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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 ± 0.12 mg 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 50 178.53 µg/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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Received 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.


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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. Microalgae are eukaryotic photosynthetic organisms that account for approximately 40% of the global photosynthesis and play a key role in aquatic ecosystems. 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. Several microalgae are reported to have antidiabetic property. 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.

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% Na₂CO₃ (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.

\[ \text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \]

2.2.2 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 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. A similar experiment was conducted with the standard drug Acarbose and the experiments were conducted in triplicate.

\[ \% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \]
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.

<table>
<thead>
<tr>
<th>Test</th>
<th>PN</th>
<th>AN</th>
<th>IN</th>
<th>MN</th>
<th>EN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for alkaloids (Mayer’s test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Test for phenol (Ferric chloride test)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Test for flavonoids (Shinoda test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Test for carbohydrates (Benedict’s test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Test for protein (Xanthoproteic test)</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Test for sterols (Liebermann Burchard’s test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Test for cardiac glycosides (Keller–Kiliani test)</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Test for coumarin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Test for tannin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

+++: 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 ± 0.12 mg 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.
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. Inhibition of the enzyme is 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.

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


