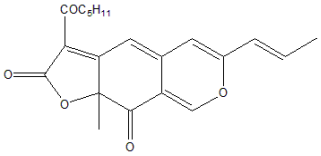
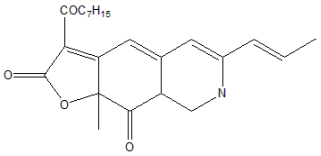
Tool

The equipment used is in the form of computer hardware and software. The device is a personal computer with the specifications of the Intel (R) Core (TM) i3 with a CPU specification of 2348M @ 2.30GHz, 2.3GHz 6.00GB (5.84GB usable) RAM, 64-bit Operating System and software that used are, MarvinSketch, ArgusLab, Molegro Molecul Viewer, LigPlot, GROMACS and web-based programs such as Pharmmapper, PdbSum, PreADMET and PRODRG.

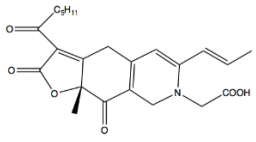
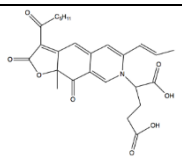
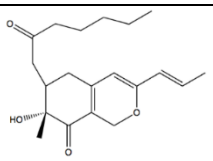
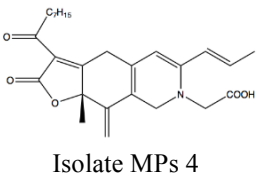
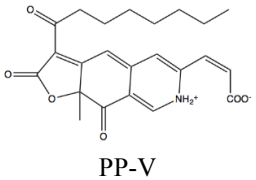
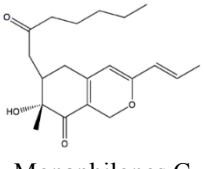
Material

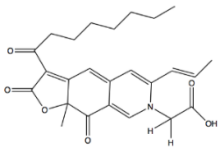
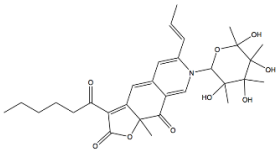
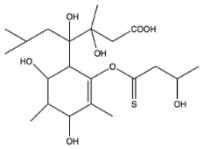
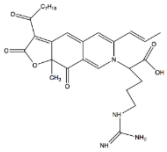
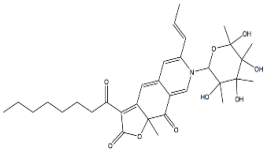
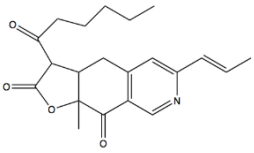
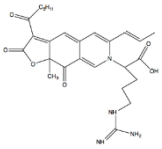
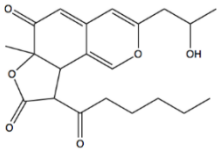
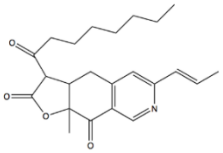
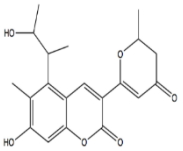
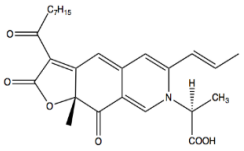
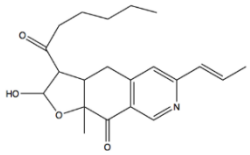
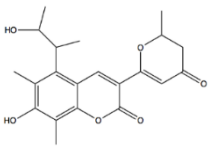
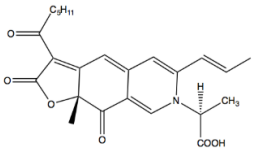
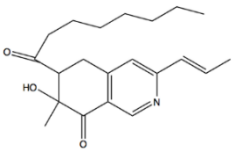
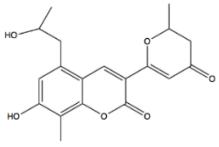
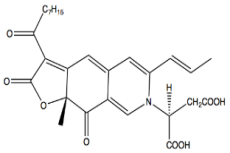
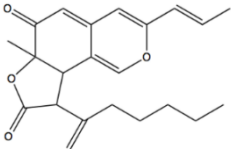
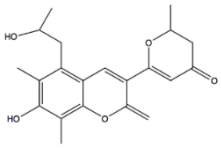
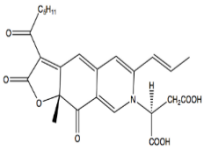
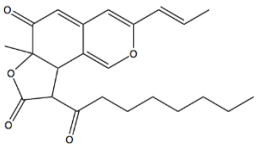
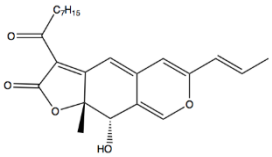
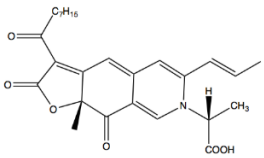
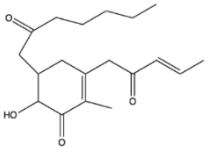
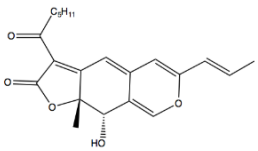
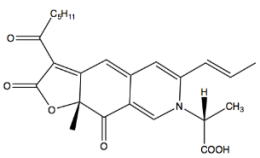
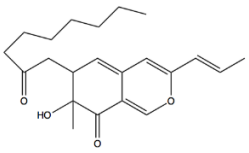
The material used in this study is the GDP receptor identification file downloaded from http://www.rscb.org and the compounds of 6 parent dyes listed in Table 1 along with 57 dyestuff compounds resulting from the isolation of azhapilone derivatives from Monascus sp mold listed in Table 2

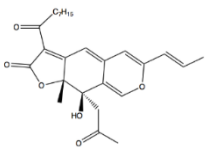
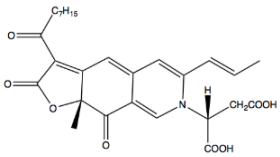
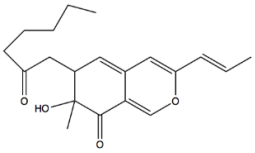
Tabel 1 Main Pigments of *Monascus sp* (Timotius, 2004)

No	Pigment	No	Pigment	No	Pigment
1	 Monascin	3	 Ankaflavin	5	 Monascorubrin
2	 Rubropunctamine	4	 Rubropunctatin	6	 Monascorubramine

Tabel 2 Derivates Azaphilone of *Monascus sp* (Yuliana et all, 2017)

No	Pigment	No	Pigment	No	Pigment
1	 Glycyl-rubropuntatin	20	 N-glutaryl Rubropuctamine	39	 Monaphilones B
2	 Isolate MPs 4	21	 PP-V	40	 Monaphilones C

3		Isolate MPs 3	22		N-glucosyl rubropuctamine
41		Y3	4		Isolate MPs 2
23		N-glucosyl monascurobramine	42		Monaspyridine A
5		Isolate MPs 1	24		Compound R3
43		Monaspyridine B	6		Monankarin A-B
25		Red Derivat 1	44		Monaspyridine C
7		Monankarin C-D	26		Red Derivat 2
45		Monaspyridine D	8		Monankarin E
27		Red Derivat 3	46		Monasfluor A
9		Monankarin F	28		Red Derivat 4
47		Monasfluor B	10		Monaphilol A
29		Red Derivat 5	48		Monashexenone
11		Monaphilol B	30		Red Derivat 6
49		Rubropuctin			

12		31		50	
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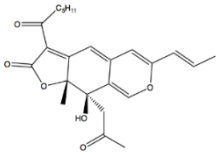
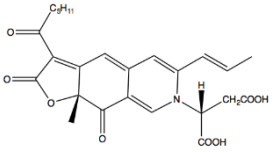
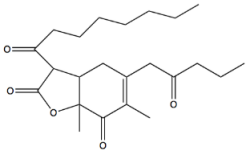
13		32		51	
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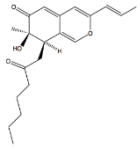
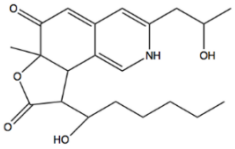
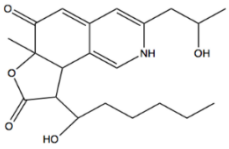
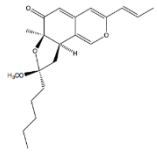
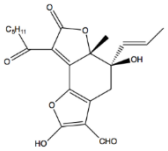
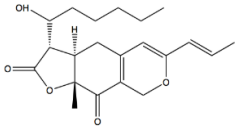
14		33		52	
	Monapurones A		Un Named		New Red Pigment
15		34		53	
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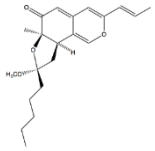
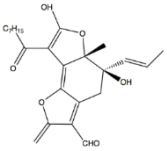
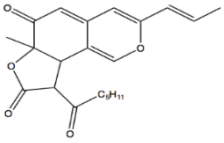
16		35		54	
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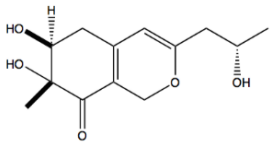
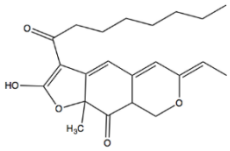
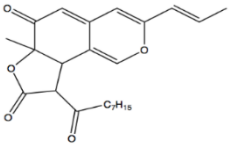
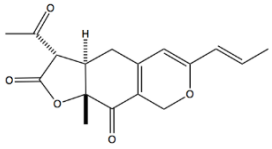
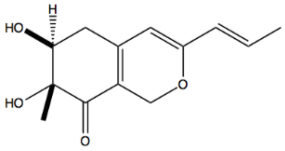
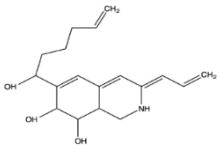
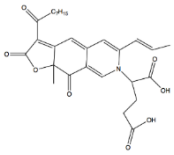
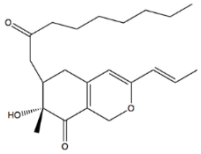
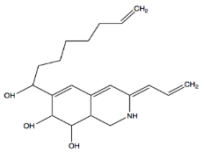
17		36		55	
	Monascusone A		Yellow II		Monascuskaodione B
18		37		56	
	Monascusone B		FK 17-P2B2		Red Shandong 1
19		38		57	
	N-glutaryl Monascorubramine		Monaphilones A		Red Shandong 2

RESULT

Ligand preparation

The Ligand compound test is described by its two-dimensional structure using the MarvinSketch software manually. Descriptions are carried out referring to references where all the ligand compounds used in the test have been successfully isolated from the color compounds of the azhaphilone fungus Monascus sp. The structure formed is carried out by optimizing the geometry to get a stable molecular structure. After geometry optimization then the test ligand is protonated so that it is present at pH 7.4 with the Major Microspecies method to obtain structures or compounds that are in accordance with the blood pH (Aryani, 2016). Next, Conformational search is

done to get the structure that best fits the target receptor or protein. This conformation determination uses conformers method and there are 10 conformations that represent all ligand positions (Agistia, 2013).

Lipinski Testing (Drug Scan)
Lipinski's rule of five is a parameter in determining the bioavailability of drugs in measuring the ability of drug candidates to interact with targets. To obtain maximum drug bioavailability, Lipinski set rules to identify the optimum physicochemical properties needed by a compound to achieve maximum bioavailability so that it can avoid the biological resistance of a drug compound before reaching its target (Omran, 2014). The results of this data objective are seen in Table 3 and Table 4

Table 3. Result of *Drug Scan* Main Pigments with *Lipinski's Rule of Five*

No	Pigments	Parameter				
		Molecule Wight	Proton Donor	Proton Aseptor	Log P	Refractory Molar
		< 500 g/mol	< 5	< 10	< 5	40 – 130
1.	Ankaflavin	384,4654	0	7	4,66	110,23
2.	Monascin	358,4281	0	7	3,67	100,50
3.	Monascorubramine	380,4568	0	7	2,90	111,56
4.	Monascorubrin	382,4495	0	7	4,54	110,48
5	Rubropunctamine	454,4196	0	7	1,94	102,35
6	Rubropunctatin	354,3964	0	7	3,65	101,28

Table 4. Result of *Drug Scan* Derivates Azaphilone Pigments with *Lipinski's Rule of Five*

No	Pigments	Parameter				
		Molecule Wight	Donor Proton	Akseptor Proton	Log P	Refractory Molar
		< 500 g/mol	< 5	< 10	< 5	40 – 130
1.	Compound R3	374,4275	1	8	2,05	101,41
2.	Fk 17-P2b2	236,2637	2	5	0,35	66,34
3.	Glycyl-Rubropunctatin	413,4636	1	10	3,33	114,09
4.	Isolat Mps 1	510,5821	5	13	2,85	152,26
5	Isolat Mps 2	538,6352	5	13	3,74	161,46
6	Isolat Mps 3	439,5009	1	10	4,08	123,54
7	Isolat Mps 4	439,5436	1	8	4,32	126,87
8	Monankarin A-B	358,3851	2	7	2,38	98,10
9	Monankarin C-D	372,4117	2	7	2,90	103,14
10	Monankarin E	344,3585	2	7	2,02	93,63
11	Monankarin F	342,3857	2	6	2,48	98,17
12	Monaphilol A	384,4654	1	6	3,62	111,10
13	Monaphilol B	356,4123	1	6	2,73	101,89
14	Monaphilol C	440,5287	1	8	3,59	125,32
15	Monaphilol D	412,4755	1	8	2,70	116,12
16	Monaphilones A	360,4871	1	6	4,16	106,90
17	Monaphilones B	318,4073	1	6	2,89	92,02
18	Monaphilones C	332,4339	1	6	3,27	97,70
19	Monapurones A	328,4452	0	5	4,44	100,82
20	Monapurones B	344,4446	0	5	3,93	101,83
21	Monapurones C	344,4446	0	5	3,93	101,83
22	Monarubrin (Y,Bf)	330,4180	1	6	3,13	97,95
23	Monascuskaodione A	356,4123	0	7	3,38	100,67

24	Monascuskaodione B	384,4654	0	7	4,27	109,87
25	Monascusone A	254,2790	3	6	-0,99	67,08
26	Monascusone B	302,3218	0	7	1,64	82,07
27	Monascuspiloin	360,4440	1	6	3,11	101,32
28	Monasfluor A	354,4394	0	5	3,98	104,30
29	Monasfluor B	384,4654	0	7	4,27	109,87
30	Monashexenone	320,4232	1	7	3,70	92,33
31	Monaspyridine A	355,4275	0	7	4,23	98,81
32	Monaspyridine B	383,4807	0	7	5,12	108,01
33	Monaspyridine C	357,4434	1	7	3,90	99,88
34	Monaspyridine D	343,4599	1	6	4,61	100,56
35	New Red Pigment	375,4587	3	7	1,16	103,91
36	N-Glucosylmonascorubramine	585,6851	4	12	3,54	159,12
37	N-Glucosylrubropunctamine	557,6320	4	12	2,65	149,92
38	N-Glutarylmonascorubramine	511,5635	2	13	4,30	138,82
39	N-Glutarylrubropunctamine	483,5104	2	13	3,41	129,82
40	PP-V	411,4477	2	9	3,26	136,05
41	Purpureusone	390,5131	0	8	5,43	107,95
42	Red Derivat 1	453,5274	1	10	4,65	126,03
43	Red Derivat 2	425,4743	1	10	3,76	118,83
44	Red Derivat 3	497,5369	2	13	4,01	134,04
45	Red Derivat 4	469,4836	2	13	3,12	124,87
46	Red Derivat 5	453,5274	1	10	4,65	128,03
47	Red Derivat 6	425,4743	1	10	3,76	118,83
48	Red Derivat 7	497,5369	2	13	4,01	134,07
49	Red Derivat 8	469,4838	2	13	3,12	124,87
50	Red Shandong 1	303,3960	4	4	0,54	91,77
51	Red Shandong 2	331,4492	4	4	1,43	100,98
52	Rubropuctin	358,4712	1	5	4,02	107,15
53	Un Named	375,4587	3	7	1,16	103,91
54	Xantomonascin A	388,4111	2	8	4	102,20
55	Xantomonascin B	414,4914	2	8	3,76	126,81
56	Y3	448,571	6	8	0,34	115,88
57	Yellow II	372,4547	1	7	4,31	116,32

Based on the results of the drugscan test in Table 3 and Table 4, all the parent compounds meet the test parameter requirements. In contrast to the isolated compounds there are 14 compounds that do not meet the requirements of drugscan so that the 14 compounds are eliminated as candidates for antimicrobial drugs. While other isolation test compounds that are not marked in red meet all the requirements of the rules of the lipinski's rule of five so that they can be candidates for good drug compounds to be followed by subsequent testing.

CONCLUSION

Based on the identification of target proteins from 6 parent compounds of Azapilone dyes namely Ankaflavin, Monascin, Monascorubrin, Monascorubramine, Rubropunctatin and Rubropunctamine obtained slices of three antimicrobial receptors namely 1YVX, 1DD6 and 1PW1 with the evaluation of 1PW1 receptors as the best receptors for test compounds.

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