RESEARCH PAPER

Evaluation of Glucose Uptake Activity of *Nitophyllum marginale* on L6 murine adipocyte cell lines

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ABSTRACT

Lower or unregulated uptake of glucose is the key characteristic feature of type 2 diabetes, which highlights the importance of the need for the development of innovative strategies in diabetes management. The red alga *Nitophyllum marginale* has been recognized as a potent source of various bioactive metabolites and has also been shown to possess antidiabetic properties therefore the aim of the present study is to investigate the effect of different extracts of *N. marginale* on cell-based assays to assess their role in glucose absorption. The samples of *N. marginale* were collected from various sites in the area of Mandapam, Tamil Nadu, and their analysis for glucose uptake activity was conducted on L6 murine adipocyte cell lines and the results of this study suggested that methanol solvent extract, due to the presence of potent bioactive components, is capable of modulating glucose metabolism better as compared to other solvent extracts, indicating its potential in managing diabetes.

Keywords: Adipocytes, Glucose uptake, L6 mouse cell line, Metabolic disorders, *N. marginale*.

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INTRODUCTION

Diabetes mellitus (DM) is a prolonged condition in which the glucose level in the blood remains high. It happens when the pancreas produces too little insulin, hormone that manages blood sugar or when the body cannot use proper insulin (Elsayed et al., 2023). Diabetes can form due to unhealthy habits like poor diet and lack of exercise, or it can be pass down from one generation to another (Rahman et al., 2021). Uncontrolled diabetes can result in major side effects such as Diabetic neuropathy, Diabetic retinopathy and Nephropathy. Riaz, 2009; Papatheodorou et al., 2018). Type 2 Diabetes Mellitus (T2DM) prevalence was estimated by the International Diabetes Federation (IDF) to be 7.5% (374 million) in 2019 and is expected to rise to 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045 (Jarrar et al., 2023; Saeedi et al., 2019). Allopathic medicine serves diabetes by helping the body to use insulin in a better way and lower blood sugar levels. In order to manage the sugar levels and enhance the muscle uptake of glucose nowadays, a combination of sodium-glucose co-transporter 2 (SGLT2), and dipeptidyl peptidase-4 (DPP4) inhibitors are used with the other routine drugs utilized to manage diabetes, such as sulfonylureas and metformin. Although these treatments work well, they may become less productive or cause health-related complications, and due to this reason, more focus is being shifted to alternative management, like the use of natural products, especially those based on seaweeds, which further enhance the body's defense system and thus help in managing diabetes (Malhotra and Cojandaraj, 2024). Seaweeds are increasingly recognized as an alternative to conventional treatments for several reasons. They are especially rich in essential minerals like iodine, calcium, magnesium, and iron, as well as vitamins such Vit. C & Vit. K (Choudhary et al., 2021; Vidyashree et al., 2024). Also they comprised of various nutrients and has a list of natural compounds that play a vital role in numerous bodily processes and can aid in correcting deficiencies when included in the diet and has also been proven to show a wide range of medicinal properties (Chen et al., 2018; A. K. Pandey et al., 2020; Lomartire & Gonçalves, 2022; Gani et al., 2016; Xu et al., 2023; Meinita et al., 2022; Malhotra et al., 2024). Studies have shown that eating seaweed can be beneficial for health in many aspects (Pérez-Lloréns et al., 2023). For instance, six varieties of seaweed can assist thyroid function due to their iodine content, protect cardiovascular health by reducing cholesterol and blood pressure, boost immune function, enhance gut health through prebiotic effects, and aid in weight management by increasing satiety and reducing fat absorption (Ryu et al., 2021; Smyth, 2021). Seaweeds are a natural and ecofriendly alternative to regular medicine. Harvesting of seaweeds is also not a big challenge, and they can be collected without affecting marine ecosystems. Also, seaweeds don't require an abundant amount of water for their growth, which enhances their utility in those areas where there is a scarcity of water (Pereira & Cotas, 2024; Sudarwati et al., 2020). Seaweeds offer a more reliable and sustainable option as compared to conventional treatment methods, as conventional methods are based on synthetic chemical formulations for their action, and on the other side, due to the presence of bioactive compounds, seaweeds display a number of health benefits, and also many of them have cultural importance (Thurstan et al., 2018). More research is needed to explore the medicinal effects of the seaweeds especially *N. marginale*. L6 myotubes, derived from rat skeletal muscle, are a widely used in vitro model for studying glucose uptake because they maintain a functional insulin signaling pathway and express glucose transporter type 4 (GLUT4), the key insulin-responsive glucose transporter. Since skeletal muscle is a major site of glucose disposal after meals, impaired insulin-stimulated glucose uptake in this tissue is a defining feature of type 2 diabetes mellitus. In response to insulin, GLUT4 moves from intracellular stores to the plasma membrane, enabling glucose to enter the cell. Since there is no study that has been reported regarding the glucose uptake activity of *Nitophyllum marginale* on cell lines, therefore, this study aims to investigate the in vitro glucose uptake activity analysis of NM on L6 cells.

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MATERIAL & METHODS

Sample collection

Samples of *Nitophyllum marginale* were collected from different sites of coastal areas of Mandapam in Tamil Nadu, India. The location from which the samples were collected possess a wide variety of algae and favourable conditions for seaweed proliferation. Samples were thoroughly washed with seawater on-site (to remove epiphytes, sand, and detritus once they were collected. (Latitudes 9º 28” 31” 0º N and longitudes 79º 15” 7” 11º E). Further samples were elated to the laboratory in cooling conditions for downstream processing (Gupta et al., 2019).

Authentication and Herbarium Submission

The collected seaweed specimens were taxonomically identified as *Nitophyllum marginale* (Kützing) J. Agardh, belonging to the family Delesseriaceae and the genus *Nitophyllum*, by an expert phycologist. A voucher specimen, along with an authenticated herbarium sample, was prepared and submitted in the herbarium with the reference number SHB-22/23-01 at Sri Herbasia Biotech Pvt. Ltd. Amritsar, Punjab, India, for future reference.

Glucose Uptake assay

Firstly, solvent extraction of *Nitophyllum marginale* was carried out by the Soxhlet apparatus to achieve the goal of extraction of bioactive molecules. To assess the impact of extracted solvents on glucose uptake, L6 murine adipocyte cell lines have been utilized. Further, different concentrations of *Nitophyllum marginale* extract were applied to the cells for a duration of the next 24 hours. Post-treatment measurement of glucose uptake was carried out, and the comparative percentage relative to the control group has been calculated (Gupta et al., 2019).

RESULTS AND DISCUSSION

Chloroform and methanolic extracts of *Nitophyllum marginale* have been analyzed to determine their effects for glucose uptake activity on L6 murine adipocyte cell lines. Results of these solvent extracts were also compared with the effects of insulin, which is used as a positive control in this study (Table 1). The results of this study indicate that the presence of bioactive components in both solvent extracts has influenced glucose metabolism in a better way even as compared to insulin, which shows the necessity of its in vivo validation and investigation of the individual molecules responsible for these effects.

Table1: Effect of extract of *N. marginale* on glucose uptake in L-6 cell line.

Sample conc.	Absorbance at 630 nm				Glucose conc. (ug/ml)	Percentage Glucose uptake
	Triplicate 1	Triplicate 2	Triplicate 3	Average		
Control (Insulin treated)	0.968	0.962	0.966	0.965	686.33	31.37
25ug/ml	0.706	0.703	0.703	0.704	425.00	57.50
50ug/ml	0.598	0.594	0.596	0.596	290.00	71..30
100ug/ml	0.392	0.397	0.394	0.394	115.33	93.47
Methanol						
25ug/ml	0.944	0.949	0.941	0.945	665.67	37.43
50ug/ml	0.918	0.911	0.913	0.914	635.00	46.12
100ug/ml	0.868	0.873	0.872	0.871	592.00	58.89
Chloroform						
25ug/ml	0.953	0.954	0.952	0.953	674.00	22.60
50ug/ml	0.945	0.942	0.941	0.943	663.67	28.63
100ug/ml	0.938	0.932	0.939	0.936	678.33	32.27

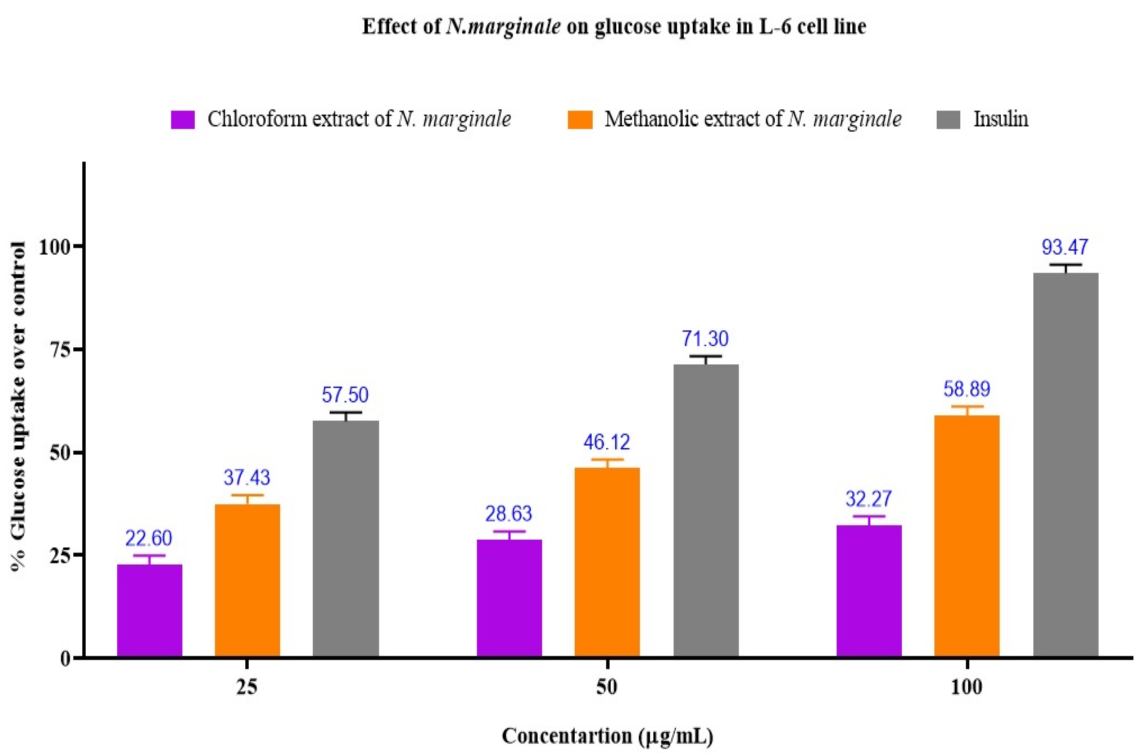

Fig 1: Glucose uptake in L-6 cell line using chloroform and methanol extract of *Nitophyllum marginale*

In vitro evaluation of glucose absorption of L6 cell lines was carried out in laboratory conditions. Figure 1 shows that chloroform and methanolic extracts of *Nitophyllum marginale* display glucose uptake at concentration of 25 µg/mL 22.60% and 37.40%, respectively while control (insulin) standard showed an uptake rate of 57.50%. The chloroform and methanol extracts had absorption rates of 32.27% and 58.89%, respectively, at a concentration of 100 µg/mL on the other hand, Insulin had the highest uptake rate at 93.47%. The research findings indicates that both methanolic and chloroform extracts facilitated glucose uptake in L6 cells in a dose-dependent manner, with maximum activity observed at highest concentration of 100 µg/mL.

Bioactive nutraceuticals like polyunsaturated fatty acids (PUFAs), carbohydrates, minerals and some pigments like phycobilins, chlorophylls, and carotenoids along with some antioxidants like tocopherols, and polyphenols are richly present in the marine algae (Ghosh et al., 2022). The various components of algae enable it to provide numerous health benefits, such as combating infections, alleviating inflammation, offering cancer protection, and functioning as an antioxidant (Menaa et al., 2021).

Red algae have different sulphated polysaccharides like carrageenans and porphyrin that exhibits antioxidant, immunomodulatory properties as well as antiviral and anticancer properties (Eladl et al., 2024). Marine algae are significant functional foods and promising possibilities for therapeutic applications aimed at preventing chronic diseases and enhancing health (A et al., 2025). The current study shows that methanolic extract of *Nitophyllum marginale* enhances glucose uptake in L6 cells, suggesting a potential effect on insulin signaling or GLUT4 translocation. Results of this study are comparable with the earlier studies utilizing murine L6 cell lines to investigate the natural antidiabetic compounds. Earlier research conducted by Zhao and coworkers demonstrated the positive effect of methanolic extract of Folium sennae by showing increased glucose uptake by 2.04-fold via activation of PKC and AMPK pathways along with enhanced intracellular calcium levels. Similar findings have revealed for hot aqueous extract of Helicteres isora which showed improved glucose uptake at a conc. of 200 µg/ml by 28.99% as compared to insulin and metformin indicating its hypoglycemic potential (Zhao et al. 2018). Moreover, extracts from the seaweed Fucus racemosa demonstrated even greater glucose uptake in L6 myotubes than orally consumed and used as first line treatment of type 2 diabetes; metformin, reinforcing the therapeutic promise of marine-derived compounds. Studies have also shown that α1-adrenoceptor activation enhances glucose uptake through phospholipase C and PKC pathways, which intersect with insulin signalling. Generally, these studies emphasize the value of the L6 cell model in identifying plant and marine extracts with glucose-lowering potential and exploring mechanisms involving GLUT4 translocation and key metabolic signalling pathways. This research will be helpful for the development of alternative therapies and managing diabetic conditions. Several research findings demonstrated the antidiabetic potential of different seaweed species, specially by showing in improving glucose uptake in the muscle cell models; L6 myotubes (Kim et al., 2021). Evidence from at least ten different species including red algae such as Gracilaria verrucosa and Gracilaria chorda; brown algae such as Undaria pinnatifida, Laminaria japonica, Hizikia fusiforme, and Ecklonia cava; and green algae such as Codium fragile and Caulerpa lentillifera indicates significant stimulation of cellular glucose absorption. In vitro lab studies have shown that extracts from species like C. fragile, U. pinnatifida, and G. verrucosa can positively increase the glucose uptake by approximately 120-132% in muscle cells relative to untreated controls (Sharifuddin et al., 2015). Also, it has been observed that the magnitude of these effects is comparable to insulin, further pointing out the therapeutic potential of seaweed derived bioactive molecules. The overall effects, including antioxidant activity, anti-inflammatory nature, and potency to inhibit carbohydrate hydrolyzing enzymes of marine algae, are beneficial and show their possibilities for the new therapeutic strategies in diabetes management (Kim et al., 2021).

CONCLUSION

This study found that extracts from the *Nitophyllum marginale*, especially the methanol extract, helped the cells to take up more glucose under in vitro conditions, which may be due to the presence of an abundance of bioactive molecules in methanol extracts. In the future, more studies are required to explore the bioactive molecules that are majorly responsible for showing better glucose uptake activity of this seaweed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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