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The polar fraction of a Sea cucumber (*Bohadschia Argus*) is a potential source of anti-inflammatory and hypoglycemic agents

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Abstract

Diabetes is a complex metabolic condition that affects the glucose homeostasis of the human body. Inflammation has been significantly associated with diabetes pathogenesis and so makes this area a popular target for the prevention and treatment of these metabolic abnormalities. In this study, we have evaluated the anti-inflammatory and anti-diabetic properties of polar methanol (SB1M) and non-polar hexane (SB1H) extracts of a sea cucumber, *B. argus*, via inhibition of albumin denaturation assay, starch-iodine and glucose-uptake assay by yeast cells assays. Whilst there was no anti-inflammatory action observed in SB1H extracts, the SB1M showed anti-inflammatory potential (84.70±1.9% to 100±1.11%) with comparable activities with a known anti-inflammatory drug Flanax (Naproxen) ($p<0.05$). For anti-diabetic activity, the average alpha-amylase percent inhibition capacity for both extracts was observed to be in the range of 60.99±1.23% to 71.26±3.33% with a moderate enhancement of glucose uptake using yeast cells. Therefore, this study provides groundwork data to indicate *B. argus* as a potential source of agents with anti-inflammatory and anti-diabetic effects.

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Introduction

Inflammation is a normal bodily defense mechanism against harmful stimuli including damaged cells, pathogens, and other toxic compounds (Chen *et al.,* 2018). However, when not controlled, it can progress to progress to either acute or chronic inflammation which is linked to various diseases including cardiovascular diseases, neurodegenerative disorders, cancer, and diabetes among others (Abdulkhaleq *et al.*, 2018).

Diabetes is a complex metabolic condition characterized by decreased glucose tolerance and hyperglycemia as a result of insulin deficiency or resistance. The 2021 reports by the World Health Organization estimated 1.5 million diabetes-related deaths in 2019. The majority (90%) of diabetic individuals have Type 2 Diabetes Mellitus (T2DM) (Oguntibeju, 2019; Tsalamandaris, 2019).

Recent research has found growing evidence that inflammatory pathways are the primary, common pathogenetic mediators in the natural course of diabetes when risk factors such as physical inactivity, obesity, high body mass index (BMI), and low socioeconomic status are linked to unhealthy lifestyle patterns and limited access to healthcare care facilities, and smoking leading to oxidative stress and inflammation, are present (Bellou *et al.*, 20018; Tsalamandaris, 2019).

For many years, natural products have been known as an important resource for biomedical research, and their significance has even further been acknowledged as the search for safer alternatives to synthetic medicine continues (Mathur and Hoskins, 2017). Recently, there has been a growing interest in the natural products harbored by marine life, particularly from the Phylum Echinodermata.

Phylum Echinodermata is made up of approximately 7000 marine invertebrates species that are widely distributed from the intertidal zone to the deepest depths of the ocean (Stabili *et al.,* 2018). It is divided into five major classes: Ophiuroidea (brittle stars), Crinoidea (Sea Lilies), Echinoidea (Sea Urchins), Asteroidea (Starfishes), and Holothuroidea (Sea Cucumbers) (H.P & W.J.A.B.N, 2016).

This Phylum has attracted great attention in Scientific Research as an unexploited source of new bioactive molecules which have potential pharmacological significance (Layson *et al.,* 2014).

This study evaluated the anti-inflammatory and anti-diabetic effects of the polar methanol and non-polar hexane extracts of *B. argus* using the bovine serum albumin denaturation and the starch-iodine and glucose-uptake assays, respectively.

Materials and methods

Sample collection and identification

The Bohadschia Argus (Jaeger 1833) species were collected from Sarangani Beach, South Cotabato, and identified in coordination with the Department of Marine Sciences, MSU-IIT.

Sample preparation and extraction

Collected samples were thoroughly washed, curated, cut, and then freeze-dried. Dried samples were then soaked in 95% methanol and hexane for 3-5 days. Afterward, this was filtered off to obtain the polar (methanol, SB1M) and the nonpolar (hexane, SB1H) fractions. Both fractions were concentrated *in vacuo* before performing the anti-inflammatory and anti-diabetic assays.

Inhibition of albumin denaturation assay

The inhibitory effect of the different fractions of the specie towards protein denaturation was assessed using the methods of Mizushima and Kobayashi and Sakat *et al.* with minor modifications. 222.5 µL of 1% Bovine Serum Albumin (BSA) were added to 27.5 µL of crude extract. This mixture was incubated at 37° C for 20 minutes, followed by heating at 57°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Flanax (Naproxen) was used as a positive control (Reshma *et al.,* 2014). The experiment was carried out in triplicates and percent inhibition for

protein denaturation was calculated using:

$$\%Inhibition = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

Where: $Abs_{control}$ is the absorbance of the positive control; and Abs_{sample} is the absobance of the sample extract.

Inhibition of alpha-amylase via starch iodine assay

Screening for α-amylase inhibition activity of the echinoderm species was carried out according to the method of Xiao *et al.* (2006) with slight modification based on the starch-iodine test. A 40-µL each of starch solution, sample extract and 1X PBS containing 0.04 units of α-amylase solution were allowed to react for 10 min at 37°C. Thereafter, 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent (5 mM I2 and 5 mM KI). The color change was noted, and the absorbance was read at 620 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included (Kumar *et al.,* 2013). Inhibition of enzyme activity was calculated as:

$$\%Inhibition = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

Where: $Abs_{control}$ is the absorbance of the positive control; and

Abs_{sample} is the absorbance of the sample extract

Glucose uptake by yeast cells

Preparation of yeast extract

Yeast cells were prepared according to this method of Cirillo. Baker's yeast was dissolved in distilled water to prepare 1% suspension. The suspension was then kept at room temperature (25°C) and then centrifuged at 3000 rpm for 5 minutes.

This process was repeated but with the addition of an appropriate amount of distilled water until a clear supernatant was already observed. Finally, 10 parts of the clear supernatant solution were mixed with 90 parts of distilled water to obtain a final 10% (v/v) suspension of yeast cells.

Glucose-uptake activity

A mixture of 96.5 µL of the sample extracts and 85.5 µL of glucose was incubated at 37°C for 10 minutes. The uptake reaction was initiated by adding 18 µL of the yeast suspension, vortexing the tubes, and incubating this further for 60 minutes.

The tubes were then centrifuged at 2000 rpm for 5 minutes. A 150 µL of the content was then transferred to the 96-well plate and the absorbance was read at 620 nm. The acarbose was used as the positive control. The experiment was carried out in triplicates and was calculated using:

$$\%Uptake = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

Where: $Abs_{control}$ is the absorbance of the positive control; and Abs_{sample} is the absobance of the sample extract.

Statistical analysis

The experiments were performed in triplicates and the results were expressed as mean ± standard deviation. Results were analyzed using Microsoft Excel 2015 software (Microsoft, Redmond, WA, USA) and GraphPad Prism (version 9.3.1). A statistical value of p <0.05 for all tests was considered significant.

Results and discussion

Anti-inflammatory activity

Preliminary analysis of the crude extracts revealed that the hexane fractions (SB1H) had not demonstrated any effects in preventing albumin denaturation. In contrast, more than 75% of heat-induced albumin denaturation was prevented by the polar methanolic fractions (SB1M) at concentrations (25-100 µg/mL). Fig. 1, generally shows how at a lower concentration (25ppm) SB1M could significantly inhibit (p <0.05) the protein denaturation.

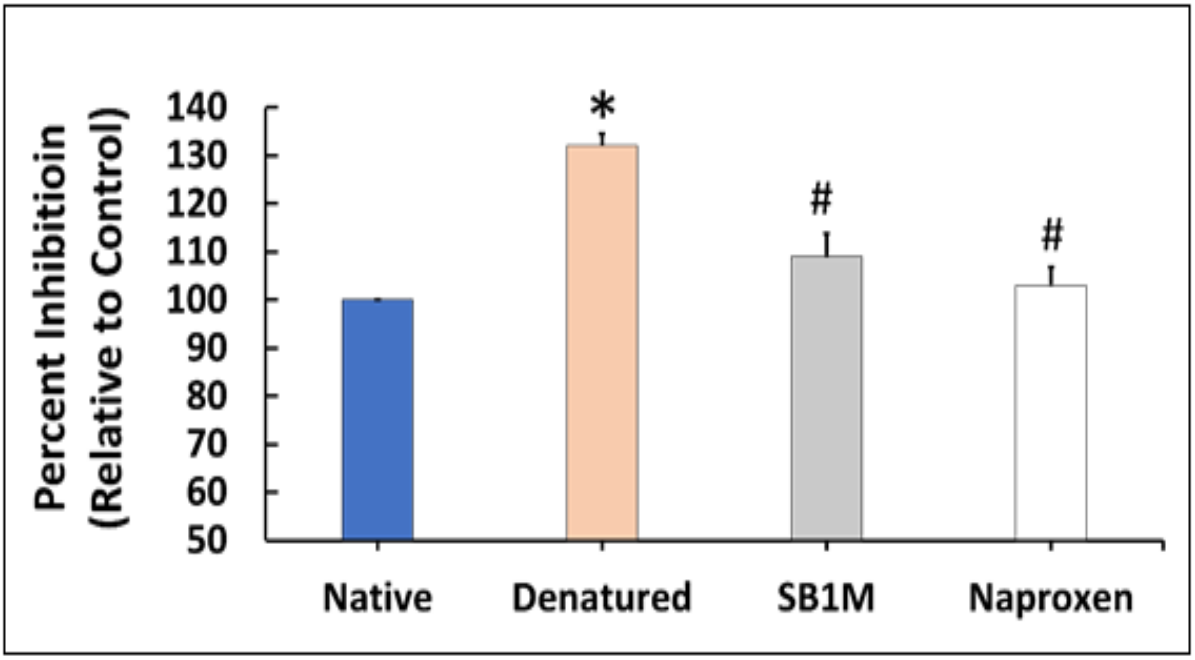

Fig. 1. SB1M prevents albumin denaturation. BSA solution ± 25ppm SB1M was incubated at 37°C and denatured at 57°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Results are expressed as percentage change relative to native control from n=3 independent experiments. [* p <0.05 wrt native albumin (N), Tukey's test; #p <0.05 wrt Denatured (D); Duncan's Test].

As observed, the extract displayed statistically the same response as that of the standard drug used. Moving forward, it was deemed necessary to further investigate to what extent the anti-inflammatory activity of the latter crude extract could potentially impact glucose homeostasis using enzyme inhibition and yeast cells to indicate its hypoglycemic effects.

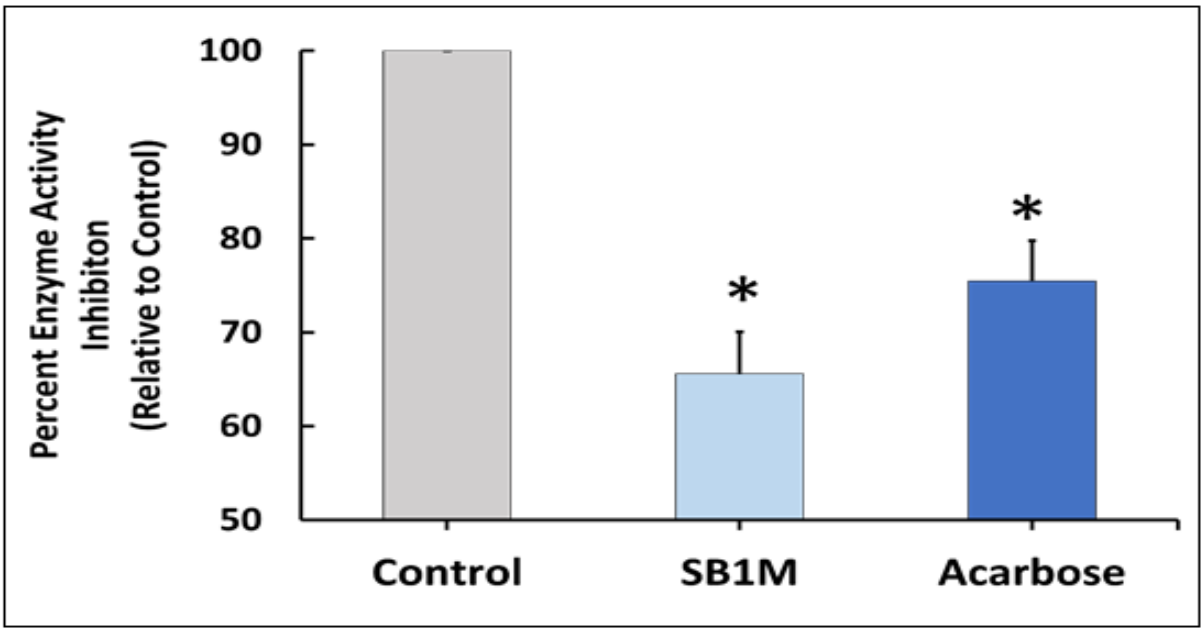

Fig. 2. Methanolic extract of SB1M is an effective inhibitor of alpha-amylase. A starch solution ± SB1M in PBS containing 0.04 units of the α-amylase solution was allowed to react for 10 min at 37°C. Inhibitory effects were determined after adding HCl and Iodine reagent at 620 nm. Results are expressed as percentage change relative to control from n=3 independent experiments. [*p <0.05 wrt Control; Duncan's Test].

Anti-diabetic activity

Inhibition of alpha-amylase: To assess the potential hypoglycemic effects of SB1M, its inhibitory activity towards amylase was conducted. At concentrations (25-100 ppm), it was observed that there was no significant difference in its capacity to inhibit amylase action suggesting the bioactivity of SB1M even at lower concentrations. The result of which is depicted in Fig. 2. The α- amylase inhibitors are also called starch blockers as they act, if not to prevent, then delay the absorption of starch into the body through blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltotriose and other simple sugars.

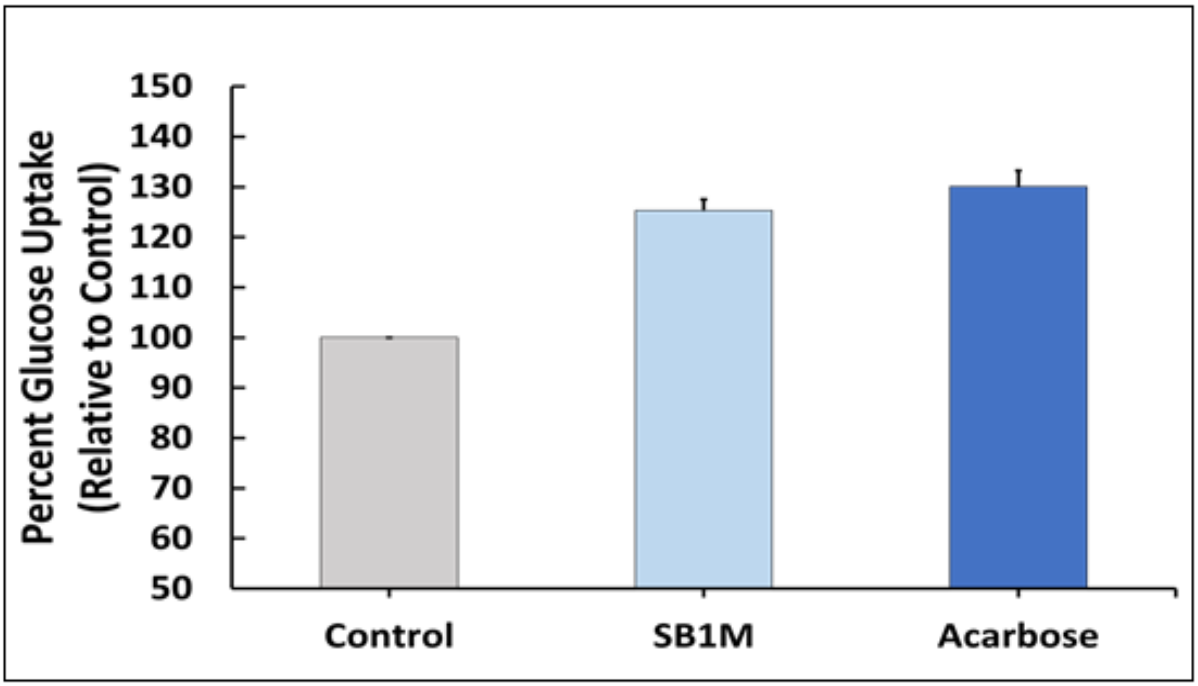

Fig. 3. SB1M enhances glucose uptake by yeast cells. Data are expressed as percent relative change wrt control from n=3 independent experiments.

It is tempting to speculate that the α-amylase inhibitory activity of SB1M might be due to the presence of polar phytoconstituents and thus, this warrants further investigation, such as isolating and characterizing the presence of pure active compounds.

Glucose-uptake assay

Peripheral insulin resistance often characterizes pre-diabetes and involves the failure of skeletal muscle to remove glucose from the bloodstream in response to insulin. We, therefore, sought to determine whether SB1M might also have a beneficial action on glucose uptake using yeast cells. Figure 3 indicates that at 25ppm of SB1M, an enhancement of glucose was observed.

The present study uses two *in vitro* anti-diabetic assays. The results obtained suggest that SB1M could be utilized as a potential source of agents in the management of blood glucose levels.

Conclusion

The present study revealed that the methanolic extract of sea cucumber exhibited significant *in vitro* anti-diabetic and protein denaturation inhibitory activities. Equally important, the results provide the basis for further investigation that can be exploited to discover what particular class of bioactive natural products in SB1M can be potentially developed for new pharmaceuticals. Lastly, purification of these promising active constituents with screening utilizing more physiologically relevant cell or animal models of type 2 diabetes is desirable next step of this study moving forward to help counteract this global health challenge.

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