Phytochemical constituents and antidiabetic activity of edible marine redseaweed (Halymenia durvilae)

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Abstract. Seaweeds have bioactive compounds with enormous health prospective which interests the pharmaceutical industries. The isolated bioactive compounds of seaweeds have been utilized as drug and food in the world. Phytochemical constituents of seaweeds have an assortment of prospective biological activity, such as antidiabetes. In worldwide the appearance of type two diabetes mellitus (T2DM) as the greatest non-transmittable disease has motivated search for new antidiabetic approaches. The purpose of the research was to determine the phytochemical properties and antidiabetic effect using α-glukosidase on methanol extract, n-hexane, chloroform, and water fraction of marine red algae *Halymenia durvilae*. The result of this study showed the phyto-constituent of *H.durvilae* includes steroids, flavonoids and triterpenoids are present in all extracts. Saponins and hyquinones showed their presence only in methanol extract. Alkaloid and tannin were not present in methanol extract and its fractions. *H.durvilae* on the extract and its fractions had antidiabetic activity. Water fraction had the highest activity to inhibit α-glukosidase (IC_{50} 4.34±0.32 mg mL) followed by chloroform, hexane and methanol extract. Therefore, it can be concluded that *H.durvilae* could be used as a dietary food source of bioactive compound especially natural antidiabetic compounds.

Keyword: antidiabetic, *Halymenia durvilae*, seaweed

1. Introduction

Over the past three decades seaweeds have included one of the wealthiest and largest amount potential resources of bio-functional compounds in industry [1]. In excess of 2400 marine ordinary bioactive compounds as we distinguished as secondary metabolites of seaweed have been isolated which possess a wide variety of bio-functional properties. Seaweeds have been utilized by human as drug and food and their extracts have yielded an immense quantity of attention in medicinal manufacturing as a fresh source of bioactive compound with enormous therapeutic prospective [2].

Edible seaweeds contain low calories and large amount of dietary fibers, unsaturated fatty acids and vitamins appropriate for administrating diabetes [3]. Seaweed bioactive compounds have important responsible in the inflection of glucose-stimulated oxidative stress, reserve of starch-digestive enzymes [4, 5].

Diabetes mellitus is one of multifarious diseases typed by chronic hyperglycemia because of shortage in insulin secretion or resistance [5, 6]. The encouragement and improvement of endogenous insulin secretion, and restrain ordinary nutritional enzymes for instance α-amylase and α-glucosidase are the presently existing therapeutic method [7]. Alga glucosidase inhibitor functioned as medically oral

antihyperglycemic agents, for example acarbose and voglibose. However they often instigate harsh gastrointestinal side impact. Consequently, the medication of postprandial hyperglycemia have developed into an attractive method for explore new α-glucosidase inhibitors from natural resources [8].

Ecklonia cava, a marine brown algae contains phloroglucinol derivatives, had capacity to inhibit α-Glucosidase and α-amylase activities [4, 9]. Polysaccharides drawn from seaweed mostly in low molecular weight oligosaccharides are also potential as stimulant of insulin secretion [10].

The majority of marine algae from North Sulawesi, Indonesia are still unexploited for bioactive substance and there are no previous report of an antidiabetic activity of *H.durvilae* that grow abundantly in North Sulawesi. This research aimed to determine the phytochemical compound qualitatively and antidiabetic effect on *H. durvilae* by *in vitro* assays using α-glucosidase enzyme.

Seaweed is an enormous source of carotenoids, pigments, polyphenols, enzymes, various useful polysaccharides and is also an admirable source of vitamin A, B1, B12, C, D and E [2]. From the previous researches, it is well known that seaweed contain antibacterial, antiviral, antidiabetic, cytotoxic and anticancer compounds [2, 10, 11]. Due to their potentially beneficial activities, seaweed have been intensive studied.

Seaweeds ethanol extracts commonly consisted of polyphenol compounds about 70-80% and polysaccharides around 20-30%, while seaweeds water extracts have extremely small quantity of polyphenols and > 90% polysaccharides. *Gracilaria* species contain phycocolloids, the major source of agar α-(1,4)-3,6-anhydro-L-galactose and β-(1,3)-D-galactose with slight esterification in cell wall. Therefore they are essential substances for the manufacturing and biotechnological purposes [12, 13].

Diabetes mellitus is one of acute diseases, recognized from hyperglycaemia, a situation characterized as a result of an extreme degree of glucose flowing in the blood [1] with unsteadiness in carbohydrate, fat and protein metabolism [8]. Diabetes mellitus categorized into two major types specifically Type one diabetes mellitus (T1DM), instigated of the total insulin manufacture nonattendance because the auto-immune refereed collapse of pancreatic β-cells. Type two diabetes mellitus (T2DM), which caused by the virtual insufficiency of the similar hormone concerning insulin defiance, peculiar forming of hepatic glucose and growing relapse of pancreatic β-cell roles [1].

T2DM reports roughly 90% of diabetes situation global, edge on epidemic magnitude impressing both developed and developing countries [11]. This ascending is featured to larger occurrence of inactive daily life, detrimental diet and increasing of obesity inside contemporary civilization, as well as a rising number of old populations [2, 14].

Diabetes is regularly associated with enlarged risk in hypertension, vascular complications, blindness and kidney malfunction [12]. Macrovascular complications have been studied to be higher in T2DM patients with the probability of emergent diseases connecting the human vascular tree for instance stroke, coronary artery disease and peripheral arterial disease to be fourfold greater, which extended reasonably formerly in diabetic than non-diabetic patients. Sufferers of T2DM usually have decreased life hope by reason of these diverse co-morbidities [11].

One of the healing methods for managing diabetes is to diminish postprandial hyperglycaemia. This can be attained by suspending the absorption of glucose by means of reducing of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase in digestive tract. The α-glucosidase can delay the relase of glucose from dietary multifarious carbohydrate and obstruction glucose absorption, consequently a diminished postprandial plasma glucose level and decreased postprandial hyperglycaemia [8].

Hyperglycemia can be treated in two ways, namely by using injections of insulin and oral antidiabetic medications such as sulfonylureas, biguanide, thiazolidinedion, and by supplementing or

functionalizing food. Functional food are like a drug and they can also inhibit the enzyme glucosidase and amylase so that it can inhibit the absorption of glucose for patients with T2DM [15].

2. Materials and Methods

2.1. *Sampling*

Red seaweed *Halimenia durvilae* was collected from North Sulawesi Coastal Area of Indonesia. The fresh sample was carefully cleaned with marine water and fresh water to eliminate epiphytes, and grime particles [16]. The sample was then delivered to the laboratory for future analysis.

2.2. *Preparation of the sample extract and fractions*

Sample (1 kg) extracted using 70% methanol (1/2 w/v) overnight for 3 times in room temperature, then filtered with Whatman paper No. I and converged down to 500 ml at 40 °C by rotary vacuum evaporator. The extract obtained was dissolved in 1 L ethyl acetate for 3 times and concentrated by rotary evaporator, and then the undissolved residue was removed. The ethyl acetate extract was subjected to the first solvent-fractionation step of using 500 mL of an aqueous 90% methanol solution and 3.5 L of n-hexane. As a result of this fractionation, bioactive ingredients were found in the aqueous methanol solution layer. Again, this aqueous solution layer was subjected to the second solvent-fractionation step of using 500 mL of an aqueous 30% methanol solution and 1 L of chloroform. Thereafter, the aqueous 30% methanol solution layer was dried in vacuo. The extract and fraction were stored in -20 °C before analysis [17].

2.3. *Chemicals and reagents*

α-glukosidase, ρ-nitrophenil-α-D-glucopyranoside, phosphate buffer, sodium carbonate were bought from Sigma-Aldrich (St. Louis MO, USA) .

2.4. *Phytochemical analysis*

The phytochemical analysis qualitatively of *Halimenia durvilae* extract consisted of alkaloids, tannins, saponins, flavonoids, triterpenoids/steroids and hydroquinon.

2.4.1. Test for alkaloids. Test for alkaloids of methanol extract and fractions were estimated by the method of [18] with slight modification. Briefly, 0.5 g of sample was added with a few drops of ammonium and dissolved in 5 mL $CHCl_3$. Filtrate was added with 2-5 drops of H_2SO_4 M and shaken until it formed 2 layers. The filtrate at upper layer (acid layer) dissolved in the three reagens. Then observated the establishment of precipitates and any color changes. Purple (Dragendroff reagent) and Brown (Wagner reagent), White creamy impetuous (Meyer reagent) imply the attendance of alkaloids.

2.4.2. Test for tannins. Test for tannins of crude methanol extract and fractions were determined based on the method of Mehdinezhad [20]. Briefly, 1 g of sample was boiled using 20 mL of distilled water for 5 minutes in water bath and filtered. The cold filtrate (1 mL) was diluted into 5 mL of aquades and added with 2-3 drops of 10% ferric chloride, and then detected the establishment of impulsive and any color transformation. Forming a bluish-black or brownish-green sudden shown the attendance of tannins.

2.4.3. Test for saponins. Test for saponins of crude methanol extract and fractions were estimated [19]. Briefly, 100 mg of sample was boiled with 1 mL of aquades and filtered. Then 0.5 mL of aquades was added in filtrate and quivered strongly for about 5 minutes. Formation of bubbles (froth) which persevered on affectionate suggested the existence of saponins.

2.4.4. Test for flavonoids. Test for flavonoids of crude methanol extract and fractions were determined according to the method of [19] with slight modification. Briefly, 1 g sample was boiled with 10 mL of aquades, added with 5-10 drops of HCL and small piece of magnesium and then boiled for few minutes. Formation of reddish-brown color designated the attendance of flavonoids.

2.4.5. Test for triterpenoid/steroid. Test for triterpenoids/steroids of crude methanol extract and fractions were determined by the method prescribed [20]. Briefly, one-grams of sample was put in a test mixer (test tube) and added with 5 ml of 50% ethanol, heated for 3 minutes in a water bath and let to cool at room temperature and filtered. The filtrate was dried a dish evaporator and it was then dissolved in 1 ml of diethyl ether and mixed for 5 minuntes. The ethyl ether fraction was then decanted and removed. After that 10 ml of chloroform was added and stirred for about 5 min, it was then removed into test mixer, added with 0.5 mg of anhydrous sodium sulphate, quaken softly and filtered, the filtrate was then alienated into two test tubes for applied to the following tests:
-Liebermann-Burchard's reaction: To test mixer I, the same volume of acetic anhydride was added and shaken tenderly. Then 1 ml pure sulfuric acid was put down to the side of the tube. The formation of a brownish-red ring at the contact region of the two liquids and a greenish color in the partition layer specified the attendance of sterols and triterpenes.
-Salwoski's test: To test mixer II, 2 to 3 drops of pure sulphuric acid was added to construct a lower layer. Formation reddish-brown color at the inter stage implies the existence of steroidal ring.

2.4.6. Test for Hydroquinones. Test for hydroquinones of crude methanol extract and fractions were determined [18], with slight modification. Briefly, 1 g sample was boiled with 2 ml of methanol for 5 minutes, filtered even as it was hot and let to cool and then subjected with 3 drops of 10% NaOH. Formation a red color designed the presence of hydroquinone.

2.5. α-glucosidase inhibition assay
The α-glucosidase inhibitory activity the extract and fractions were performed following to the method reported [21], with a slight modification. Briefly, sample (10 µl) at diverse concentration (0.156, 0.312, 0.625, 1.25, 2.5 and 5 mg/mL), 20 µl α-glucosidase (0.5 unit/ml), 120 µl of 0.1M phosphate (pH 6.9) were mixed. The solution was incubated in 96-well plates at 37°C for 15 minutes (pre-incubation). After incubating the enzymatic reaction was instigated by adding 20 µl of 5 mM phosphate buffer (pH 6.9) and again incubated for 15 minutes at 37°C. The reaction was ceased by adding 80 µl of 0.2 M sodium carbonate, the absorbance was determined at 405 nm by ELISA (Epoch, Biotech, USA. Positive control using glucobay (0.1; 1; 5 and 10 μ/mL). the reaction system exclusive of extract was performed as control and the system exclusive of α-glucosidase was performed as blank for adjusting the surroundings absorbance. The percent inhibition of α-glucosidase was calculated using the following equation (1).

$$\% \text{ inhibition} = \left(\frac{\text{control absorbance} - \text{sample absorbance.}}{\text{control absorbance.}}\right) \text{x } 100 \quad (1)$$

3. Results and Discussion

3.1. Phytochemical constituents
Phytochemical constituents of crude methanol extract of *Halymenia durvilae* and its n-hexane, chloroform, and water fraction is depicted in table 1. The phyto-constituent present in the extract and all fractions includes steroids, flavonoids and triterpenoids, saponin and hydroquinone showed its presence only in methanol extract. Alkaloid and tannin were not attendancein the extracts and fractions.

Saponins are essential to avoid disease offensive to plants by parasitic fungi and this property may be accountable for antimicrobial activity of seaweed extracts [19]. In traditional chinese medicines saponins are respected as a key ingredient and are accountable for the the majority of the detected biological activity. Saponins give respons on inflammation and are exported commercially as nutritional complement [2]. Saponins contain distinctive residue like 2, 3-dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), which permits saponins to scavenge superoxides by resulting hydroperoxide intermediates which avoids bio-molecular injury [22].

Table 1. Phytochemical constituents in methanol extract, hexane, chloroform and water fraction of *H. durvilae*.

Phytochemical Constituents		Extract and fractions			
		Methanol	Hexane	Chloroform	water
Alkaloids	- Wagner	-	-	-	-
	- Mayer	-	-	-	-
	- Dragondorf	-	-	-	-
Steroids		+	+	+	+
Flavonoids		+	+	+	+
Tannins		-	-	-	-
Saponins		+	-	-	-
Triterpenoids		+	+	+	+
Hidroquinons		+	-	-	+

Steroids, flavonoids and triterpenoids showed its presence in all of the extracts. Plant are source of steroids which are recognized to be the principal by reason of insecticidal, antimicrobial, antiparasitic and cardiotonic capacities. Steroids also have essential purpose in diet, herbal drug and cosmetics [2]. Flavonoids are also an important antimicrobial agent found in marine and terrestrial plants. Flavonoids, particularly isoflavonones, have higher capacity in contrary to gram positif bacteri than gram negative bacteria. Isoflavanones, isoflavons, and isoflavonones have activity against fungal pathogens [19]. Flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins are proven have anti-tumour and antioxidant activity [23].The composition of steroid, flavonoids and triterpenoids, saponins and anthroquinones in red algae *Champia parvula*, each were 24.30±0.11, 10.17±01, 55.33±0.14, 22.50±0.11 and 10.43±0.00 mg g^{-1} [23].

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3.2. Antidiabetic activity

Alga glukosidase inhibition (% inhibiton) of crude methanol extract of *H. durvilae* and its n-hexane, chloroform, and water fraction are shown in figure 1. The result showed all the test concentration in extract and fractions have α-glucosidase inhibition activity. The inhibition activities of extract methanol and hexane, chloroform and water fraction (5 mg mL^{-1}) each were 18.71±5.4, 17.53±3.55, 33.9±2.41, 44.56±1.37%. Water fraction showed the highest inhibition (IC_{50} 4.34 ±0.32 mg mL^{-1}). Aqueous extract of red seaweed *G. edulis and G. corticata* showed good α-glucosidase inhibitory property [8].

In human body α-amylase and α-glucosidase react simultaneously to digest by breaking down starch via pancreatic α-amylase and the absorption of glucose by intestinal α-glucosidase. Pancreatic α-amylase is a key enzyme that ascertains the degree of starch digestion by the hydrolysis of inner α-1,4-glucosidic linkages, and yields linear and branched malto-oligosaccharides. These are then acted on by α-glucosidases, which have important function in the exchange of carbohydrates into glucose, and this may initiate postprandial hyperglycemia [5].

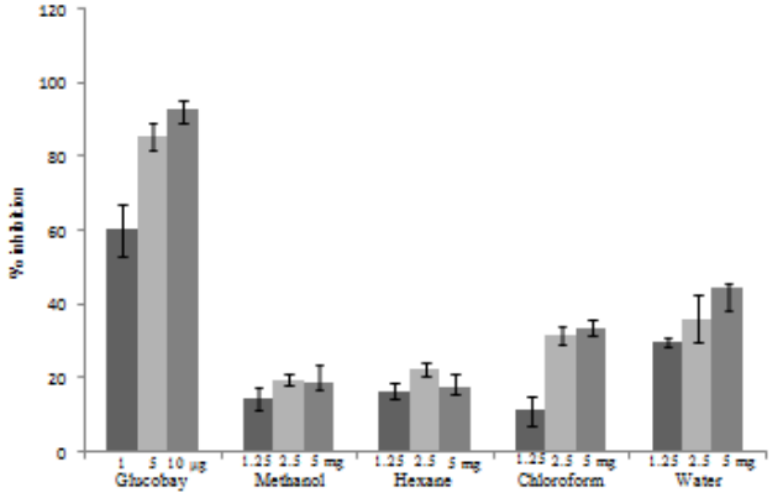

Figure 1. α-glucosidase inhibition activity of glucobay, methanol extract, hexane, chloroform and water faction of *H.durvilae*.

A valuable approach for diminishing postprandial hyperglycaemia is the reserve of pancreatic α-amylase and α-glucosidase, which holdups the carbohydrate digestion and glucose absorption appreciably [5, 24]. Many algae which were extracted with several organic solvents were potential inhibit α-amylase and α-glucosidase. Acetone crude extract of *Caulerpa racemosa* and *Spatoglossum schroederi* restrained the α-amylase activity, ED_{50} of 0.09 mg/mL and 0.58 mg mL, respectively [7]. Fucoidan which extracted from *Ascophyllum nodosum* was capable to obstruct α-glucosidase and α-amylase activity, while fucoidan from *Fucus vesiculosus* is barely active against α-glucosidase. Fucoidan from *A. nodosum* also inhibit α-amylase activity between 7% and 100% (5 mg/mL) (IC_{50} 0.12 to 4.64 mg/ mL) [25]. Phenolic compounds in seaweed *Ascophyllum nodosum* reported have capacity to against α-glucosid [26], as well as fatty acid in *Spatoglossum macrodontum* [27]. In the present study it is shown that inhibitory activity against α-glucosidase of methanol extract of *H.durvilae* (5 mg mL^{-1}) was 18.71±5.4 %. Total phenolic content of 70% methanol extract of *H.durvilae* was 18.83±0.77 g GAE/100 g [2 and the ethanol extract was 7,605±0.383 μg GAE g^{-1} [29]. Two pure compounds of bromophenols from red seaweed *Grateloupia elliptica* prevented α-glucosidase activity to diverse organisms. Inhibitory capacity of 2,4,6-tribromophenol was more intense than 2,4-dibromophenol against *Bacillus. stearothermophilus* and *Saccharomyces.cerevisiae* α-glucosidase particular, while 2,4-dibromophenol was comparably stronger against rat-intestinal maltase and sucrase but relatively more delicate than acarbose and voglibose, perhaps because of variances in particular substrates [25].

4. Conclusion

The result of the study can be concluded that various various phytochemical constituents detected in *H. durvilae* and showed potential to inhibit α-glucosidase activity. Therefore, it be utilized as a functional food especially for the treatment of diabetes. Future studies are required to evaluate the effect of the extracts in diabetic rats and to isolate the active compound which is responsible for antidiabetic effect.

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