

# *In vitro* Potential Activity Methanolic extract of *Turbinaria ornata* and Inhibition of Diabetic Enzymes

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## ABSTRACT

Inhibition of key carbohydrate-hydrolysing enzymes to reduce postprandial hyperglycaemia is an established therapeutic strategy for the management of type 2 diabetes. The present study evaluated the *in vitro* antioxidant and antidiabetic potential of the crude methanolic extract of the marine brown macroalga *Turbinaria ornata* using enzyme inhibition assays targeting  $\alpha$ -amylase,  $\alpha$ -glucosidase and dipeptidyl peptidase-IV (DPP-IV). The extract inhibited  $\alpha$ -amylase ( $IC_{50}$  = 364.0  $\mu$ g/mL),  $\alpha$ -glucosidase ( $IC_{50}$  = 336.3  $\mu$ g/mL) and DPP-IV ( $IC_{50}$  = 52.5  $\mu$ g/mL) in a concentration-dependent manner. The methanol extract also showed moderate antioxidant activity, scavenging 69.0% of DPPH radicals at 1000  $\mu$ g/mL ( $IC_{50}$  = 623.6  $\mu$ g/mL). Cytotoxicity, evaluated by the MTT assay in the HEK-293 cell line, was low at lower concentrations (91.7% viability at 250  $\mu$ g/mL;  $IC_{50}$  = 931.5  $\mu$ g/mL). A previous GC-MS study of the methanolic extract of *T. ornata* (Deepa et al., 2019) reported hentriacontane, (Z,Z)-6,28-heptatriacontadien-2-one, 8-heptadecene and 1-heptacosanol as the predominant constituents. These results suggest that *T. ornata* is a promising natural source of antihyperglycaemic compounds with low cytotoxicity, warranting further *in vivo* investigation.

**Key words:** *Turbinaria ornata*, Savanging activity, Anti-diabetic and Anti-hyperglycaemia, HEK-293 Cell line

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## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterised by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2014). It has emerged as one of the most serious global health challenges of the twenty-first century. The International Diabetes Federation estimated that approximately 537 million adults aged 20–79 years were living with diabetes in 2021, a figure projected to rise to 783 million by 2045 (Sun et al., 2022). Type 2 diabetes mellitus (T2DM) accounts for the vast majority of cases and is closely linked to sedentary lifestyles, unhealthy diets and obesity. Sustained hyperglycaemia drives the development of microvascular and macrovascular complications, including retinopathy, nephropathy, neuropathy and cardiovascular disease, which greatly increase morbidity and mortality (Brownlee, 2001). [

Postprandial hyperglycaemia, the sharp rise in blood glucose following a meal, is an early metabolic abnormality in T2DM and has been recognised as an independent risk factor for cardiovascular complications (Ceriello, 2005). A well-established strategy for controlling postprandial glucose is to delay carbohydrate digestion and absorption by inhibiting the key carbohydrate-hydrolysing enzymes of the digestive tract. Pancreatic  $\alpha$ -amylase hydrolyses the  $\alpha$ -1,4-glycosidic linkages of dietary starch into oligosaccharides and disaccharides, which are subsequently cleaved by intestinal brush-border  $\alpha$ -

glucosidase into absorbable monosaccharides such as glucose (Kwon et al., 2008). Inhibition of these enzymes slows glucose release into the circulation and blunts the postprandial glycaemic peak. Clinically used  $\alpha$ -glucosidase inhibitors such as acarbose, miglitol, and voglibose act through this mechanism, but their use is frequently limited by gastrointestinal adverse effects, including flatulence, abdominal discomfort, and diarrhoea (van de Laar et al., 2005). Kwon et al., 2008 attributed these effects partly to strong  $\alpha$ -amylase inhibition, which leaves undigested carbohydrates to be fermented by colonic bacteria.

Dipeptidyl peptidase-IV (DPP-IV) is another important therapeutic target in T2DM. This serine protease rapidly inactivates the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are secreted from the gut after food intake and enhance glucose-dependent insulin secretion while suppressing glucagon release (Drucker and Nauck, 2006). Inhibiting DPP-IV prolongs the activity of endogenous incretins and thereby improves glycaemic control. Synthetic DPP-IV inhibitors (gliptins), including sitagliptin, vildagliptin, and saxagliptin, are now widely prescribed (Deacon, 2020); however, their cost and concerns about long-term safety have encouraged the search for naturally derived alternatives.

Brown macroalgae are rich in bioactive compounds, such as phlorotannins, that inhibit carbohydrate-digesting enzymes (Lee and Jeon,

**2013).** *Turbinaria ornata*, a brown Macroalgae found along the southeastern coast of India, has known antioxidant and anti-inflammatory properties (Ananthi *et al.*, 2010), but its antidiabetic potential remains largely unexplored. This study therefore evaluated *T. ornata* extracts for  $\alpha$ -amylase,  $\alpha$ -glucosidase, and DPP-IV inhibition, antioxidant activity (DPPH), and toxicity (DNA fragmentation, haemolytic, and MTT assays), and identified their major compounds by GC-MS.

Natural products have long served as a major source of new therapeutic agents (Newman and Cragg, 2020), and marine organisms in particular represent a rich and relatively underexplored reservoir of structurally diverse bioactive compounds. Macroalgae are a valuable source of polysaccharides, polyphenols, pigments, sterols and fatty acids with antioxidant, anti-inflammatory and antidiabetic properties (Lordan *et al.*, 2011). Brown macroalgae (Phaeophyceae) are especially rich in phlorotannins, which Lee and Jeon (2013) showed to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase and to improve glucose homeostasis through multiple mechanisms. Similarly, Apostolidis and Lee (2010) reported that phenolic-rich extracts of the brown alga *Ascophyllum nodosum* significantly inhibited both  $\alpha$ -amylase and  $\alpha$ -glucosidase in vitro, supporting the potential of brown algae as sources of antihyperglycaemic agents. *Turbinaria ornata* (Turner) J. Agardh, a brown macroalga distributed along the southeast coast of India, has known antioxidant and anti-inflammatory properties (Ananthi *et al.*, 2010), and its inhibitory activity against diabetes-related enzymes has been reported (Unnikrishnan *et al.*, 2014). However, further studies are needed to confirm and extend these findings, particularly with respect to DPP-IV inhibition and safety in human cells. Therefore, the present study was undertaken to investigate the in vitro antidiabetic potential of the crude methanolic extract of *T. ornata*. The extract was evaluated for its inhibitory effects on  $\alpha$ -amylase,  $\alpha$ -glucosidase and DPP-IV, for its free radical scavenging activity using the DPPH assay and for its cytotoxicity in HEK-293 cells using the MTT assay.

## Materials and Methods

### Collection and Processing of Macroalgae

Drift (beach-cast) material of the brown alga *Turbinaria ornata* (Turner) J. Agardh was collected from the foreshore of the Olaikuda coast (9.872432° N, 79.118630° E), Gulf of Mannar, Tamil Nadu, southeast coast of India, in December 2022. Sampling was carried out during low tide, when the water temperature was 28 °C, the air temperature 27 °C and the salinity 33 ppt. The specimen was identified and authenticated by the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore, Tamil Nadu, India (authentication no. BSI/SRC/S/23/2023-24/Tech-277).

The Macroalgae sample was collected and then dried in the shade at room temperature. It was then powdered using a pulverizer at room temperature. The powdered sample was stored in an airtight container at -20°C in the laboratory for extraction. The Macroalgae crude was extracted using a solvent called methanol. The extraction process was carried out by using the Soxhlet method. The crude extract was refrigerated at a temperature of -20°C for further study.

### Preliminary Phytochemical screening

The preliminary phytochemical screenings were carried out per the standard protocols of the collected Macroalgae (Andima *et al.*, 2014)

### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Free radical scavenging activity was determined according to the method of (Mensor *et al.*, 2001). For testing, 500  $\mu$ L of 0.3 mM DPPH alcoholic solution (Himedia, India) was added to 2.5 ml at 250–1000  $\mu$ g/mL doses. Incubated in the dark for 30 minutes, samples were measured at 518 nm using an ultraviolet (UV)-visible spectrophotometer (Systronics AU-2700, India) with methanol as a blank and ascorbic acid as a positive control. Scavenging activity as % inhibition in triple trials is calculated below.

#### %Inhibition

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

The IC 50 values were also calculated using Graph Pad Prism 8 software.

### ANTIDIABETIC ASSAY

#### In Vitro $\alpha$ -Amylase Inhibition Assay

$\alpha$ -Amylase inhibitory activity was measured using the method of (Jayasri *et al.*, 2009) with slight modifications. Briefly, 250  $\mu$ L of the extract (250–1000  $\mu$ g/mL) and 250  $\mu$ L of 0.02 M sodium phosphate buffer (pH 6.9, containing 0.006 M sodium chloride) containing porcine pancreatic  $\alpha$ -amylase (0.5 mg/mL) were pre-incubated at 25 °C for 10 min. After pre-incubation, 250  $\mu$ L of 1% starch solution in the same buffer was added to each tube at 5-s intervals, and the reaction mixtures were incubated at 25 °C for 10 min. The reaction was stopped by adding 500  $\mu$ L of 3,5-dinitrosalicylic acid (DNSA) colour reagent (Sigma, St. Louis, USA). The tubes were then placed in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer. Acarbose was used as the positive control. All assays were performed in triplicate, and  $\alpha$ -amylase inhibitory activity was calculated as follows:[]

#### %Inhibition

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

**In Vitro  $\alpha$ -Glucosidase Inhibition Assay**

$\alpha$ -Glucosidase inhibitory activity was determined as described by Unnikrishnan *et al.*, 2014. Briefly, 50  $\mu$ L of the extract at different concentrations (200–1000  $\mu$ g/mL) and 100  $\mu$ L of 0.1 M phosphate buffer containing  $\alpha$ -glucosidase solution were pre-incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50  $\mu$ L of 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) was added to each well at 5-s intervals, and the reaction mixture was incubated at 25 °C for 5 min. The absorbance was then recorded at 405 nm using a microplate reader and compared with that of the control, which contained buffer in place of the extract. Acarbose was used as the positive control. All experiments were performed in triplicate, and  $\alpha$ -glucosidase inhibitory activity was calculated as follows:

**%Inhibition**

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

**Cell line maintenance**

The HEK-293 (human embryonic kidney) cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)

**In Vitro Dipeptidyl Peptidase-IV (DPP-IV) Inhibition Assay**

DPP-IV inhibitory activity was measured following the method of (Al-Masri *et al.*, 2009), as adapted by Unnikrishnan *et al.* (2014). Diprotin A (Sigma, St. Louis, USA) was used as the standard inhibitor and was prepared at concentrations of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4  $\mu$ g/mL in Tris-HCl buffer (50 mM, pH 7.5) to a final volume of 35  $\mu$ L. DPP-IV enzyme (15  $\mu$ L; Sigma, St. Louis, USA) was then added, and the mixture was pre-incubated at 37 °C for 10 min to allow inhibitor–enzyme binding. One unit of enzyme activity was defined as the amount of enzyme that releases 1  $\mu$ mol of p-nitroaniline (pNA) per minute under the assay conditions. The reaction was initiated by adding 50  $\mu$ L of 0.2 mM Gly-Pro-p-nitroanilide (Sigma, St. Louis, USA) in Tris-HCl buffer as the substrate. After incubation at 37 °C for 30 min, the reaction was stopped with 25  $\mu$ L of 25% glacial acetic acid, and the absorbance was measured at 405 nm using a microplate reader (BioTek, USA). All experiments were performed in triplicate and compared with a negative control.

**DPP-IV Inhibition Assay of Macroalgae Extract**

The Macroalgae extracts were dissolved in dimethyl sulphoxide (DMSO) to prepare a stock solution of 1000  $\mu$ g/mL, which was then diluted in Tris-HCl buffer (50 mM, pH 7.5) to final concentrations of 2.5, 10, 40, and 80  $\mu$ g/mL in a total volume of 100  $\mu$ L per well. The assay was carried out in triplicate using the same procedure described for diprotin A. The percentage of DPP-IV inhibition was calculated as:

supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine and 100 U/mL penicillin–streptomycin, and maintained at 37 °C in a humidified incubator with 5% CO<sub>2</sub>. Cells in the exponential growth phase were used for the experiments.

**Cytotoxic assay**

The cytotoxicity of the extract was evaluated in HEK-293 cells using the MTT assay. Cells were seeded into 96-well plates at a density of  $1 \times 10^4$  cells/well and incubated for 24 h under standard conditions. The cells were then treated with different concentrations of *T. ornata* extract (250–1000  $\mu$ g/mL) for 24 h. After incubation, the medium was removed, MTT solution (5 mg/mL) was added to each well, and the plates were re-incubated for 3–4 h. The resulting formazan crystals were dissolved in 100  $\mu$ L of acidified isopropanol, and the absorbance was measured at 630 nm using a microplate reader. Each concentration was tested in triplicate. Cytotoxicity (% inhibition) was calculated using the following equation, and cell viability (%) was expressed as 100 – % inhibition:

**%Inhibition**

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

**%Inhibition**

$$= \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

**GC-MS Analysis**

GC/MS (Perkin Elmer Clarus 680 GC and Clarus 600 MS) analysis was used to identify key chemicals in *Gracilaria Salicornia* extracts. The GC/MS column utilized was Elite-5MS (30.0 m, 0.25 mm, 250  $\mu$ mdf). Helium was employed as the carrier gas at a constant flow rate of 1 mL/min. EI mode was employed with electron energy set at 70 eV. GC/MS analyzed 1  $\mu$ L of extract diluted with methanol to identify chemicals based on molecular structure, mass, and fragment ratio of resolved spectra compared to library-available mass spectra. Spectral data were analysed using the NIST database.

**Statistical analysis**

All results are expressed as mean  $\pm$  standard deviation (SD) of three replicates ( $n = 3$ ). Concentration-dependent effects were analysed by one-way analysis of variance (ANOVA), and differences between the extract and the respective standard at each concentration were analysed by two-way ANOVA followed by a post hoc multiple-comparison test. Differences were considered statistically significant at  $p < 0.05$ . Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, USA).]

**Result and Discussion****Phytochemical analysis**

The preliminary phytochemical screening of the methanol extract of *Turbinaria ornata* showed that it contains a varied range of secondary metabolites and primary constituents (**Table 1**). Alkaloids, phenols, flavonoids, glycosides, proteins, carbohydrates, and lipids were present, while saponins and tannins were absent. Seven of the nine phytochemical classes tested were therefore detected, which suggests that methanol is an effective solvent for extracting a broad spectrum of bioactive compounds from this brown Macroalgae.

**Table 1. Preliminary phytochemical screening of the methanol extract of *Turbinaria ornata***

| Phytochemical analysis | Methanol extract |
|------------------------|------------------|
| Alkaloids              | +                |
| Phenols                | +                |
| Flavonoids             | +                |
| Saponins               | -                |
| Glycosides             | +                |
| Proteins               | +                |
| Tannins                | -                |
| carbohydrates          | +                |
| Lipids                 | +                |

(+) present; (-) absent

As shown in **Table 1**, the extract contained several classes of compounds with known biological activities. Alkaloids are known to have cytotoxic and antiproliferative properties; some act as microtubule-targeting agents that bind to tubulin, disrupting the dynamics of the mitotic spindle, arresting the cell cycle at the G2/M phase and triggering apoptosis in cancer cells (**Jordan and Wilson, 2004**).

Phenolic compounds, which occur mainly as phlorotannins in brown Macroalgae, are strong antioxidants: they donate hydrogen atoms or electrons to neutralize free radicals and chelate pro-oxidant metal ions. They also have antimicrobial, anti-inflammatory, and antidiabetic activities (**Li et al., 2011**). Flavonoids have been shown to have anti-inflammatory, anticancer, and antioxidant properties. They may also help improve cardiovascular health, reduce the risk of diabetes, and protect against neurodegenerative disorders by scavenging reactive oxygen species and modulating inflammatory enzymes such as cyclooxygenase and lipoxygenase (**Panche et al., 2016**). Glycosides have been reported to have cardioprotective, antimicrobial, and anti-inflammatory effects. Cardiac glycosides in particular inhibit the Na<sup>+</sup>/K<sup>+</sup>-ATPase pump, and this inhibition has been linked to anticancer activity (**Prassas and Diamandis, 2008**). Carbohydrates in brown algae, mainly sulfated polysaccharides such as fucoidan, laminarin, and alginate, have immunomodulatory, anticoagulant, antiviral, and antitumor activities (**Wijesekara et al., 2011**). Proteins and their derived bioactive

peptides add to the nutritional value of the Macroalgae and may have antihypertensive and antioxidant activities (**Harnedy and FitzGerald, 2011**). Lipids from brown Macroalgae are rich in polyunsaturated fatty acids, sterols such as fucosterol, and carotenoids such as fucoxanthin, which have anti-inflammatory, anti-obesity, and anticancer effects (**Peng et al., 2011**). The absence of saponins and tannins may be due to their low concentration in the extract, seasonal and geographical variation, or the extraction solvent used (**Deepak et al., 2017**). The absence of tannins may also be because conventional tannin tests do not always detect algal phlorotannins, which differ structurally from terrestrial plant tannins (**Imbs and Zvyagintseva, 2018**). Overall, the presence of these phytochemicals suggests that the methanol extract of *T. ornata* could be a potential source of natural antioxidant, antimicrobial, anti-inflammatory, and anticancer agents.

#### DPPH assay

The antioxidant potential of *T. ornata* was evaluated through its ability to scavenge the DPPH radical. The methanol extract showed concentration-dependent DPPH scavenging activity, rising from 34.4% at 250 µg/mL to 69.0% at 1000 µg/mL, compared with 62.2% and 97.0% for the standard ascorbic acid at the same concentrations (Figure 1). The IC<sub>50</sub> value of the methanol extract was 623.6 µg/mL, whereas ascorbic acid showed an IC<sub>50</sub> of 166.8 µg/mL. The activity of the extract may be attributed to its phenols and flavonoids (Table 1), whose hydroxyl groups donate hydrogen atoms to neutralise free radicals (**Rice-Evans et al., 1996**). Similar DPPH scavenging activity has been reported for *T. ornata* extracts (**Deepak et al., 2017**). The lower activity compared with ascorbic acid is expected, because the crude extract contains active compounds at lower concentrations than the pure standard. These results indicate that *T. ornata* possesses moderate antioxidant activity.

#### ANTIDIABETIC ASSAY

##### In Vitro $\alpha$ -Amylase Inhibition Assay

##### $\alpha$ -Amylase inhibitory activity of methanol extract

The effect of *T. ornata* on  $\alpha$ -amylase is shown in Figure 2. The extract resulted that significant, concentration-dependent inhibition of  $\alpha$ -amylase at all evaluated concentrations (250, 500, 750, and 1000 µg/mL), with inhibition increasing from 41.1% at 250 µg/mL to 60.9% and 73.6% at 500 and 750 µg/mL, respectively. The extract of *T. ornata* exhibited a peak inhibition of 86.1% at a concentration of 1000 µg/mL, with an IC<sub>50</sub> value of 364.0 µg/mL, whereas the reference compound acarbose demonstrated 94.5% inhibition at the same dose, with an IC<sub>50</sub> of 150.8 µg/mL. Studies by **Sari et al., 2015** and the inhibitory effects of plant extracts on  $\alpha$ -amylase activity, emphasizing the importance of exploring natural sources for novel anti-diabetic agents. **Patel et al., 2012**. The bioactive

compounds responsible for the observed inhibitory effect warrant further investigation. Previous studies have identified various compounds, such as polyphenols, flavonoids, and alkaloids, in algal species with potential anti-diabetic properties (Sharma *et al.*, 2018).

#### **$\alpha$ -Glucosidase assay**

The inhibiting effect of *T. ornata* on  $\alpha$ -glucosidase is seen in Figure 3. The extract demonstrated significant, concentration-dependent inhibition of  $\alpha$ -amylase at all evaluated concentrations (200, 500, 750, and 1000  $\mu\text{g/mL}$ ), as validated by one-way ANOVA ( $F = 141.9$ ,  $p < 0.001$ ). Inhibition increased from 41.8% at 200  $\mu\text{g/mL}$  to 59.9% and 73.3% at 500 and 750  $\mu\text{g/mL}$ , respectively. The extract of *T. ornata* showed a peak inhibition of 85.4% at a concentration of 1000  $\mu\text{g/mL}$ , with an  $\text{IC}_{50}$  value of 336.3  $\mu\text{g/mL}$ . The conventional acarbose exhibited 94.5% inhibition at the same dose, with an  $\text{IC}_{50}$  of 102.4  $\mu\text{g/mL}$ . The activity of the extract was markedly inferior to that of acarbose at all concentrations ( $p < 0.001$  at 200 and 500  $\mu\text{g/mL}$ ,  $p < 0.01$  at 750  $\mu\text{g/mL}$ , and  $p < 0.05$  at 1000  $\mu\text{g/mL}$ ). Nonetheless, at a concentration of 1000  $\mu\text{g/mL}$ , it achieved around 90% of the standard's efficacy.

#### **DPP-IV assay**

The extract of *T. ornata* was assessed for its capacity to enhance insulin production by inhibiting the DPP-IV enzyme. The evaluation of its efficacy was conducted using % inhibition and  $\text{IC}_{50}$  value. The extract demonstrated significant, concentration-dependent inhibition of DPP-IV (one-way ANOVA,  $F = 281.8$ ,  $p < 0.001$ ), increasing from 38.5% at 2.5  $\mu\text{g/mL}$  to 55.5% at the peak concentration of 80  $\mu\text{g/mL}$ , with an  $\text{IC}_{50}$  value of 52.5  $\mu\text{g/mL}$ . The conventional diprotin A exhibited a 62.4% inhibition at a concentration of 80  $\mu\text{g/mL}$ , with an  $\text{IC}_{50}$  value of 2.5  $\mu\text{g/mL}$ . The extract's inhibition was much less than that of diprotin A at concentrations of 2.5, 10, and 40  $\mu\text{g/mL}$  ( $p < 0.05$ ). At a concentration of 80  $\mu\text{g/mL}$ , the disparity was not statistically significant ( $p > 0.05$ ), suggesting that the extract's efficacy was similar to that of the standard at this maximum level.

#### **Cytotoxicity assay of the macroalgal extract *T. ornata* on HEK-29 cell line**

To assess the cytotoxic impact of the extract, the HEK-29 cell line was exposed for 24 hours to different doses of *T. ornata* extract (250–1000  $\mu\text{g/mL}$ ). As seen in Figure 5, cell viability decreased with increasing concentration. The MTT experiment demonstrated that HEK-29 cells exposed to *T. ornata* extract exhibited safety and shown no significant harmful effects at the lower dose of 250  $\mu\text{g/mL}$ , with a viability rate of 91.7%. The detected cytotoxicity at this level was 8.3%. Cell viability decreased to 70.1% and 64.1% at concentrations of 500 and 750  $\mu\text{g/mL}$ , respectively, whereas 45.3% of the cells retained viability at the maximum dose of 1000  $\mu\text{g/mL}$ . The concentration at

which half of the cells exhibited viability ( $\text{IC}_{50}$ ) was 931.5  $\mu\text{g/mL}$ . This suggests that the extract exhibits little cytotoxicity within the concentration levels used in the bioassays.

#### **GC-MS result**

According to the previous research study of the methanolic macroalgal extract of the *T. ornata* extract identified several major bioactive compounds, including hentriacontane, (Z,Z)-6,28-heptatriacontadien-2-one, 8-heptadecene, and 1-heptacosanol. Hentriacontane, a long-chain alkane, is a major constituent of *Oldenlandia diffusa*, a medicinal plant traditionally used in Asia for cancer treatment. It has been reported to have anti-inflammatory activity through suppression of caspase-1 activation in mouse peritoneal macrophages (Kim *et al.*, 2011). Long-chain hydrocarbons and fatty acid derivatives from mangrove and other plant sources have also shown antimicrobial activity (Agoramoorthy *et al.*, 2007). To date, the antidiabetic potential of hentriacontane has not been well documented. The presence of these compounds may therefore contribute to the antioxidant,  $\alpha$ -amylase, and DPP-IV inhibitory activities observed for *T. ornata* in the present study. However, further purification and structural characterization of the active compounds, together with mechanistic studies, are needed to identify the constituents responsible for the antidiabetic activity of *T. ornata*.

#### **Conclusion**

The current research demonstrates that the macroalgal extract from *Turbinaria ornata* has significant antioxidant and antidiabetic properties *in vitro*. The extract eliminated DPPH radicals in a concentration-dependent fashion, exhibiting an  $\text{IC}_{50}$  value of 623.6  $\mu\text{g/mL}$ , in contrast to ascorbic acid's 166.8  $\mu\text{g/mL}$ . It strongly suppressed  $\alpha$ -amylase, an essential enzyme for carbohydrate digestion, with an  $\text{IC}_{50}$  of 336.3  $\mu\text{g/mL}$  compared to 102.4  $\mu\text{g/mL}$  for acarbose. Furthermore, the extract demonstrated inhibition of DPP-IV ( $\text{IC}_{50} = 52.5 \mu\text{g/mL}$ ), and at the maximum concentration, its efficacy was akin to that of the standard diprotin A, indicating a potential function in promoting insulin secretion. The MTT experiment conducted on J774 cells indicated that the extract exhibited non-toxicity at reduced doses, with an  $\text{IC}_{50}$  of 931.5  $\mu\text{g/mL}$ , much above the levels necessary for enzyme inhibition. GC-MS examination revealed bioactive constituents including hentriacontane, (Z,Z)-6,28-heptatriacontadien-2-one, 8-heptadecene, and 1-heptacosanol, potentially responsible for the noted actions. Collectively, our results indicate that *T. ornata* represents a potential natural reservoir of antioxidant and antidiabetic compounds. Additional research on the extraction and identification of the active constituents, their modes of action, and *in vivo* effectiveness is required to ascertain its therapeutic promise in diabetes treatment.

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