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Research Article

Antioxidant and Antiviral Potential of Brown Algae (Phaeophyceae)

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ABSTRACT

We reviewed potential antioxidant and antivirus activities of brown algae. It's known as Phaeophyceae, a plant-like protists and commonly found at the bottom of waters, especially in cold regions. Thousand types of brown algae have been identified. It is widely used as food and medicine ingredients, to use as natural nutrition addition and treatment for many diseases. Brown algae is a source of bioactive compounds because it is able to produce secondary metabolites that vary with extensive biological activity, including as an antioxidant to stop the chain reaction of free radicals. There have been many studies that prove that brown algae has antioxidant and antiviral activity. However, every species of brown algae and its thallus parts may have different levels of antioxidant and antiviral activity. The highest antioxidant was found in the apical of thallus, getting down to basal the antioxidant activity become lesser.

Keywords: Brownalgae, antioxidant, antiviral

INTRODUCTION

Brown algae or known as phaeophyceae is a class of algae Heterokontophyta.[1,2] Brown algae is a plant-like protist that has a multicellular thallus, so that it can be seen macroscopically (visible).[3] The thallus has a sticking device to attach its body to the substrate, while the rest of the body parts are floats on water surface.[4,5] In general, brown algae are macroscopic, it can reach more than 30 meters in size and have air bubbles as a float.[5] Some species of Phaeophyta Phylum such as Sargassum, Macrocystis, and Nereocystis have air bubbles that function to store nitrogen gas and to float above the water surface.[6] The name of this algae is taken from dominant pigments, they are brown pigment (xanthophyll), chlorophyll a and c. The number of xanthophyll pigments is the most dominant, it's causes the brown color in talus and the algae called brown algae.[7,8,9] Other Phaeophyceae's pigments are chlorophyll and carotene.[10] Its food reserves are stored as laminarin. In general, brown algae are multicellular.[11,12] Its body shape resembles a seed bearing plants because it has roots, stems and leaves, this makes algae easy to recognize.[13]

Almost all types of Phaeophyta lives in the sea (especially in cold regions), can be found on rocks at the bottom of the sea as deep as 1.5 - 5 meters from the water surface.[14] All brown algae are threads or sheets and they are autotrophic (capable of producing their own food).[15] All Phaeophyta lives in colonies in various shapes from simple to large and complex cell organization. In large colonized Phaeophyta, the real organ has not yet formed, although in some species there are shapes that resembles roots, stems, and leaves. But that whole part is called as thallus.[16,17] The protection mechanism of brown algae to against light is through the xantofyl cycle[18] and can oxidize plants which depends on ascorbate existence, that is an enzymatically epoxy release from xanthophyl that occurs in strong light conditions (de-epoxidation). The

enzymes that plays this role are de-epoxidase and epoxidase.[19,20]

Since a long time ago, many living creatures from each kingdom in the taxonomy have been used as food and medicine, for example the use of sea cucumber and milkfish,[21,22] shiitake mushrooms, rice,[23] and

also this brown algae which are used to treat various types of diseases. Brown algae is a source of bioactive compounds because it is able to produce secondary metabolites that vary with extensive biological activity, including as an antioxidant to stop the chain reaction of free radicals.[24,25,26] Antioxidants are substances that can fight the harmful effects of free radicals that formed as a result of oxidative metabolic (the result of chemical reactions and metabolic processes that occur in human body).[27]

About 1.500, or even more, types of Phaeophyta or brown algae are already identified.[1,28] Below is the classification of brown algae with some examples of species that found in a research by Ode et al in Ambon Island:[29,30]

Table 1: Classification of Brown Algae

Kingdom	Phylum	Subfilum	Infraphylum	Superclass	Class	Order	Family	Genus	Species
Chromista	Ochrophyta	Phaeista	Limnista	Fucistia	Phaeophyceae	Dictyotales	Dictyotaceae	Padina	australist
						Fucales	Sargassaceae	Sargassum	crassifolium
									cinereum
									vulgare
								Hormophysa	cuneiformis
								Turbine	ornata

RESEARCHES IN ANTIOXIDANT ACTIVITY OF BROWN ALGAE

Table 2: Below is a comparison of several studies of brown algae's antioxidant activity based on the subjects, species, and methods.

No	Title (Author)	Subject	Method	Results				
1	Antioxidant effect of Sargassum polycystum (Phaeophyceae) against acetaminophen induced changes in hepatic mitochondrial enzymes during toxic hepatitis (Hanumantha Rao Balaji Raghavendran, Arumugam Sathivel, Thiruvengadum Devaki)	S. polycystum extract and male Wistar albino strain rats.	Rats were divided into 4 groups: (1) control, (2) only given acetaminophen 800 mg / kg, (3) only given S. polycystum extract 200 mg / kg, (4) given S. polycystum extract 200 mg / kg then acetaminophen 800 mg / kg.	Enzyme	1	2	3	4
				Lipid peroxide	168 ± 15.43	285.78 ± 25.16	164.33 ± 13.19	188.94 ± 17.18
				Glutathione	3.20 ± 0.28	2.21 ± 0.17	3.29 ± 0.31	3.16 ± 0.29
				Superoxide dismutase	42.14 ± 3.28	28.8 ± 2.11	43.60 ± 3.74	38.6 ± 1.91
				Catalase	128.71 ± 11.31	68.81 ± 5.99	125.67 ± 10.97	101.46 ± 7.94
				Conclusion: S. polycystum extract significantly reduces the severity of hepatitis due to acetaminophen, marked by increased the levels of superoxidase dismutase and catalase as well as a decrease in lipid peroxide as a cause of free radical formation.				
2	Intra-thallus	Cystose	Phenol extraction	Conclusion:				

<table>
<tr><td></td><td>variation of phenolic compounds, antioxidant activity, and phenolsulphatase activity in Cystoseira tamariscifolia (Phaeophyceae) from southern Spain (Roberto Teófilo Abdala-Díaz, Alejandro Cabello-Pasini, Eugenia Márquez-Garrido, Félix López Figueroa)</td><td>ira tamaris cifolia</td><td>from the collected algae that was carried out, then phenolsulfatase activity and antioxidant activity was analyzed using DPPH with the free radical method.</td><td>• The highest antioxidant activity was found in the apical part of the thallus which had the highest phenolic content.
• As getting down to basal part, phenolic levels and antioxidant activity and phenolsulfatasenya decreases.</td></tr>
<tr><td>3</td><td>Phytochemical Composition and Evaluation of Marine Algal Sargassum polycystum for Antioxidant Activity and In Vitro Cytotoxicity on Hela Cells (Ade Arsianti, Anton Bahtiar, Vincent Kharisma Wangsaputra, Norma Nur Azizah, Wilzar Fachri, Lince Dameria Nadapdap, Ajeng Megawati Fajrin, Hiroki Tanimoto, Kiyomi Kakiuchi)</td><td>S. polycystum and cervical cancer cells (HeLa cells)</td><td>Alga S. polycystum is extracted through maceration process with n-hexane, chloroform, ethyl acetate and ethanol. HeLa cells were cultured in the medium and given algae extracts.</td><td>Antioxidant activity against DPPH free radicals
Extract preparation | IC50 | Category
Ascorbic Acid (positive control) | 6.47 | Very strong
Ethylacetate extract of S. polycystum | 289.32 | Weak
Ethanol extract of S. polycystum | 624.76 | No antioxidant activity
Cytotoxicity of S. polycystum Extract against HeLa Cells
Extract preparation | IC50 | Category
Cisplatin (positive control) | 14.5 | Strong
n-hexane | 60.9 | Middle
ethyl acetate | 112.0 | Weak
chloroform | 38.3 | Middle
ethanol | 112.8 | Weak
Conclusion:
• Only ethylacetate S. polycystum extract has antioxidant activity.
• The anticancer strength of S. polycystum chloroform extract and n-hexane against HeLa cells is classified as moderate, the ethyl acetate and ethanol extracts are classified as weak anticancer.
• S. polycystum extract can be a natural source of antioxidants and anti-cervical-cancer.</td></tr>
<tr><td>4</td><td>Antihepatotoxic potential of Sargassum polycystum (Phaeophyceae) on antioxidant defense status</td><td>• Brown algae extract (S. polyc</td><td>Mice were divided into 4 groups: (1) control, (2) normal mice given 125 mg / kg of algae extract orally for 15 days, (3) mice</td><td>Group | ALT | AST | LDH
1 | 84.5 ± 7.26 | 95.2 ± 7.11 | 164 ± 11.2
2 | 86.9 ± 7.05 | 88.7 ± 7.48 | 152 ± 12.4
3 | 329 ± | 376 | 344 ±</td></tr>
</table>

	in Dgalactosamines induced hepatitis in rats (B. Meena, R. Anbin Ezhilan, R. Rajesh, A. Sheik Hussain, B. Ganesan, R. Anandan)	ystum) • Male albino rats Wistar strain	injected with GalN on intraperitoneal to induce hepatitis, (4) mice were given algae extracts orally 125 mg / kg for 15 days and then injected with GalN		28.6	± 32.3	27.3
				4	118 ± 9.41	135 ± 9.94	187 ± 14.1
				Conclusion: In a dose of 125 mg/kg algae extract contains antioxidant capacity that is able to neutralize free radicals and works as hepatoprotector.			

RESEARCHES IN ANTIVIRUS ACTIVITY OF BROWN ALGAE

Table 3: Below is a comparison of several studies of the brown algae extracts antiviral activity based on the subjects, species, and methods.

No	Title (Author)	Subject	Method	Results		
1	In vitro antiviral activity of diterpenes isolated from the Brazilian brown algae Canistrocarpus cervicornis (Magui Aparecida Vallim1, Juliana Eymara Barbosa1, Diana Negrão Cavalcanti, Joel Campos De-Paula, Viveca Antonia Giongo Galvão da Silva, Valéria Laneuville Teixeira and Izabel Christina Nunes de Palmer Paixão)	• Extracts of 2 types of diterpene from brown algae Canistrocarpus cervicornis: dolastane diterpenes 4-hydroxy-9,14-dihydroxydolasta-1 (15), 7-diene (diterpene 1) and 4,7,14-trihydroxydolasta-1 (15), 8 -diene (diterpene 2). • Kidney cells from African monkeys (cultured in Dulbecco's modified Eagle's medium), known as Vero cells	Vero cells are given diterpene 1, diterpene 2, and acyclovir for 72 hours.	Intervention	CC_{50} µM	Inhibition (%)
				Diterpene 1	1423 (± 83)	90
				Diterpene 2	706 (± 100)	99
				Acyclovir	860 (± 32)	99
				Conclusion: Both types of diterpene, especially diterpene 2, have significant inhibition of HSV-1 replication, even equal to the antiviral ability of acyclovir.		
2	Therapeutic efficacy in BALB / C mice of extract from	• C. cervicornis extract	Mice received intervention in the form of an incision with a sterile scalpel	Group	Treatment Results	
				1	This group has the highest	

<table>
<tr><td></td><td>marine algae Canistrocarpus cervicornis (Phaeophyceae) against herpes simplex virus type 1 (Caroline de Souza Barros, Valeria Garrido, Vanessa Melchiades1, Rafaela Gomes, Max Willian Lisboa Gomes, Valeria Laneuville Teixeira, Izabel Christina Nunes de Palmer Paixão)</td><td>• Four groups of BALB /C mice: (1) did not receive any therapy, (2) received regular topical medication containing 70% vaseline, 29.97% lanolin and 0.03% hydroxytoluene butylate, (3) received topical medication containing 2% of C. cervicornis extract, (4) received a topical drug containing 5% acyclovir</td><td>and then inoculated 10 microliters of HSV-1 KOS. Management of topical drugs each given since 1 hour after inoculation of the virus, repeated three times a day for 16 days.</td><td colspan="5"><table>
<tr><td></td><td>disease score, even experiencing back limb paralysis (disease score 8)</td></tr>
<tr><td>2</td><td>This group has the most severe lesion. The lesions began to improve on the eleventh to fourteenth day.</td></tr>
<tr><td>3</td><td>This group experienced a peak of infection within six days (disease score 2.8), improvement starting from the seventh day.</td></tr>
<tr><td>4</td><td>As a control group and comparison of effectiveness with alga extract.</td></tr>
<tr><td colspan="2">Conclusion:
Significantly better improvement was found between the groups that given algae extract and acyclovir compared to the group that did not receive any treatment.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Anti-HSV1 activity of brown algal polysaccharides and possible relevance to the treatment of Alzheimer's disease (Matthew Wozniak, Tracey Bell, Ádám Dénes, Ruth Falshaw, Ruth Itzhaki)</td><td>Five species of brown algae: Scytothamnus australis, Marginariella boryana, Papenfussiella lutea, Splachnidium rugosum and Undaria pinnadifida.</td><td>Six algae extracts were prepared: (1) M. boryana resproductive extract with 1% H2SO4, (2) M. boryana vegetative extract with 1% H2SO4, (3) P. lutea extract with 1% H2SO4, (4) Scy australis extract with 1% H2SO4, (5) Spl. rugosum extract with 0.2 M HCl and (6) U. pinnatifida extract with 0.2 M HCl. Antiviral activity was analyzed using plaque reduction assay (PRA) and immunocythocemistry (ICC).</td><td colspan="5"><table>
<tr><td>Extract</td><td>PRE</td><td>ICC</td><td>A-beta</td><td>P-tau</td></tr>
<tr><td>1</td><td>> 20</td><td></td><td></td><td></td></tr>
<tr><td>2</td><td>3.75</td><td>7.57</td><td>0.68</td><td>3.16</td></tr>
<tr><td>3</td><td>0.75</td><td>0.98</td><td>0.72</td><td>0.86</td></tr>
<tr><td>4</td><td>> 20</td><td></td><td></td><td></td></tr>
<tr><td>5</td><td>0.87</td><td>1.18</td><td>1.81</td><td>2.18</td></tr>
<tr><td>6</td><td>0.72</td><td>0.77</td><td>0.63</td><td>0.83</td></tr>
<tr><td>ACV</td><td></td><td>> 20</td><td></td><td></td></tr>
<tr><td>ACVa</td><td>0.19</td><td>19.4</td><td></td><td></td></tr>
<tr><td colspan="5">Conclusion:
Extract 2, 3, 5 and 6 have strong antiviral activity, the rest are weak.
Between those four types of extracts, all are significantly effective to against A-beta, but only extract 3 and 8 are effective at reducing P-tau.</td></tr>
</table></td></tr>
</table>

DISCUSSION

Many studies have been conducted using different species of brown algae to find out its beneficial and potential content for humans. Fucoidan from several types of brown algae effectively works as an antimicrobial.[31] Padina has antibacterial abilities, while the Sargassum besides working as an antibacterial, it also has antitumor activity.[32] In general, antiviral activity, antioxidants, immunomodulators and many other activity have been found in fucoidan from this brown algae,[33] it is a sulfation polysaccharide compound.[34]

Raghavendran et al examined the effect of antioxidants from Sargassum polycistum on mitochondrial enzymes in mice with hepatitis due to the excess dose of acetaminophen. The results significantly showed hepatitis mice that given S. polycystum extract had higher levels of superoxidase dismutase and catalase and lower level of lipid peroxide which caused free radical formation than hepatitis mice that did not get S. polycystum extract.[35]

Abdala-Diaz et al found that the antioxidant and phenolsulfatase activity of 3 parts of brown algae thalus Cystoseira tamariscifolia were significantly related to the levels of phenolic compounds they contained. The higher phenolic compounds, will produce a better protection system against ultraviolet radiation. The highest antioxidant and phenolsulfatase activity was found in the apical part of the thallus which had the highest phenolic content. Getting down to basal, phenolic levels, antioxidant and phenolsulfatase activity are reduced.[36] This concentration difference in each parts is caused by many factors, including due to the season, the level of photosynthetic radiation and ultraviolet exposure level.[37,38]

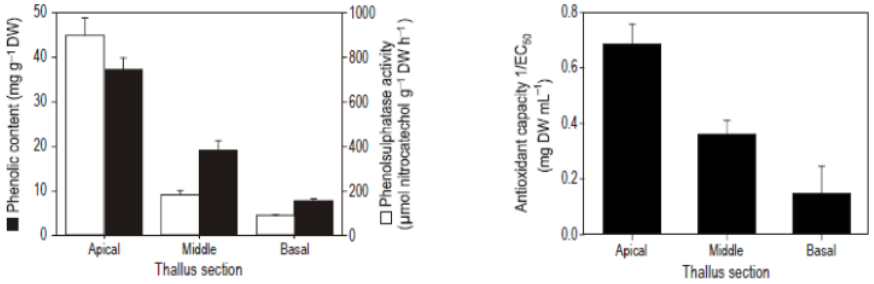

Fig.1: The difference of phenolic levels in each parts of thallus and its relation to the levels of antioxidant activity[36]

Arsianti et al was found the same result in their research, that phenolic concentration were directly proportional to the antioxidant activity produces. He conducted an analysis of the Sargassum polycystum antioxidant capacity and observed its anticancer activity against cervical cancer cells or called HeLa cells. The results showed only its ethylacetate extract has antioxidant activity. Meanwhile, as an anticancer, the ability of S. polycystum chloroform and n-hexane extracts to against HeLa cells are moderate, while the ethyl acetate and ethanol extracts are weak. Arsianti et al conclude that S. polycystum extract can be a natural source of antioxidants and anti cervical cancer.[39]

Meena et al in their study in mice found that Sargassum polycysticum extract at the certain dose was also effective in hepatitis induced by D-galactosamine treatment. The hepatitis mice group was given 125 mg/kg S. polycystum extract for 15 days. As the result, its antioxidant activity inhibits lipid peroxidation and decreases levels of liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in mice with hepatitis due to D-galactosamin. Meena et al concluded in a dose of 125 mg/kg algae extract contains antioxidant capacity that is able to neutralize free radicals and works as hepatoprotector.[40]

Infection can occur due to various pathogens. One of infection markers is an increase in leukocytes level, including in viral infection.[41,42,43,44] Many studies have analyzed the antiviral capacity in brown algae. Vallim et al examined two types of diterpene in Canistrocarpus cervicornis, namely dolastane diterpenes 4-hydroxy-9,14-dihydroxydolasta-1 (15), 7-diene (diterpene 1) and 4,7,14-trihydroxydolasta-1 (15) 8-diene (diterpene 2) as anti herpes simplex virus 1 (HSV-1). He used kidney cells from African monkeys that were cultured in Dulbecco's modified Eagle's medium (called Vero cells), then the Vero cells were given diterpene 1, diterpene 2 and acyclovir for 72 hours. The results showed that replication of HSV-

1 virus was inhibited by both types of diterpenes, especially diterpene 2 that has the same antiviral ability as acyclovir (99%).[45]

Barros et al conducted a similar study, using Canistrocarpus cervicornis extract and BALB/C mice. Group of mice infected with HSV-1 by dissected their skin. The skin lesions were treated with topical medication containing algae extract 3 times per day for 16 days. The results showed the result was as good as the group of mice that were given acyclovir. Both groups were significantly better than another that did not receive any treatment.[46]

Another study by Wozniak et al on the potential anti-herpes simplex 1 activity in five species of brown algae as an alzheimer's disease drug. The cells that infected with HSV-1 cause beta amyloid deposition and AD-like tau (P-tau). Beta amyloid deposits are the main cause of senile plaques that found in alzeimer patients, this is a significant marker for this disease.[47] The algae extract used in the study were Scytothamnus australis, Marginariella boryana, Papenfussiella lutea, Splachnidium rugosum and Undaria pinnadifida. Six kinds of algae extracts, namely (1) M. boryana reproductive extract with 1% H_2SO_4, (2) M. boryana vegetative extract with 1% H_2SO_4, (3) P. lutea extract with 1% H_2SO_4, (4) Scy. australis extract with 1% H_2SO_4, (5) Spl. rugosum extract with 0.2 M HCl and (6) U. pinnatifida extract with 0.2 M HCl, their antiviral activities were analyzed using a plaque reduction assay (PRA) and immunocythocemistry (ICC). The results showed extract 2, 3, 5 and 6 have strong antiviral activity, the rest are weak. Between those four extract types, all are effective against A-beta, but only extract 3 and 8 are effective at reducing P-tau.[48]

CONCLUSION

There have been many studies that prove that brown algae have antioxidant and antiviral activity. However, it's activity level can be differ between each brown algae species and depending on part of the thallus.

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