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Screening Anti-Acne Potency of Microalgae: Antibacterial and Antioxidant Activities

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ABSTRACT

Sixteen microalgae isolates were screened for their potency as the sources of anti-acne agents. The methanol and hexane extract of the isolates were assayed for their antibacterial bioactivity against Propionibacterium acnes and Staphylococcus aureus and theirantioxidant activities as free-radical-scavenging bioactivity by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) test. The results showed that the extract of Micractinium sp. Ehime was the most potential as the source of anti-acnes agent based on their combined activities: antibacterial against P. acnes (clear zone 28.6 mm) and antioxidant activity (IC_{50} 361.23 µg/ml).

Keywords: Anti-acne, Antibacterial activity, Microalgae, Antioxidant

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INTRODUCTION

Skin is the largest body organ that serves as an important environmental interface providing a protective envelope that is crucial for homeostasis. On the other hand, the skin is major target for toxic insult by a broad spectrum of physical (i.e., UV radiation) and chemical (xenobiotic) agents that are capable of altering its structure and function [1]. Acne vulgaris is extremely common skin disorder that effect virtually all individuals at least once during life. Acne is a disorder of the sebaceous follicles, which special pilosebaceous units located on face, chest, and back. They consist of sebaceous glands associated with small hair follicles. Several factors contribute to the pathogenesis of acne-sebum includingabnormal follicular differentiation, *Propionibacterium acnes* infection and inflammation. Each of these factors provides a potential target for treatment [2,3].

P. acnes, ananaerobic Gram-positive normally inhabits the skin and implicates in the inflammatory phase of acne [3,4]. This bacterium plays a central role in the current concept of acne pathogenesis [3,5] and appears to be the target of oral and topical antibiotic usage. The reduction of their numbers is a valid parameter for the therapeutic effectiveness of an antibiotic [3,6].

Recently it was reported that the most chronic medical conditions of acne are characterized by both oxidative stress and inflammation. It is likely that the blood levels of antioxidants are used up readily in those with acne because there is a greater demand to deal with free radicals. Compounds targeting acne, therefore, should be able to inhibit *P. acnes* growth and inhibit the oxidative stress. In other words, compounds or materials advocated for acne control should possess antibacterial and antioxidant activities [3].

Medicinal plants have been used in medicine for thousands of years. However, their efficacies proven with scientific methods, which can be employed to give a better understanding of their mechanisms of action, were only established in recent times. The antioxidant effects of antioxidant-rich polyphenols such as green tea, turmeric, and berries were reported to have good activity in reducing the oxidative stress of acne patients [3]. In this study, the antimicrobial activity against *P. acnes*, *Staphylococcus aureus* and antioxidant properties of some microalgae were investigated.

MATERIALS AND METHODS

Microalgae materials

Sixteen microalgae isolates used in this study were collected from West Sumatera, Indonesia. Microalgae isolate Kili 1 and Kili 2 were collected from spring water Bukit Kili, Solok, *Micractinium sp. CCAP*, *Micractinium sp. Ehime*, and *Mychonastes rotundus* were collected from the palm oil mill effluent (POME) ofMutiara Agam Company, West Sumatera, Indonesia [7]. Other microalgae isolates were the collection of Biochemistry Laboratory, Faculty of Mathematic and Natural Science, Universities Andalas, Padang, Indonesia. The voucher specimens were collected at the Biomedicine Laboratory, Faculty of Medicine, Universitas Andalas, Padang.

Preparation of microalgae extracts

All samples were dried before being extracted with methanol and hexane. Briefly, the dried microalgae materials were extractedthree times with solvents (ratio of 1 g sample: 10 mL solvent), for 24 h. The extract were then dried in ovenat 40°C.

Antibacterial assay

The test organism used in this study were *Propionibacterium acnes* from Microbiology Laboratory, Faculty of Medicine, Indonesia University and *Staphylococcus aureus* from Bacteriology Laboratory, Balai Veteriner Bukittinggi. The medium consisted of blood agar with yeast extract for *P. acnes* and blood agar for *S. aureus*. Microalgae extract were diluted in dimethyl sulfoxide (DMSO) at concentration 10-15 mg/ml. *P. Acnesn* and *S. Aureus* were inoculated by equally distributed on the blood agar media, and then the paper discs were placed on the top of media. The DMSO sample solution (0.03 ml) were then dripped on paper disc. Incubation was performed in anaerobic condition for 72 h for *P. acnes* and in aerobic condition for 24 h for *S. aureus*. The zones of inhibition formed around the paper discs demonstrate the sample extract ability to inhibitthe growth of bacteria. The standard drug used was clindamycin.

Antioxidant assay

The antioxidant assay used in this study adopted a free-radical-scavenging activity using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) test [8]. Samples were diluted in methanol to make concentration of 62.5, 125, 250, 500 and 1000 ppm. An aliquot of sample (100 µl), 100 µl of 118 mg/L DPPH solution in methanol were added to each well of a 96-well plate. After 30 min incubation in dark room, the absorbance of the mixture was measured at 514 nm. The positive control was ascorbic acid, while methanol was used as the blank. The inhibitory activity was calculated according to the following equation:

$$\text{Inhibition } (\%)=[1-(A_{sample}-A_{control})/(A_{blank}-A_{control}) \times 100\%$$

Where A_{sample} is the absorbance of the sample, $A_{control}$ is absorbance of ascorbic acid as control and A_{blank} is the absorbance of ethanol as the blank. Each sample concentration of the samples and positive control were tested in triplicate.

Preliminary phytochemical analysis

Preliminary analysis of phytochemical constituents of microalgae sample were carried out according to standard method described by Harborne [9]. The chemical tests to analyze the presence of steroid/terpen was by Salkowski; Libermann-Burchardand Libermann's test, phenol compounds were by ferric chloride, saponin were by frothing and haemolysis tests and flavonoids were by Shinoda test. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

RESULT AND DISCUSSION

Sixteen microalgae were collected for anti-acne potency screening. The anti-acne potency of microalgae was analyzed based on antibacterial, and antioxidant activity. The data showthat all of methanol extracts were active to inhibit the growth of *Propionibacterium acne* and *Staphylococcus aureus.* On the other hand, most of the hexane extract were only active as growth inhibitor of most of *P. acne* but non to *S. Aureus* (Table 1). The most effective inhibitor for *P. acne* were the methanol extracts of *Micractinium* sp. Ehime, and *Uncultured oscillatoria* sp. IPOME4, while to the *S. Aureus*, all of the active extract shown only a minor inhibitor activity.

The diameter zone of inhibition values of the *Micractinium* sp. Ehime methanol extracts were 28.6 mm, which was closer to diameter zone of inhibition of the positive control clindamycin which was 31.3 mm. Several studies have shown that the compounds have high antibacterial ability is largely a polar compounds than non-polar compounds [10].

In this study, phytochemical screening shown that the methanol extracts of several microalgae contain flavonoids, steroids, terpenoids and phenolic compound (Table 2). The content of secondary metabolites is comparable with antibacterial capabilities. Generally, from microalgae methanol extract containing flavonoids, terpenoids, phenolic and steroids show good antibacterial abilities.

Table 1: Antibacterial and antioxidant properties of microalgae extracts

S. No.	Sample	Solvent	Antibacterial (zone of inhibition) in mm		Antioxidant Activity IC_{50} (µg/ml)
			Propionibacterium acnes	***Staphylococcus aureus***	
1.	Kili 1	Methanol	10.3 ± 1.0	12.1 ± 1.3	649.85 ± 0.12
		Hexane	10.3 ± 3.3	NI	
2.	Kili 2	Methanol	20.1 ± 7.7	10.0 ± 0.0	888.66 ± 0.02
		Hexane	11.3 ± 0.5	NI	
3.	*Chroococcus dispersus*	Methanol	15.7 ± 7.8	11.1 ± 0.8	>1000
		Hexane	10.7 ± 1.0	NI	
4.	*Chlorella vulgaris*	Methanol	24.7 ± 2.2	10.3 ± 0.9	>1000
		Hexane	10.0 ± 0.0	NI	
5.	*Diatom*	Methanol	9.3 ± 1.0	11.5 ± 0.8	>1000
		Hexane	9.7 ± 0.5	NI	
6.	*Dunaliella salina*	Methanol	9.7 ± 0.5	10.9 ± 0.8	913.49 ± 0.29
		Hexane	NI	NI	
7.	*Uncultured oscillatoria*sp. IPOME4	Methanol	**26.9 ± 5.6**	10.2 ± 0.3	>1000
		Hexane	9.3 ± 0.5	NI	
8.	*Micractinium*sp. CCAP	Methanol	10.0 ± 0.9	12.4 ± 0.8	508.94 ± 0.02
		Hexane	11.0 ± 0.0	NI	
9.	*Micractinium*sp.Ehime	Methanol	**28.6 ± 3.2**	12.4 ± 1.2	**361.23 ± 0.17**
		Hexane	10.0 ± 0.0	NI	
10.	*Mychonastes rotundus*	Methanol	9.7 ± 0.5	11.7 ± 1.4	546.36 ± 0.09
		Hexane	10.0 ± 0.0	NI	
11.	*Nannochloropsis oculata*	Methanol	9.0 ± 0.9	9.7 ± 0.4	>1000
		Hexane	5.7 ± 4.3	NI	
12.	*Scenedesmus bijuga*	Methanol	8.7 ± 1.0	12.2 ± 0.7	748.89 ± 0.08
		Hexane	NI	NI	
13.	*Scenedesmus dimorphus*	Methanol	9.7 ± 0.5	9.9 ± 1.3	772.07 ± 0.07
		Hexane	6.3 ± 4.8	NI	
14.	*Spirulina platensis*	Methanol	8.3 ± 0.5	11.1 ± 0.6	771.87 ± 0.24
		Hexane	NI	NI	
15.	*Spirulina* sp.	Methanol	10.0 ± 0.0	12.6 ± 0.9	650.10 ± 0.01
		Hexane	9.0 ± 0.9	NI	
16.	*Tetraselmis chuii*	Methanol	10.3 ± 0.5	**13.1 ± 1.2**	989.34 ± 0.38
		Hexane	10.3 ± 0.5	NI	-
17.	Clindamycin	-	31.3 ± 3.2	30.2 ± 0.6	-
18.	Ascorbic acid	-	-	-	<62.5

NI: Not Inhibition

Table 2: Phytochemical screening of microalgae extracts

S. No.	Sample	Phenolic	Flavonoid	Steroid	Terpene	Saponin
1	Kili 1	-	-	-	-	-
2	Kili 2	+	-	+	+	-
3	*Chroococcus disperses*	+	+	+	+	+
4	*Chlorella vulgaris*	+	+	+	+	+
5	*Diatom*	-	-	+	+	-
6	*Dunaliella salina*	+	+	+	+	-
7	*Uncultured oscillatoria* sp. IPOME4	-	-	+	+	-
8	*Micractinium* sp. *CCAP*	+	+	+	+	-
9	*Micractinium* sp. *Ehime*	+	+	+	+	-
10	*Mychonastes rotundus*	-	-	-	-	-
11	*Nannochloropsis oculata*	-	-	+	+	-
12	*Scenedesmus bijuga*	+	-	+	-	-
13	*Scenedesmus dimorphus*	-	-	-	-	+
14	*Spirulina platensis*	+	+	+	+	-
15	*Spirulina* sp.	-	-	+	+	-
16	*Tetraselmis chuii*	-	-	-	-	-

For antioxidant activity, 11 samples had IC_{50} values lower than 1000 µg/ml and *Micractinium* sp. Ehime had IC_{50} values 361.23 µg/ml, the lowest among all samples, but much higher than positive control, ascorbic acid (<62.5 µg/ml). On the other hand, 5 samples at a concentration of 1000 µg/ml could not inhibit the oxidation reaction of DPPH by 50% (Table 1). Although there is still the possibility of microalgae which has antioxidant potential is developed by isolating and purifying the active compound that has antioxidant activity.

From the data illustrated in Table 2. It was observed that phenolic, flavonoid, steroid, terpene and saponin were present in the extracts some microalgae samples studied.Almost all of microalgae which has high antibacterial and antioxidant activity contains phenolic and flavonoids compounds, and most of microalgae that contains no flavonoids and phenolic compounds haslow antioxidant and antibacterial activity.

Based on the two activities, *Micractinium* sp. Ehimeextracts (methanol) have the highest potential as an anti-acne agent. Among all samples, these extracts had the best antimicrobial activity and good antioxidant activity. It is important that the active anti-acne compounds from these species be isolated, purified and identified.

CONCLUSION

The data shown that from 16 microalgae isolate that were collected from Sumatera Barat, Indonesia, the sample with the best antibacterial activity were *Micractinium* sp. Ehime MeOH extracts to inhibit *P. acnes.* All of methanol extract shown their antibacterial activity against *S. aureus*, but the activity were very low compare to the control (clindamycin). The sample that had the best antioxidant activities was *Micractinium*sp. Ehime. Based on the two activities, *Micractinium* sp. Ehime extracts (methanol) have the best potential as an anti-acne agent.

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