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Tyrosinase Inhibitory Activity of Ethyl Acetate Extracts from Marine Sponge-Derived Fungi *Haliclona fascigera*

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Tyrosinase plays an important role in catalyzing the biosynthesis of melanin which can be used as an approach to the skin hyperpigmentation and cosmetic. Finding natural resources of tyrosinase inhibitor were done through marine-derived fungi from the sponge *Haliclona fascigera* from South Coast of West Sumatera. Ethyl acetate extract of the marine-derived fungi was tested as a tyrosinase inhibitor. The inhibition assay was tested against diphenolase (DOPA), and kojic acid was used as a positive control (IC_{50}, 29.61 µg/ml). Four out of 20 the extract, WR_3, WR_4, WR_9, and WR_{13}, exhibited high inhibition (>50%) against diphenolase at the highest screening concentration with IC_{50} values 25.58, 72.70, 198.85 and 300.34 µg/ml, respectively. In additional, phytochemical screening of the potential extracts showed that they contain phenolic compounds. This study gave as an overview of marine-derived fungi of sponge *H. fascigera* could be exploited as potential natural resources of tyrosinase inhibitors.

Keywords: *Haliclona fascigera*, marine sponge-derived fungi, Tyrosinase-inhibition.

INTRODUCTION

Sunlight exposure has an important role in improving the organism's life on earth. It accelerates melanin production by increasing the activity of tyrosinase enzyme while human skin gets sunlight exposure. Tyrosinase is the rate-limiting enzyme involved in melanin synthesis. Melanin is the main pigment that determines the skin color which is synthesized in melanosome, the specific organelle in melanocyte located in the basal layer of the epidermis (Graillet et al., 1997; Park et al., 2007). Despite its advantages, abnormal production or distribution of melanin is the cause of various dermatological disorders such as melasma, lentigines, age spots and post-inflammatory hyperpigmentation (Kim and Umaya, 2005). The biosynthesis pathway of melanin was started from the tyrosine oxidation to be dopaquinone which was catalyzed by the main enzyme, tyrosinase (Schallreuter et al., 2007). This enzyme was important as a catalyst in the biosynthesis of melanin (Park et al., 2007). Therefore, an approach to inhibit tyrosinase catalytic activity could be applied to prevent abnormal pigmentation caused by tyrosinase (Chang, 2009).

The secondary metabolite of fungus is one of the largest sources of tyrosinase inhibitors. Kojic acid as a derivative of the tyrosinase inhibitor derived from *Aspergillus* and *Penicillium fungus*. It has prompted the discovery of more natural tyrosinase inhibitors (Tsuchiya et al., 2008). Furthermore, a new compound was found from *Trichoderma viride* Strain H1-7 which is potential as a tyrosinase inhibitor. From *Myrothecium* sp. was isolated compound 6-n-pentyl-α-pythonic and

Cunninghamella elegans also produce a 17-hydroxylation steroid compound as potential tyrosinase inhibitor (Tsuchiya et al., 2008; Chang, 2009).

As a continuation of our research on chemical constituents and biological activity of marine sponge-derived microorganism, in this study, we report the tyrosinase inhibitory activity of ethyl acetate extracts of fungi from marine sponge *H. fascigera* (Handayani et al., 2018; Handayani et al., 2016; Handayani et al., 2015a. Handayani et al., 2015b).

MATERIALS AND METHODS

Fungi Material

Twenty isolated fungi obtained from marine sponge-derived fungi of *H. fascigera* which was collected from the South Coast of West Sumatera, Indonesia, in the depth of ± 13 m (Handayani et al., 2018).

Cultivation of Isolated Fungi in Medium of SDA

The pure culture stock of fungal isolates from the previous study was taken one loop and then grown on Sabouraud Dextrose Agar (SDA) as a medium. The fungi were grown for 5-7 days. Each fungus was then cultured in rice as medium and incubated at room temperature for 4-6 weeks until the volume of rice in the Erlenmeyer was overgrown by the fungi (Kjer et al., 2010).

Extraction of Fungal Isolates

After fungi isolates have optimally grown, each fungus was macerated with 100 ml ethyl acetate (EtOAc). The ethyl acetate macerates were collected and evaporated *in vacuo* using rotary evaporator. All EtOAc fungi extracts were tested for tyrosinase inhibitors activity.

Screening for Tyrosinase Inhibitor Activity

The tyrosinase inhibitory activity was done *in vitro* following the procedure of Momtaz (2008). Each extract was diluted in DMSO solvent to obtain a concentration of 2500 ppm as the stock solution. The assay was applied to 96-well microplate, each well was added 50 µL of each extract, 20 µL of tyrosinase enzyme (250 U/mL in buffer phosphate pH 6.5) and 90 µL buffer phosphate. Subsequently, the sample was incubated at room temperature for 5 minutes. The absorbance value was calculated using the microplate reader at 492 nm. Then added 40 µL L-DOPA substrate (5.07 mM in buffer phosphate pH 6.5) to procure the final concentration of the sample was 625 ppm. This mixture was incubated 30 minutes at room temperature and measured for absorbance value at 492 nm using the microplate reader. The percentage of tyrosinase inhibitors calculated using the formula:

% tyrosinase inhibitors = ((A-B))/A ×100 %

where:

A is the absorbance value without the addition of the test compound

B is the absorbance value by the addition of the test compound.

The IC_{50} values were determined by the regression method in five different concentrations. Kojic acid is used as a positive control of tyrosinase inhibitor.

Identification and Phytochemical Analysis of Symbiotic Fungi

Macroscopic and microscopic identification was only conducted on symbiotic fungi which have tyrosinase inhibitor activity. The macroscopic examination included visual observation of the shape and color of the fungus colonies. While microscopic examination, the observation was done by using a microscope based on the pure isolate of fungi. Pure isolate than identified based on Brigitte (1980). Phytochemical examinations were carried out for all the ethyl acetate extracts of fungi. Phenolic, alkaloid, steroid, and terpenoid test were performed to identify a group of the compound in each extract (Tiwari et al., 2011).

RESULTS AND DISCUSSION

A harmful side effect of market whitening agent and trending back to nature become the reason of researcher to find other natural tyrosinase inhibitor resources (Parvez et al., 2007). For example, a high concentration of kojic acid may be hepatocarcinogenic and may cause erythema and allergic contact dermatitis as well as hydroquinone and azelaic acid may lead to carcinogenic (Lin et al., 2008). Several studies had reported which some fungi produce substituents having potential as tyrosinase inhibitors. However, studies of fungi which symbiotic with the marine organism are still minimally reported.

The isolated fungus from *H. fascigera* was cultivated using rice medium because this medium can produce a higher amount of metabolite than other natural media such as corn, oatmeal, and wheat germ (Molen et al., 2013). In addition, the production of spores using rice medium gives more results than sago, yeast, wheat, and corn medium (Derakhsan et al., 2008).

In our research, using rice as a medium for growing the fungus could obtain the ethyl acetate extract of symbiotic fungi from sea sponge *H. fascigera* in range 211.7 mg to 1979 mg. This amount was higher than research conducted by Handayani (2018) by using the Malt Extract Broth (MEB) as a medium. Rice medium is also one of optimization method of the medium that we are doing in our lab since rice is the main food of Indonesian.

The assay using L-DOPA as the substrate on 20 EtOAc Extract of the fungi yielded four ethyl acetate extracts which had acted as tyrosinase inhibitors. Four out of 20 ethyl acetate extracts at a concentration of 625 µg/mL showed a tyrosinase inhibitor percentage greater than 100%. The extract of WR_3 (82.96%), WR_4 (82.71%), WR_9 (66.92%), and WR_{13} (52.36%) showed higher inhibition of tyrosinase inhibitory at a concentration of 312.5 µg/m. The fourth extract is then continued for IC_{50} testing, due to its ability to inhibit enzyme tyrosinase more than 50% at concentrations below 500 µg/mL (Young et al., 2007). Furthermore, the four extracts tested for IC_{50} values by using the regression method in 5 different concentrations. Kojic acid was used as a positive control because kojic acid is the most potent inhibitor of tyrosinase inhibitory activity (Table 1). Based on the results of the test, the WR_3 extract showed the best results compared to other extracts. When compared to the IC_{50} of kojic acid, then the WR_3 inhibitory activity is stronger than kojic acid. The IC_{50} value of ethyl acetate extract WR_3 was 25.58 µg/mL while the IC_{50} value of kojic acid was 29.61 µg/mL. Further research is required to determine the active compound containing in extract symbiotic fungi, which potential as new whitening agents.

Identification of potential fungi of tyrosinase was known as *Penicillium* sp. (WR_3), *Aspergillus niger* (WR_4), *Penicillium* sp.3 (WR_9), and *Trichophyton megninii* (WR_{13}). Research on tyrosinase inhibitor from the same fungi had also conducted. Kim (2004) had obtained a compound from *Penicillium sp* 20135 that have a strong inhibitory activity on melanin formation. This fungus was isolated from a soil sample in Sokcho-city, Korea. *Aspergillus niger* from soil samples of Agumbe forest, India was also a potential source for producing a tyrosinase inhibitor compound (Vasantha et al., 2014).

The phytochemical screening was carried out based on secondary metabolite of higher plant, four extracts of ethyl acetate symbiotic fungi retrieved positive result with $FeCl_3$ 1% which means it was contained phenolic compounds (Table 2).

Table 1; IC_{50} value of ethyl acetate extract which has the potential of producing tyrosinase inhibitor compound

Code of Samples	Concentration (µg/mL)	% tyrosinase inhibitors	IC_{50} value (µg/mL)
WR_3	25	48.78 ± 3.77	25.58
	50	57.84 ± 3.2	
	100	68.29 ± 6.96	
	200	70.03 ± 7.11	
	400	79.09 ± 4.79	
WR_4	25	23.53 ± 6.35	72,70
	50	40.14 ± 1.2	
	100	66.09 ± 1.14	
	200	71.28 ± 0.6	
	400	81.31 ± 1.04	
WR_9	25	12.11 ± 8.39	198.85
	50	33.56 ± 6.32	
	100	41.52 ± 2.4	
	200	50,87 ± 2.6	
	400	78.55 ± 1.58	
WR_{13}	25	5.38 ± 8.47	300.34
	50	12.31 ± 8.56	
	100	21.54 ± 11.09	
	200	40 ± 6.15	
	400	62.31 ± 8.9	
Kojic Acid	7.825	9,16 ± 6.85	29.61
	15.65	28.03 ± 2.8	
	31.3	52.02 ± 2.45	

Table 2: Phytochemical Examination of ethyl acetate extracts for potential fungi isolate

Secondary metabolite constituent	WR_3 (*Penicillium* sp.)	WR_4 (*Aspergillus* niger)	WR_9 (*Penicillium* sp.3)	WR_{13} (*Trichophyton megninii*)
Alkaloid	-	+	-	-
Phenol	+	+	+	+
Steroid	**+**	**-**	**-**	**+**

The hydroxy substituent (OH) plays an important role in the compound act as a tyrosinase inhibitor. The content of phenol group compounds is suspected to have a substantial inhibitory effect of tyrosinase enzymes, thus preventing the risk of skin hyperpigmentation due to exposure to ultraviolet radiation from sunlight (Ohguchi et al., 2003). Phenolic compounds with hydroxyl functional groups (-OH) and carboxylic acids (COOH) are structurally like the tyrosinase substrate of L-tyrosine or L-DOPA (Ha et al., 2012; Park et al., 2013). This study has a potential finding of tyrosinase inhibitory activity of the ethyl acetate extracts from marine-derived fungi from the sponge *H. fascigera* and it would be useful as an inhibitor agent of enzymatic oxidation of tyrosinase enzyme. The application could be applied to the pharmacy, food, cosmetic and living systems, especially as a skin whitening agent for a human.

CONCLUSION

Marine-derived fungi of sponge *H. fascigera* and phenolic compounds in EtOAc extract have the possibility to be explored. The next investigations need to be done in order to have a comprehensive potential resource of natural tyrosinase inhibitors agent, especially the four potential ethyl acetate extracts that have smaller IC_{50} value than kojic acid.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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REFERENCES

Brigitte G. Kompendium der medizinischen mykologie. 1980, Berlin-Hamburg. Verlag Paul Parey.

Chang TS. An updated review of tyrosinase inhibitors. International Journal of Molecular Sciences, 2009; 10: 2440-2475

Derakhsan A, Rabindra RJ, Ramanujam B, and Rahimi M. Evaluation of different media and methods of cultivation on the production and viability of entomopathogenic fungi, Verticillium lecanii (Zimm) Viegas. Pakistan Journal of Biological Sciences, 2008; 11: 1506-1509.

Graillet CR, Aberdam E, Clement M, Ortonne JP, Ballotti R. Nitric oxide produced by ultraviolet-irradiated keratinocytes stimulates melanogenesis. J. Clin. Invest., 1997; 99: 635-642.

Ha YM, Park YJ, Kim JA, Park D, Park JY, Lee HJ, Lee JY, Moon HR and Chung HY. Design and synthesis of 5-(substituted benzylidene) thiazolidine-2,4-dione derivatives as novel tyrosinase inhibitors. European Journal of Medicinal Chemistry, 2012; 49: 245-252.

Handayani D, Ahdinur, RF, and Rustini R. Antimicrobial Activity of Endophytic Fungi from Marine Sponge *Haliclona fascigera*. J App Pharm Sci, 2015a; 5(10), 154-156

Handayani D, Murniati M, Rustini R, In vitro inhibitory activity of ethyl acetate extract of symbiotic bacteria isolated from the marine sponge Haliclona fascigera against multidrug resistant organism (MDRO). J App Pharm

Sci, 2016; 6(11):218-222

Handayani D, Rasyid W, Rustini, Zainudin EN, Hertiani T. Cytotoxic Activity Screening of Fungal Extracts Derived from the West Sumatran Marine Sponge Haliclona fascigera to Several Human Cell Lines: Hela, WiDr, T47D, and Vero. J App Pharm Sci, 2018; 8 (01): 055-058, 31 (8): 1618 – 1620.

Handayani D, Sandrawaty N, Murniati M, and Regina R. Screening of Endophytic Bacteria Isolated from Marine Sponge *Haliclona fascigera* for Inhibition against Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA). J App Pharm Sci, 2015b; 5(9): 139-142.

Kim YJ, Uyama H. 2005. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism, and perspective for the future. Cellular and Molecular Life Sciences, 62:1707–1723.

Kjer J, Debbab A, Aly AH, Proksch P. Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. Nature Protocols, 2010; 5(3): 479-490.

Lin JW, Chiang HM, Lin YC, and Wen KC. Natural products with skin-whitening effects. Journal Food and Drugs Analysis, 2008; 2(16): 1-10.

Molen KM, Raja HA, Elimat TE, and Oberlies NH. Evaluation of culture media for the production of secondary metabolites in a natural products screening program. AMB Express a Springer Open Journal, 2013; 3:71-77.

Momtaz S, Mapunya BM, Houghton PJ, Edgerly C, Hussein A, Naidoo S, Lall N. Tyrosinase inhibition by extract and constituents of Sideroxylon inerme L. stem bark, used in South Africa for skin lightening. Journal of Ethnopharmacology, 2008; 199: 507-512.

Ohguchi K, Tanaka T, Iliya I, Iro T, Iinuma M, Matsumoto K, Akao Y, and Nozawa Y. Gnetol as a Potent Tyrosinase Inhibitor from Genus Gnetum. Biosci, Biotechnol, Biochem. 2003; 67(3): 663-665.

Park HY, Pongpudpunth M, Lee J, Yaar M. Biology of Melanocytes. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffel DJ, editors. Fitzpatrick's Dermatology in General Medicine. McGraw Hill, New York, 2007; pp. 591–608.

Park JW, Ha YM, Moon KM, Kim SR, Jeong HO, Park YJ, Lee HJ, Park JY, Song YM, Chun P, Byun P, Byun Y, Moon HR, and Chung HY. De novo tyrosinase inhibitor: 4-(6,7-Dyhidro-5H-indeno[5,6-d] thiazol-2-yl) benzene-1,3-diol (MHY1556). Bioorganic & Medicinal Chemistry Letters. 2013; 23: 41

Parvez S, Kang M, Chung HS. and Bae H. Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health, cosmetics, and agriculture industries. Phytother. Res. 2007; 21: 805-816.72-4176.

Parvez S, Kang M, Suck HC, Cho C, Chang MH, Kyu MS, Bae H. Survey and Mechanism of Skin Depigmenting and Lightening Agent. Phytother. Res, 2006; 20: 921-934.

Schallreuter KU, Kothari S, Chavan B and Spencer JD. Regulation of melanogenesis-controversies and new concepts. Exp. Dermatol, 2007; 17: 395–404.

Tiwari P, Kumar B, Kaur M, Kaur G, and Kaur H, Phytochemical screening and Extraction: A Review, Internationale Pharmaceutica Sciencia, 2011; 1(1): 98-106.

Tsuchiya T, Yamada K, Minoura K, Miyamoto K, Usami Y, Kobayashi T, Hamada NS, Imada C, Tsujibo H. Purification and Determination of the Chemical Structure of the Tyrosinase Inhibitor Produced by *Trichoderma viride* Strain H1-7 from a Marine Environment. Biol. Pharm. Bull, 2008; 31 (8): 1618 – 1620.

Vasantha, KY., CS Murugesh., AP Sattur. A tyrosinase inhibitor from *Aspergillus niger. J Food Sci Technol.* 2014; 51:2877-2880

Young, JM, Young EY, Song G, Ho NL, and Gu CH. Screening of elastase and tyrosinase inhibitory activity from Jeju Island plants. Eurasian Journal of BioSciences. 2010; 4:41-53.