ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Potency of Microalgae as Tyrosinase Inhibitor.

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ABSTRACT

Tyrosinase is a determinant enzyme for modulating melanin production as its abnormal activity can result in an increase amount of melanin. Reduction of tyrosinase activity has been targeted for preventing and healing hyperpigmentation of skin, such as melanoma and related spots. The aim of this study is to screen tyrosinase inhibitor potency of 16 microalgae. All microalgae extracted with methanol. The methods for screening is based on tyrosinase inhibitor potency using mushroom tyrosinase. Out of 16 extracts, *Scenedesmus dimorphus* are the most potent extracts as tyrosinase inhibitor, but IC_{50} values are significantly different with kojic acid as positive control.

Key words: Microalgae, tyrosinase inhibitor

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ISSN: 0975-8585

INTRODUCTION

Melanin production is principally responsible for skin color and plays a significant role in protecting the skin from ultraviolet (UV) light; however, overproduction and accumulation of melanin can result in various dermatological disorders including melisma, freckles, age spots, and sites of actinic damage or other hyperpigmentations. Thus, melanogenic inhibitors have become increasingly important ingredients in medication and cosmetic to prevent hyperpigmentation. (Kao et al. 2013). Tyrosinase or polyphenol oxidase (EC.1.14.18.1) is a copper protein that uses molecular oxygen to catalyse the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) (Muñoz-Muñoz et al. 2008) in the first stages of melanin biosynthesis (Kao et al. 2013). Tyrosinase is widespread enzyme, in the phylogenetic scale, that produces melanin, from bacteria to man, by using as substrates monophenol, o-diphenols and molecular oxygen (Cesare et al. 2016). The study of enzymatic inactivation by suicide substrates or mechanism-based inhibitors is of growing importance because of possible pharmacological applications.

Many tyrosinase inhibitors have been tested as a way of preventing overproduction of melanin in epidermal layers from natural resources (Batubara et al. 2010). However, there is still a need to search for other potential compounds such as tyrosinase inhibitors from microalgae.

Microalgae are capable producers of food, feed supplement, chemicals and biofuels. In this study, we focused on 16 different microalgae species. We tried to find microalgae with the most potent tyrosinase inhibitor. Previous study have shown that methanol extract of microalgae contain natural antioxidants (Amri, Dharma, and Tjong 2017). While there is one study to investigate the inhibition of tyrosinase of microalgae methanol extract.

MATERIAL AND METHODS

Microalgae materials: Sixteen microalgae species used in this study were collected from Bukit Kili hot spring, Solok, West Sumatera Indonesia (Kili 1 and Kili 2), Palm Oil Mill Effluent (POME) from Mutiara Agam Company, Agam Regency, West Sumatera, Indonesia (Unculture oscillatoria sp./IPHOME 4, Micractinium sp. CCAP, Micractinium sp. Ehime, Myconastes rotundus) (Sekatresna et al. 2015), and other place in Indonesia (Chroococcus dispesrsus, Chlorella vulgaris, Diatom, Dunaliella salina, Nannochloropsis oculata, Scenedesmus bijuga, Scenedesmus dimorphus, Spirulina platensis, Spirulina sp., Tetraselmis chuii).

Preparation of microalgae extracts: are samples were dried before submitted to methanol. The dried microalgae were extracted with solvents (1 g sample: 10 ml solvent), sonication and for 24 h for three times. Solvent objected to dry using oven with temperature 60ºC.

Bioactivity test: Inhibition of tyrosinase activity (monophenolase). This assay was performed using methods as describe earlier (Curto et al., 1999; nerya et al, 2003). Extracts were dissolved in DMSO (dimethyl sulphoxide) to final concentration of 20 mg/ml. this extract stock solution was then diluted to 600 µg/ml in 50 nM potassium phosphate buffer (pH 6.5). the extracts were tested at the concentrations ranging from 625 to 10000 µg/ml. Kojic acid, which was used as positive control was also tested at concentration 0.625 to 10 µg/ml. In a 96-well plate, 70 µl of each extract dilution was combined with 30 µl of tyrosinase (Sigma, 333 Units/ml in phosphate buffer) in duplicate. After incubation at room temperature for 5 min, 110 µl of substrate (2nM L-tyrosine) was added to each well. Incubation commenced for 30 min at room temperature. Optical densities of the wells were then determined at 510 nm with a multi-well plate reader. The concentration of microalgae extract at which half the original tyrosinase activity was inhibited (IC50) was determined for each plant extract. Kojic acid (Sigma, Checz Republic) was used as positive control.

RESULT AND DISCUSSION

Tyrosinase is important enzyme for melanin production. Hence, to evaluate the skin whitening effects of microalgae methanol extract, their ability to inhibit tyrosinase activity was investigated. Tyrosinase inhibition rates of the different concentrations and IC_{50} were measured. In the current study, kojic acid was used as the positive control group. Tyrosinase inhibition rates displayed by 625, 1250, 2500, 5000 and 10000

ISSN: 0975-8585

µg/ml for sample and 0.625, 1.25, 2.5, 5 and 10 µg/ml to kojic acid. (Tabel 1.). This finding shows that tyrosinase inhibition rates are elevated by increase in microalgae methanol extract concentrations.

Based on tyrosinase inhibitor activity, Scenedesmus dimorphus methanol extract (IC_{50}: 1,799 mg/ml) are the most potent, but value significantly different with kojic acid (IC_{50}: 0,002 mg/ml) as positive control (Tabel 1.). If we compered these report from Batubara et al. (2010), many plant extract had inhibiton activity at low concentration, the best sample I. palembanica at concentration 125 µg/ml had inhibition level about 97.7%, while our sample Chroococcus dispersus at concentration 625 µg/ml had inhibition level about 24,41%.

Tabel 1. Inhibition Result of Enzyme Tyrosinase Extract of Microalgae and Kojat Acid

Methanol extract	Concentration	% Inhibition			Function	IC_{50} (mg/ml)
	(µg/ml)	U1	U2	Average		
Kili 1 (*Dunaliella* sp.)	625	16,62	18,18	17,40	y = 14,87ln(x) – 82,57	8,574
	1250	21,82	20,78	21,30	R^2 = 0,948	
	2500	26,05	37,41	31,73		
	5000	47,01	38,18	42,60	y = 12,32ln(x) – 63,10	
	10000	56,58	52,21	54,40	R^2 = 0,932	
Kili 2 (*Oscillatoria* sp.)	625	16,62	14,81	15,72	y = 17,5ln(x) – 95,88	4,277
	1250	21,56	20,80	21,18	R^2 = 0,768	
	2500	57,66	57,92	57,79		
	5000	42,60	43,12	42,86	y = 17,53ln(x) – 97,00	
	10000	66,75	64,42	65,59	R^2 = 0,766	
Chroococcus disperses	625	25,45	23,37	24,41	y = 24,72ln(x) – 135,9	2,109
	1250	34,81	32,73	33,77	R^2 =0,868	
	2500	68,57	52,21	60,39		
	5000	59,74	61,56	60,65	y = 22,13ln(x) – 122,0	
	10000	97,66	84,68	91,17	R^2 = 0,974	
Chlorella vulgaris	625	21,55	23,38	22,47	y = 31,67ln(x) – 187,3	1,840
	1250	34,29	35,32	34,81	R^2 = 0,980	
	2500	55,58	61,30	58,44		
	5000	83,64	77,14	80,39	y = 25,06ln(x) – 139	
	10000	95,06	78,44	86,75	R^2 = 0,876	
Diatom	625	17,92	17,92	17,92	y = 22,25ln(x) – 132,6	3,948
	1250	26,23	21,82	24,03	R^2 = 0,840	
	2500	32,72	34,29	33,51		
	5000	44,94	44,94	44,94	y = 30,49ln(x) – 121,1	
	10000	85,71	77,40	81,56	R^2 = 0,888	
Dunaliella salina	625	29,09	19,22	24,16	y = 30,46ln(x) – 176,2	1,867
	1250	37,40	33,51	35,46	R^2 = 0,836	
	2500	55,84	50,91	53,38		
	5000	65,19	69,35	67,27	y = 30,10ln(x) – 179,6	
	10000	118,2	102,1	110,15	R^2 =0,954	
Iphome 4	625	9,610	7,792	8,70	y = 24,80ln(x) – 155,4	3,909
	1250	17,92	17,40	17,66	R^2 = 0,964	
	2500	35,58	30,91	33,25		
	5000	51,17	58,70	54,94	y = 26,26ln(x) – 166,9	
	10000	78,96	78,18	78,57	R^2 = 0,967	
Micractinium sp. CCAP	625	8,312	9,351	8,83	y = 20,31ln(x) -124,2	6,063
	1250	18,96	15,58	17,27	R^2 = 0,923	
	2500	28,57	18,70	23,64		
	5000	58,96	32,21	45,59	y = 20,38ln(x) – 129,9	
	10000	58,70	71,69	65,20	R^2 = 0,797	
Micractinium sp. Ehime	625	15,32	12,99	14,16	y = 14,38ln(x) – 76,63	5,312
	1250	22,59	20,52	21,56	R^2 = 0,866	
	2500	37,66	27,79	32,73		
	5000	55,32	65,97	60,65	y = 23,04ln(x) – 140,8	
	10000	48,83	70,13	59,48	R^2 = 0,898	
Mychonastes rotundus	625	6,753	10,13	8,44	y = 14,65ln(x) – 90,94	11,95
	1250	11,43	16,88	14,16	R^2 = 0,965	
	2500	21,04	29,09	25,07		

ISSN: 0975-8585

Methanol extract	Concentration (µg/ml)	% Inhibition			Function	IC_{50} (mg/ml)
		U1	U2	Average		
	5000	31,95	51,69	41,82	y = 15,29ln(x) – 88,91	
	10000	47,27	45,71	46,49	R^2 = 0,876	
Nannochloropsis oculata	625	17,66	20,66	19,16	y = 36,10ln(x) – 221,9	1,814
	1250	32,73	35,84	34,29	R^2 = 0,946	
	2500	48,05	50,39	49,22		
	5000	85,19	87,53	86,36	y = 34,25ln(x) – 206,0	
	10000	104,9	102,1	103,50	R^2 = 0,954	
Scenedesmus bijuga	625	12,73	15,58	14,16	y = 29,04ln(x) – 185,8	3,021
	1250	17,92	25,71	21,82	R^2 = 0,871	
	2500	22,34	31,43	26,89		
	5000	62,86	50,65	56,76	y = 32,30ln(x) – 205,0	
	10000	90,91	115,1	103,01	R^2 = 0,792	
Scenedesmus dimorphus	625	18,18	14,29	16,24	y = 42,24ln(x) – 266,0	1,799
	1250	25,71	30,13	27,92	R^2 = 0,904	
	2500	54,29	49,09	51,69		
	5000	84,16	91,69	87,93	y = 41,78ln(x) – 263,7	
	10000	123,9	115,1	119,50	R^2 =0,904	
Spirulina platensis	625	16,62	14,29	15,46	y = 20,31ln(x) – 118,5	9,232
	1250	24,42	19,22	21,82	R^2 = 0,956	
	2500	36,62	26,49	31,56		
	5000	49,87	37,40	43,64	y = 12,06ln(x) -65,52	
	10000	74,29	47,01	60,65	R^2 = 0,980	
Spirulina sp.	625	11,17	16,10	13,64	y = 27,20ln(x) – 168,6	3,388
	1250	24,16	22,60	23,38	R^2 = 0,948	
	2500	41,30	32,73	37,02		
	5000	53,77	42,86	48,32	y = 24,05ln(x) – 147,5	
	10000	90,65	89,35	90,00	R^2 = 0,825	
Tetraselmis chuii	625	10,13	14,55	12,34	y = 21,99ln(x) – 136,8	5,103
	1250	21,56	21,82	21,69	R^2 = 0,878	
	2500	27,01	35,58	31,30		
	5000	40,52	45,97	43,25	y = 17,87ln(x) – 103,3	
	10000	76,88	64,42	70,65	R^2 = 0,979	
Asam Kojat	0,625	3,117	7,273	5,20	y = 31,00ln(x) + 23,70	0,002
	1,25	30,65	45,71	38,18	R^2 = 0,935	
	2,5	58,96	61,30	60,13		
	5	85,97	71,17	78,57	y = 26,23ln(x) + 30,14	
	10	85,19	85,45	85,32	R^2 = 0,921	

CONCLUTION

Out of 16 microalgae species collected from Bukit Kili, Solok West Sumatera, Palm Oil Mill Effluent (POME) from Mutiara Agam Company, Agam Regency, West Sumatera, and other place in Indonesia, the most potencial species as tyrosinase inhibitor is Scenedesmus dimorphus, but the activity significantly different with the positive control.

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