Original Article

Hypolipidemic activity of ethanolic extract of *Caulerpa recemosa*

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Abstract

Background: *Caulerpa recemosa, also known as sea grape, is a stout straggling prostrate shrubby plant with spinous stipules and uniaxial siphonous thallus, mostly divided into a creeping axis (stolon), with rhizoids and erect shoots (fronds), either nude, leaf-like or with grape or feather-like ramuli. Caulerpa racemosa has erect fronds up to 9-13 cm high bearing un-crowded vesiculate ramuli that are radially arranged. Fronds are slightly inflated above the attachment to the stolon which are fixed to the substrate by thin short rhizoids. It is collected from St. Martin's Island locally called 'Narikel Jinjira', located on the southernmost tip of Bangladesh for the present study, ethanol extract of* Caulerpa recemosa *has been screened for its hypolipidemic activity.*

Methods: *Hypolipidemic activity was screened by inducing hyperlipidemia with the help of atherogenic diet in wistar albino rats and serum levels of various biochemical parameters such as total cholesterol, triglycerides, low density lipoprotein and high density lipoprotein cholesterol were determined. Atherogenic index shows the measure of the atherogenic potential of the drugs.*

Results: *Ethanol extract showed significant ($p < 0.01$) hypolipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, low density lipoprotein and increase in high density lipoprotein level which was similar to the standard drug atorvastatin. Ethanol extract exhibited significant atherogenic index andpercentage protection against hyperlipidemia. Preliminary phytochemical analysis revealed the presence of phytoconstituents such as steroids, flavonoids, glycosides, alkaloids, phenoliccompounds.*

Conclusion: *The overall experimental results suggest that the biologically active phytoconstituents such as flavonoids, glycosides alkaloids present in the ethanolic extract of Caulerpa racemosa may be responsible for the significant hypolipidemic activity and the results may justify the use of Caulerpa racemosa as a significant hypolipidemic agent.*

Keywords: *Hypolipidemic activity, atherogenic potential, Caulerpa racemosa, low density lipoprotein, high density lipoprotein.*

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Introduction

Seaweeds are wonder plants of the sea and considered as medicinal food of the 21st century. Marine macroalgae, popularly known as seaweeds are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and are grouped into green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae. Marine algae from Indian coasts amounting to 844 species (including forma and varieties) are distributed among 217 genera. Seaweeds are primitive non-flowering plants without true root, stem and leaves. They grow in the intertidal, shallow and deep sea areas up to 180 meter depth and also in estuaries, backwaters and lagoons on

solid substrates such as rocks, dead corals, pebbles, shells, mangroves and other plant materials.[1]Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities[2] with antiviral, antibacterial and antifungal activities which acts as potential bioactive compounds of interest for pharmaceutical applications. The identification of bioactive compounds present in marine algae is a new potential area.[3] Seaweeds or macroalgae belong to the lower plants, meaning that they do not have roots, stems and leaves. Instead they are composed of a thallus (leaf-like) and sometimes a stem and a foot. Some species have gas-filled structures to provide buoyancy. They are subdivided in three groups, the red, green and brown macroalgae. Most of these bioactive substances isolated from marine algae are chemically classified as brominates, aromatics, nitrogen-heterocyclic, nitrosulphuric heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated-polysaccharides.[4] The polysaccharides are an important component of algae. The immense interest in them is because of their broad spectrum biological activity. Polysaccharides exhibiting anticoagulant, antitumor and other activities have been isolated from green algae *Caulerpa racemosa*, *Caulerpa racemosa* mainly grow in tropical regions, although some varieties may be found in subtropical regions. In South East Asian countries, it is usually served raw as a salad or eaten cooked. In the literature, among which the important one to mention are the antitumor, anti-inflammatory and growth regulator.[5] The preliminary investigation on the crude methanol extract and phases from *Caulerpa racemosa* showed antinociceptive activity.[6] Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, peroxyl radical and nitric oxide radical attack biological molecules such as lipids, proteins, enzymes, Deoxy ribonucleic acid and Ribonucleic acid, leading to cell or tissue injury.[7] The genus *Caulerpa* has attracted the attention of researchers due to its important secondary metabolite caulerpenyne (CYN) that is reported to exhibit the antineoplastic, antibacterial and antiproliferative activities.[8,9] The main objective of the present study was to evaluate the hypolipidemic effect of *Caulerpa racemosa* green seaweed.

These may be used in combination if a single drug is not effective in reaching target levels. Fibrates and extended-release niacin may be used to lower triglycerides or raise high density lipoprotein cholesterol levels.[10]

Hyperglycemia and dyslipidemia are significant and independent risk factors for the vascular complications and suggested to cause cardiovascular pathologic changes in diabetic states through the following molecular mechanisms: formation and accumulation of advanced glycation products, increased oxidative stress, activation of protein kinase C pathway, increased activity of hexosamine pathway, and vascular inflammation and the impairment of insulin action in the vascular tissues.[11]

Methods

Plant material

Caulerpa racemosa (Forsskal) J. Agardh was collected from Saint Martin Island, Bangladesh in March 2017.St. Martin's Island locally called 'Narikel Jinjira' is located on the southernmost tip of Bangladesh, roughly between *20°341- 20°391* N and 92°181 - 92°211 E, separated from the mainland by a channel that is about 9 km wide. The island is located in the Northeastern part of Bay of Bengal and while being within the tropical belt; its weather is heavily influenced by the subtropical monsoon climate that prevails over Bangladesh.The organoleptic studies indicated the useful diagnostic features of *Caulerpa* species. It has a uniaxial siphonous thallus mostly divided into a creeping axis (stolon) with rhizoids and erect shoots (fronds) either nude, leaf-like or with grape-or feather-like ramuli. *Caulerpa racemosa* has erect fronds up to 9 -13 cm high bearing un-crowded vesiculate ramuli that are radially arranged. Fronds are slightly inflated above the attachment to the stolon which are fixed to the substrate by thin shortrhizoids.[12]

Caulerpa racemosa distributed in Maharashtra (Bombay, Malwan), Gujarat (Dwarka, Okha, Sauraqshtra, Veraval), Tamil Nadu (Tirunelveli, Idinthkarai; Kuttapuli, Palk Bay, Pamban, Mandapam, Tuticorin, Krusadai Island, Gulf of Mannar, Kerala (Kovalam), Andhara Pradesh (Visakapatnam), Goa, Karnataka, Lakshadweep Island and Andaman and Nicobar Islands.[13]

Preparation of extract

After subsequent washing in fresh water the algae was shade dried for two weeks continuously. The shade-dried seaweed was partially powdered using domestic blender

and stored in air tight container for further experiments. From these stock, secondary metabolites of seaweed (100g), was extracted successively using (150mL) solvent ethanol. The sample was kept in dark for 96 hours. After incubation, the extract thus obtained was decanted and filtered. The clear extract was subsequently concentrated using rotary vacuum evaporator and kept in dark bottles in 4° C until use.[4]Coarse powders (100 g) of marine algae was individually extracted with sufficient quantityof solvent ethanol for 48 hours by maceration and then filtered to obtain crude extract. The extract was stored in air tight glass container at 4-8°C for further analysis.

Preliminary phytochemical test for extracts

Preliminary phytochemical test was performed for the identification of different class of chemical constituents present in *Caulerpa racemosa.* Results of preliminary phytochemical screening are compiled in Table I.Overall screening of extract indicated the presence of carbohydrate, glycoside, alkaloids, tannins, saponin, steroid and triterpenoid.

Table I Preliminary phytochemical analysis

Class of phytoconstituents	Ethanol extract
Alkaloids	++
Carbohydrates	-
Glycosides	-
Tannin / Phenolics	+
Flavonoids	+
Steroid / Triterpenoids	+
Amino acids	-
Saponins	+

Hypolipidemic activity[14]

The animals were divided in to four groups with six animals in each group. In order to render the hyperlipidemia in rats, they were given an atherogenic diet comprising of corn flour base, milk powder, butter, salt, groundnut oil, sucrose, and vitamin mixture. In addition 400 *mg* of cholesterol powder per kg body weight was dissolved in coconut oil and administered orally for 45 days. Group I was considered as control which received 0.5% sodium carboxy methylcellulose; Group II was considered asatherogenic group and received the atherogenic diet; Group III was the test group which received the test extract that is ethanol extract of *Caulerpa recemosa* at the dose of 200 *mg/kg* body weight per oral along with the atherogenic diet and Group IV was considered as standard group which received the standard drug atorvastatin (dose of1.2 *mg/kg* body weight per oral) along with the atherogenic diet. At the end of 45th day, blood was withdrawn from the retro orbital plexus after overnight fasting for the study of biochemical parameters. Serum was estimated for the total cholesterol, triglycerides, low density lipoprotein and high density lipoprotein cholesterol. Atherogenic index (AI), which is a measure of the atherogenic potential of an agent, was calculated using the following formula and the results were tabulated.

Atherogenic index=Total serum triglyceride/Total serum high density lipoprotein cholesterol

Ethical issue

Appropriate ethical approval was taken before starting the study for performing the animal study was acknowledged from the Medical Research Ethics Committee, Department of Biochemistry and Molecular Biology, Tejgaon College, Dhaka.

Statistical analysis

Results were presented as mean±SD. The significance of difference among the groups were assessed using one way analysis of variance (ANOVA) followed by Dunnet's test. A p value of <0.05 was considered significant.

Results

The preliminary phytochemical screening revealed the presence of phytoconstituents such as

glycosides, alkaloids, flavonoids and phenolic compound in the ethanolic extract of *Caulerpa recemosa*.

Hypolipidemic activity

A marked increase in the level of serumcholesterol, triglycerides and low density lipoprotein were found in the animals which received atherogenic diet and high density lipoprotein levels were decreased. Administrations of ethanolic extract at the dose of 200 mg/kg showed significant reduction in the level of serum cholesterol, triglyceride, low density lipoprotein and increase in high density lipoprotein level which was similar to the standard atorvastatin and are almost near the levels of normal control. A potent hypolipidemic effect of ethanol extract was evident by a statistically

Table II Effect of *Caulerpa recemosa* on serum lipid levels

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	High density lipoprotein (mg/dl)	Low density lipoprotein (mg/dl)
Group I (Control)	96.68 ± 2.99	127.44 ± 7.89	56.82 ± 2.77	89.84 ± 3.18
Group II (Atherogenic diet)	186.03 ± 2.44	187.28 ± 5.63	22.35 ± 2.85	129.55± 9.73
Group III (Standard) (Atorvastatin)	122.52 ± 4.42	102.55 ± 7.15	46.06 ± 5.44	92.66 ± 4.76
Group IV*(Caulerpa recemosa* extract)	107.65 ± 2.22	87.06 ± 3.56	58.26 ± 4.22	84.49± 2.79
p value	< 0.01	< 0.01	< 0.01	< 0.01
	Significant	Significant	Significant	Significant

significant (p <0.01) reduction in the level of serum cholesterol, low density lipoprotein and triglycerides in the cholesterol treated animals and also marked increase in the high density lipoprotein level (Table II). The atherogenic index was considerably decreased (p <0.01) in the plant extract group which was also comparable with the standard group atorvastatin against hyperlipidemia (Table III).

Table III Atherogenic index of different groups

Group	Atherogenic index	p value
Group I (Control)	2.24	—
Group II (Atherogenic diet)	8.37	—
Group III (Standard) (Atorvastatin)	2.22	<0.01
Group IV *(Caulerpa recemosa* extract)	1.49	<0.01

Discussion

The present study was performed to assess the hypolipidemic activity and to prove its claim in folklore practice against various disorders. Dyslipidemia contributes to atherosclerosis, a disease in which fatty deposits called 'plaque' build up in the arteries over time. If plaque narrows the arteries, there is high likelihood to suffer from heart disease, heart attack, peripheral artery disease (reduced blood flow in the limbs, usually the legs), and stroke. People with diabetes are more likely to develop atherosclerosis, heart disease, poor circulation, and stroke than those who do not have diabetes. Many people with diabetes have conditions called 'risk factors' that contribute to atherosclerosis and its complications. These include high blood pressure, excess weight and high blood glucose levels. Dyslipidemia further raises the risk of atherosclerosis in people with diabetes. Dyslipidemia affects people with type 2 diabetes more often than those with type 1 diabetes. The most common dyslipidemia in diabetes is the combination of high triglycerides and low high density lipoprotein levels. People with diabetes may also have elevated low density lipoprotein cholesterol. Among the drugs available to treat dyslipidemia, statins are often the first choice for lowering total and low density lipoprotein cholesterol levels. Other drugs that lower cholesterol include cholesterol- absorption blockers, bile acid sequestrants, and nicotinic acid. There are several powerful antioxidants present in *Caulerpa recemosa*, which inhibits the oxidation of cholesterol in low density lipoproteins; this slows the formation of foam cells, which contribute to atherosclerotic plaques. Similarly, flavonoids present in the plant *Caulerpa recemosa* may be responsible for its hypolipidemic action and as already reported significant antioxidant activity of chloroform extract further confirms its significant hypolipidemic activity.[15]

In addition, the past decade saw a series of remarkable studies that suggested oxidative systems; particularly oxidation of low density lipoprotein is a risk factor and plays a role at several steps of atherosclerosis.[16,17]A decrease in oxidative stress and protection of low density lipoprotein from oxidation might therefore be a strategy with great promise for prevention of atherosclerosis associated cardiovascular disease. The intense interest in this area stems in part fromthe generally low toxicity of antioxidants and the hope that treatment with antioxidants might be additive with cholesterol lowering

regimes. It is well known that low density lipoprotein plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of low density lipoprotein receptors.

Conclusion

In conclusion, it can be said that the ethanol extract of *Caulerpa recemosa* exhibited a significant hypolipidemic effect at the dose of 200 *mg/kg* body weight.Efforts are in progress to isolate and characterize the active principle, which is responsible for the hypolipidemic efficacy of this valuable medicinal plant and further studies are required to establish the efficacy of the *Caulerpa recemosa* as a hypolipidemic drug.

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