ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.4.L36-40

International Journal of Life science and Pharma Research

Research Article

Marine Biology for Medical Therapy

Phytochemical Screening and Evaluation of Antidiabetic Activity of the Marine Microalgae: *Nannochloropsis* Sp.

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Abstract: Diabetes mellitus is a metabolic disorder and is one of the major challenges on health of people all over the world. The phytochemicals of various marine algae are found to possess antioxidant, anti-inflammatory and anti-diabetic properties. Microalgae are photosynthetic organisms that produce oxygen and are capable of producing beneficial secondary metabolites. The bioactive metabolites like pigments, phenolics, amino acids, polyunsaturated fatty acids etc. produced by them have a positive effect on human health. In the present investigation, the marine microalgae *Nannochloropsis* sp. was screened for phytochemicals qualitatively and quantitatively. The evaluation of antidiabetic activity was determined against the enzymes such as alpha amylase and alpha glucosidase. The extracts were prepared in five different solvents such as petroleum ether, aqueous, isopropanol, methanol and ethyl acetate. The qualitative analysis for phytochemicals showed the presence of alkaloids, phenols, flavonoids, carbohydrates, proteins, cardiac glycosides, sterols, coumarin and tannins. The maximum total phenolic, flavonoid and sterol content was present in ethyl acetate extract of *Nannochloropsis* sp. and the values are found to be 42.17 ± 0.12mg GAE/g, 60.23 ± 0.09 mg QE/g, 55.34 ± 0.07 mg cholesterol/g of extract respectively. The ethyl acetate extract showed highest inhibitory effect of 78.52 ± 0.56 % (IC50 121.96 µg/ml) on α-amylase and 80.42 ± 0.13 % (IC 50 178.53µg/ml) on α-glucosidase enzyme at a concentration of at 1mg/ml which implies the microalgae possess anti-diabetic property. The results indicate that the phytochemicals present in the microalgae contribute the antidiabetic activity. Hence *Nannochloropsis* sp. can be applied in pharmaceuticals for the development of antidiabetic drugs which have the benefits over side effects by the use of synthetic drugs.

Keywords: marine microalgae, *Nannochloropsis* sp., phytochemicals, antidiabetic, bioactive compounds

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Recieved On 12 February 2020
Revised On 20 March 2020
Accepted On 26 May 2020
Published On 02 October 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Deepa P. K, Subramanian A and Manjusha W.A. , Phytochemical Screening and Evaluation of Antidiabetic Activity of the Marine Microalgae: Nannochloropsis Sp..(2020).Int. J. Life Sci. Pharma Res.10(4), L36-40 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.4.L36-40

1. INTRODUCTION

Diabetes mellitus is one of the most prevalent diseases among the world. It is due to lack of insulin secretion (relative and absolute) and insulin resistance, which upsets fat, protein and carbohydrate metabolism and genetic factor may be one of the causes. One of the major symptoms is hyperglycemia and major complications include hypertension, blindness, and kidney failure and so on. It is a metabolic disorder which has induced substantial morbidity and mortality due to micro vascular (neuropathy, retinopathy and nephropathy) and macrovascular (stroke, heart attack and peripheral vascular disease) complication.[1] Microalgae are eukaryotic photosynthetic organisms that account for approximately 40 % of the global photosynthesis and play a key role in aquatic ecosystems.[2] Diverse varieties of functional metabolites are produced by them. They are rich sources of phenols, flavonoids and pigments which have several roles in health ailments. Nowadays researchers show interest on algae because of its ability to produce several beneficial natural products used as anti-inflammatory, antimicrobial, antimalarial, antiproliferative, and anticancer agents.[3] Several microalgae are reported to have antidiabetic property.[4] Hence the present study was aimed to screen the phytochemicals and to evaluate the antidiabetic property of marine microalgae, *Nannochloropsis* sp.

2. MATERIALS AND METHODS

10 gms of dried microalgae cells were extracted in 100ml of different organic solvents specifically ethyl acetate, methanol, aqueous, isopropanol and petroleum ether for 7 days at room temperature. The solution was filtered through Whatman No.1 filter paper. The crude extract was kept in a sealed container and stored in a refrigerator for further studies.

2.1 Phytochemical screening

The *Nannochloropsis* sp. extracts of petroleum ether (PN), Aqueous (AN), Isopropanol (IN), Methanol (MN), and Ethyl acetate (EN) were subjected to phytochemical analysis for the presence of bioactive constituents.[5]

2.2 Quantitative analysis of Phytochemicals

2.2.1 Estimation of total phenol content

Various solvent extract (0.5g) was extracted in 10 ml of 80% ethanol. Supernatant was evaporated to dryness and the extract was dissolved in 5 ml water. Different aliquots 0.1-1 ml was taken and the final volume was made to 3 ml with water. Folin's reagent of 0.5ml was added followed by 2ml of 20% Na_2CO_3.(2 and 3 to be written subscript) Tubes were vortexed and kept in boiling water for one minute. The tubes were cooled and measured the absorbance at 650nm against blank. The standard curve of gallic acid was obtained using the same procedure and the results were expressed as gallic acid equivalent GAE/g dry weight of microalgae .[6]

2.2.2 Estimation of total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric method was used with some modifications. Various solvent extract (1ml) was added to 3 ml of methanol followed by 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and was incubated at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard (1 mg/ml). Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound).[7]

2.2.3 Estimation of steroid

A solution of 2.5 g (ml) of various solvent extract was prepared in 50 ml of distilled water after vigorous shaking for 1 hour. From that, 2ml of extract solution was washed with 3 ml of 0.1 M NaOH (pH 9) and with 2 ml of chloroform was added and 3 ml of ice-cold acetic anhydride followed by adding two drops of concentrated H_2SO_4 cautiously. Cholesterol was taken as blank. The absorbance was measured spectrophotometrically at 420 nm.[8]

2.3 Antidiabetic Activity

2.3.1 Inhibition of alpha amylase enzyme

A starch solution of 0.1% w/v was prepared by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 ml of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartrate solution and 3, 5 di nitro salicylic acid solution (96 mM). The various concentrations of the algal extract (100 to 1000 µg/mL) were added to 1 ml of starch solution and left for 10 min. Further, the reaction was initiated by the addition of the enzyme solution and allowed to react for 10 min under alkaline condition at 25°C. Finally, the reaction was terminated by adding 1 mL of calorimetric reagent and then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in a similar way by replacing algal extract with DMSO. A similar experiment was conducted with the standard drug Acarbose and the experiments were conducted in triplicate.[9]

Percentage of inhibition = Absorbance of control – Absorbance of test/Absorbance of

2.3.2 Inhibition of alpha-glucosidase enzyme

To various concentrations of algal extract, 1ml of starch substrate (2% w/v maltose or sucrose) and 1 ml with 0.2 M Tris buffer pH 8.0 were added and incubated it at 37°C for 5 minutes. Then 1 ml of alpha glucosidase enzyme (IU/ml) was added followed by incubation for 40 minutes at 37°C. The reaction was terminated by the addition of 2 ml of 6N HCl. The intensity of colour was measured at 540 nm. Control experiment was done by replacing the extract with DMSO. 10 Percentage of inhibition was calculated by using the following formulae,

% of inhibition = Absorbance of control – Absorbance of test/Absorbance of control × 100

3. STATISTICAL ANALYSIS

The data were subjected to two way and one-way ANOVA using statistics software package (SPSS, ver.22) to analyze the statistical significance. The data were analyzed and expressed as means ± SD. The IC50 values were calculated from linear regression analysis.

4. RESULTS AND DISCUSSION

4.1 Phytochemical screening

Various solvent extracts of *Nannochloropsis* sp. were screened for phytochemicals and are shown in table 1.

Table 1: Phytochemical screening of various solvent extracts of *Nannochloropsis* sp.

Test	PN	AN	IN	MN	EN
Test for alkaloids (Mayer's test)	-	-	-	++	+
Test for phenol (Ferric chloride test)	-	-	+	-	+++
Test for flavonoids (Shinoda test)	-	-	-	+	++
Test for carbohydrates (Benedict's test)	-	-	-	+++	++
Test for protein (Xanthoproteic test)	+	-	+++	+	++
Test for sterols (Liebermann Burchard's test)	-	-	-	+	++
Test for cardiac glycosides (Keller–Kiliani test)	-	-	+++	-	++
Test for coumarin	-	-	-	++	+
Test for tannin	-	-	+	+++	+

+++: highly present, ++: moderately present: +: trace amount *Abbreviation to be given for PN, AN, IN, MN and EN for this tabular column) is given below the materials and methods: phytochemical screening

4.2 Quantitative analysis of phytochemicals

The qualitative analysis of phytochemicals showed the presence of most of the analyzed phytochemicals. Of them total phenol, flavonoid and sterols were quantified. The total phenolic content of EN and IN was found to be 42.17 ± 0.12mg GAE/g and 11.64 ± 0.06 mg GAE/ g of extract respectively. The flavonoid content was found to be 60.23 ± 0.09 mg QE/ g in EN and 14.13 ± 0.21 mg QE/ g of extract in MN. The Sterol content was found to be 55.34 ± 0.07 mg cholesterol/g and 12.31 ± 0.12 mg cholesterol/g of extract in EN and MN accordingly. All experiments were performed in triplicates and the results were expressed as mean ± SD. These phytochemicals are well known sources of antioxidants. Many studies proved that flavonoids and phenols are capable of scavenging activity. Various phenolic compounds were reported to inhibit in vitro α-glucosidase activity and thereby diabetes mellitus can be controlled. Flavonoids are a family of polyphenolic compounds which are found to possess antihyperglycemic activity.[11]

4.3 Anti-diabetic activity

Diabetes is defined as a state imbalance in homeostasis of carbohydrate and lipid metabolism due to improper regulation by pancreatic hormone, leading to hyperglycemia. The regulation of enzymes such as alpha amylase and alpha glucosidase is one of the major tasks involved in the treatment of Diabetes.

4.3.1 Inhibition of alpha amylase activity

α-amylase is one of the key enzymes that breaks down starch to simpler sugars and increase the absorption rate of glucose. The highest level of inhibition was exhibited by EN of about 78.52 ± 0.56 % at 1000 µg/ml and the IC50 was found to be 121.96 µg/ml. The inhibitory effect of acarbose was found to be 98.10 ± 0.10 % with an IC50 of 103.69 µg/ml. The inhibition level was found to be 3.89 ± .08 to 78.52 ± 0.56 % on varying extract at different concentrations. The dose dependent activity of various extract against alpha amylase is shown in figure 1. Variance among various levels of extract was $P<0.01$ is statistically significant. Maintenance of blood glucose level near normal during fasting as well as postprandial state is one of the major goals involved in the treatment of diabetes mellitus.[12] These α-amylase inhibitors inhibit the action of α-amylase enzyme leading to a reduction in starch hydrolysis which shows beneficial effects on glycemic index control in diabetic patients.[13]The inhibition of these enzyme is very important to regulate the release of glucose during digestion. Sangeetha *et al* found methanolic extract of *Nannochloropsis oculata* is a potent inhibitor of alpha amylase with the IC50 of 762.613 µg/ml.[14] The EN, in the present study exhibited better result with a significant inhibitory effect.

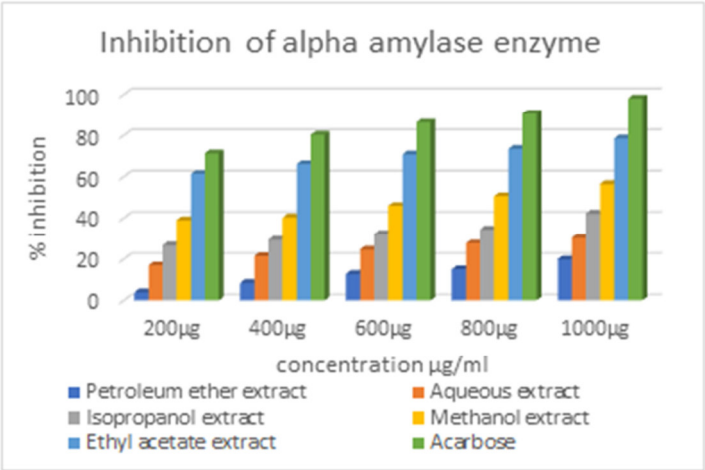

Fig 1.Inhibition of alpha amylase

4.3.2 Inhibition of alpha-glucosidase activity

In the present study, EN exhibited a significant inhibitory action on α-glucosidase enzyme followed by MN, IS, AN and PN. The EN, at the concentration of 1000 µg/ml exhibited α-glucosidase inhibitory activity of 80.42 ± 0.13 % with an IC50 of 178.53 µg/ml when compared to the remaining extracts. The inhibition level was found to be 12.02 ± 0.09 to 80.42 ± 0.13%. The standard drug acarbose showed an inhibition of 97.81 ± 0.04 % and the IC50 was found to be 103.69 µg/mL at the highest concentration. The dose dependent activity of various extract against alpha glucosidase is shown in figure 2. Variance among various levels of extract was found to be statistically significant ($P<0.01$). Alpha glucosidase breaks down starch and disaccharides to glucose.[15] Inhibition of the enzyme is the one of the strategies to avoid hyperglycemia. The *Nannochloropsis* sp. present study showed the higher α-glucosidase inhibition when compared to the study reported by Sangeetha *et* al, in which methanolic extract of *Nannochloropsis oculata* showed an IC50 of 613.72 µg/ml.[14]

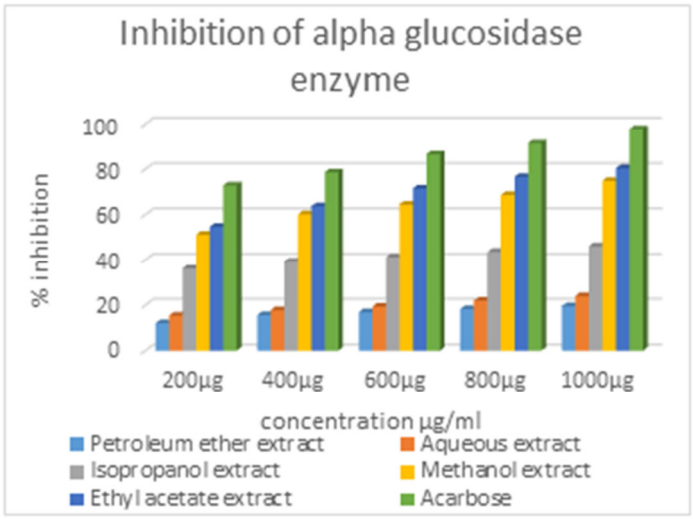

Fig 2. Inhibition of alpha-glucosidase

5. CONCLUSION

The marine green microalgae, *Nannochloropsis* sp. was found to contain various phytochemicals. The major compounds with bioactive potential such as phenols, flavonoids and sterols were present in significant quantity. The ethyl acetate extract of the microalgae possesses effective inhibitory activity on alpha amylase and alpha glucosidase enzyme which implies that the extract has antidiabetic effect. The phytochemicals present in the microalgae delivers the antidiabetic property and hence the *Nannochloropsis* sp. can be explored in pharmaceutical industry for the development of antidiabetic drug.

6. AUTHORS CONTRIBUTION STATEMENT

Professor Dr. A Subramanian gives the guidelines for the research study and Deepa. P.K. carried out the research work. Dr. W.A. Manjusha evaluated the results and supported in manuscript preparation.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. Moreno Garrido I. Microalgae immobilization: current techniques and uses. Bioresour. Technol. 2008; 99(10):3949-64. DOI: 10.1016/j.biortech.2007.05.040
2. Enwereuzor UP, Onyeagoro GN. A novel aeration method for the preparation of algae (Dunaliella salina) biomass for biofuel production. AJER. 2014; 3(9):209-214. Available from: http://www.ajer.org/papers/v3(9)/ZB03902090214.pdf
3. Chiara Lauritano, Adrianna Ianora. Marine organisms with anti-diabetes properties. Mar Drugs. 2016; 14(12): 220. DOI: 10.3390/md14120220
4. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012; 2(4): 320-330. DOI: 10.1016/S2221-1691(12)60032-X
5. Sani Ali Audu, Ilyas Mohammed, Haruna Abdul Kaita. Phytochemical screening of the leaves of Lophira lanceolata (Ochnaceae). Life Sci J. 2007; 4(4):75-79.
6. Malik CP Singh MB. Plant enzymology and histo enzymology. New Delhi: Kalyani Publishers; 1980.
7. Aiyegoro OA, Okoh AI Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complement Altern Med. 2010; 10(1):21. DOI: 10.1186/1472-6882-10-21
8. Trease GE, Evans MD. A Textbook of Pharmacognosy. London: Macmillan Publishers ;1989. Bailliere Tindall Ltd. 1983;12.
9. Bhatt UP, Sati SC, Subhash Chandra, Sudhir Kumar, Amit Anthwal, Rajendra Singh. Evaluation of in vivo and in vitro antidiabetic activity of Roylea cinerea. Int. J. Pharm Sci. Rev. Res. 2015; 32(2):210-213.
10. Murugesan S, Anand Babu M, Bhuvaneswari S, Kotteswari M, Thennarasan S. In vitro antidiabetic activity of methanolic extracts of selected marine algae. ejpmr . 2015; 2(6):256-260.
11. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G. Blood pressure is reduced and insulin sensitivity increased in glucose intolerant, hypertensive subjects after 15 days of consuming high polyphenol dark chocolate. J Nutr. 2008; 138(9): 1671-76. DOI: 10.1093/jn/138.9.1671
12. Bailey CJ. Potential new treatments for type 2 diabetes. Trends in pharmacol sci. 2000; 21(7): 259-265. DOI: 10.1016/S0165-6147(00)01506-6
13. Notkins L. Immunologic and genetic factors in type I diabetes. Journal of Biological Chemistry. 2002; 277 (46): 43545–43548.
14. Sangeetha P, Anuradha V, Suganya V, Bhuvana. WJPPS. 2012;10(3):307-317.
15. Sindhu S Nair, Vaibhavi Kavrekar, Anshu Mishra. In vitro studies on alpha amylase and alpha glucosidase inhibitory activity of selected plant extracts. Eur J Exp Biol. 2013; 3(1):128-132. Available from: https://www.semanticscholar.org/paper/In-vitro-studies-on-alpha-amylase-and-alpha-of-Nair-Kavrekar/29b016810c3e1cc0f1182b4d5bd79b6b0af75ccc