Phytochemical assessment, and estimation of hypoglycemic potentials of the dichloromethane extract of *Araucaria heterophylla* leaves

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ABSTRACT

Diabetes mellitus (DM), a chronic metabolic illness, is described by long term imbalance in glucose homeostasis. This study was carried out to identify the various phytochemicals, and to evaluate the hypoglycemic endeavor of crude dichloromethane extract of *Araucaria heterophylla (A. heterphylla)* leaves. Extraction was carried out using dichloromethane as a solvent. Preliminary phytochemical assessment of the crude dichloromethane extract of *A. heterophylla* (DAH) indicated the existence of diverse phytochemicals, including flavonoids, sterols, terpenoids, phenols, and carbohydrates. The DAH exhibited α-amylase inhibitory capacity comparable to that of the standard acarbose. Additionally, DAH effectively adsorbed glucose in both low, and high glucose concentrations used in the study.

These findings indicate the potent hypoglycemic effects of the DAH *in-vitro*. The presence of the bioactive constituents may be related to the hypoglycemic activity of the plant extract. To detect the specific compounds responsible for these novel findings, more detailed study is required.

1. Introduction

Diabetes, a worldwide health risk, manifests persistent hyperglycemia. Hyperglycemia is indicated when blood glucose level is consistently high (World Health Organization, 2018). This complex disease mainly disturbs the metabolism of carbohydrates, and protein, and fat. In recent years, the number of patients with hyperglycemia has augmented worldwide (Manikandan *et. al.,* 2013). Diabetes mellitus (DM), characterized by chronic hyperglycemia, encompasses several types based on underlying causes. Type 1 diabetes appears from an obvious deficiency of insulin due to permanent damage of pancreatic β-cells. While type 2 diabetes**,** the most prevalent form globally, results from inadequate insulin secretion, and/or the body's reduced sensitivity to insulin. Type 3 diabetes is secondary to other medical conditions such as pancreatitis, pancreatectomy, or because of certain drug therapies. Type 4 diabetes**,** commonly referred to as gestational diabetes, occurs during pregnancy when blood glucose levels exceed normal ranges but do not meet the diagnostic threshold for diabetes.

Diabetes poses a significant global health burden, and is a leading cause of severe diabetic related disorders like blindness, kidney failure, stroke, cardiovascular disease, and lower limb amputation. According to the WHO, diabetes was the lead cause for 1.6 million deaths in 2016 alone (World Health Organization, 2018). While various pharmacological treatments are available for managing diabetes, many are associated with limitations, including adverse side effects, and diminished long-term efficacy. Consequently, there is a growing interest in identifying, and developing novel therapeutic agents, particularly from natural sources, that offer improved safety profiles, and enhanced effectiveness in glycemic control.

Araucaria heterophylla, most known as Norfolk Pine, Norfolk Island Pine, Star pine, Triangle tree, Christmas tree, Living Christmas tree, Christmas Plant, and House Pine plant belongs to Araucariaceae family (Vennell, 2015). It is a large conical shaped tree with a huge erect stem reaching almost 30-80 metres. It has small, and narrow needle like leaves spreading horizontally (Patil *et. al.*, 2013) which is composed of essential oils, alkaloids, biflavone, and phenolic compounds (Michael *et. al.*, 2010). Young leaves are light-green, incurved, and about 1 cm long; adult leaves are dark

green, incurved, toothache (Bussmann, 2008). It has shown activity against toothache, and extracting teeth (Aslam *et. al.*, 2013).

Numerous studies have highlighted the diverse pharmacological activities of *A. heterophylla*. The resin extracted sequentially with chloroform, and a chloroform/methanol mixture has demonstrated cytotoxic properties, exhibiting moderate anticancer effects against colon, and breast cancer (Sattar *et. al.,* 2009). In addition, dose-dependent antiulcerogenic effects of the resin have been observed in ethanol-induced gastric ulcer models using Sprague Dawley rats, as testified by Sattar *et. al.,* (Sattar *et. al.,* 2009). The natural polysaccharide gum derived from *A. heterophylla* (AHG), obtained via aqueous extraction, and acetone precipitation, was found to be non-toxic in albino mice, with an LD_{50} value of 2000 mg/kg body weight (Divvela *et. al.,* 2016). Furthermore, the leaf powder has shown promising potential as a bioadsorbent, effectively removing Pb^{2+} ions from aqueous solutions, suggesting its utility as a cost-effective method for purifying contaminated water (Sarada *et. al.,* 2013). Antimicrobial properties of *A. heterophylla* leaves extract against pathogenic *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris* was also reported (Goud *et. al.,* 2017). Additionally, studies have shown that the leaves can efficiently remove hexavalent chromium from aqueous solutions within 15 to 30 minutes, reinforcing their value as an economical, and effective bioadsorbent (Shukla *et. al.,* 2012). Previous investigations also reported mild antibacterial properties of *A. heterophylla* leaves extract against gram-negative bacterial strains (Sadia *et. al.,* 2019).

Given this wide range of bioactivities, this study aims at further exploration of the phytochemical constituents of the dichloromethane extract of *A. heterphylla* leaves, and also evaluation of the hypoglycemic activity *in-vitro*.

2. Materials and Methods

2.1 Extraction

The taxonomy identification of the plant (*Araucaria heterophylla*) was done by botanist from National Herbarium Bangladesh, and the identification code is DACB 48435. The plant *Araucaria heterophylla* Franco is a member of Araucariaceae family. The plant leaves were collected from R. P. Shaha University campus. Leaves were dedusted gently, and shade dried after cleaning. Dried leaves were then crushed into small powders by using a laboratory grinder. About 260 g of plant material was extracted with 2300 mL of dichloromethane. Approximately, 3.8% yield from 260 g plant parts.

2.2 Chemicals

Acarbose (Sugatrol, Pacific Pharmaceuticals Ltd.), α-amylase (Sigma-Aldrich), dimethyl sulfoxide (DMSO), glucose, hydrochloric acid, iodine, methanol, phosphate buffer, potassium iodide, and starch were bought from reliable local suppliers.

2.3 Phytochemical screening

The DAH initial phytochemical screening tests were carried out using Trease *et. al.,* methodology (Trease *et. al.,* 1989).

2.4 *α-amylase inhibition assay*

Modified starch-iodine colorimetric technique as described by Sudha *et. al.,* (Sudha *et. al.,* 2011) was used for *in-vitro* α-amylase inhibition assay of DAH. In the reaction blend contained 6.0 mL phosphate buffer at pH 6.9, 0.2 mL α-amylase solution at 100 µg/mL, and graded concentrations (250, 500, 1000, and 2000 µg/mL) of the test materials. After pre-incubation of this mixture at 37 °C for 10 minutes, 0.2 mL of 1% (w/v) solution of starch was added to initiate the enzymatic reaction, which was then kept for 15 minutes at 37 °C to complete the reaction. The enzymatic reaction was ended by adding 200 µL 1 M hydrochloric acid, followed by adding 0.3 mL of iodine reagent (10 mM iodine, and 2.5 mM potassium iodide). After the color change occurred, absorbance was taken at 620 nm using a UV-visible spectrophotometer. A dark-blue coloration indicated the presence of unhydrolyzed starch, a yellow color denoted complete starch hydrolysis, and a brownish hue suggested partial degradation.

In this study, no starch degradation was observed across the test samples. Acarbose, as the standard inhibitor, was tested at the same concentrations as that of test samples. The control reaction, which characterized 100% enzyme activity, was carried out without any test material. Additional control samples excluding the enzyme were included to account for any background absorbance contributed by the test compound itself.

Enzyme activity, and % α-amylase inhibition of the test compounds was calculated following formula:

$$Enzyme\ activity\ (EA) = Absorbance\ of\ test\ control - absorbance\ of\ test\ samples$$

$$\%\ \alpha - amylase\ inhibition\ = \frac{EA_{\ control} - EA_{\ test}}{EA_{\ control}} \times 100$$

2.5 Glucose adsorption capacity assay

The glucose adsorption capability of the sample was assessed based on the technique designated by Ou *et. al.,* (Ou *et. al.,* 2001), with minor modifications. At first, a 0.25% (w/v) solution of DAH was prepared, and added to 20 mL of glucose solutions having 10, 20, 50, 100, and 200 mM concentrations. After thorough stirring, the mixtures were kept at 37 °C using temperature controlled water bath for 6 hours to facilitate adsorption. Following incubation, the samples were centrifuged at 4800 rpm for 20 minutes. The amount of glucose remaining in the supernatant was then quantified by UV-visible spectrophotometry at 540 nm, as outlined by Yakoob *et. al.,* (Yakoob *et. al.,* 2016).
The bound glucose content was estimated based on the formula below:

$$Glucose\ bound = \frac{(G_1 - G_6)}{Weight\ of\ the\ sample} \times volume\ of\ solution$$

Here, G1 denotes the initial glucose concentration of the solution, while G6 refers to the glucose concentration measured after 6 hours of incubation.

3. Results

3.1 Phytochemical screening

The result of phytochemical screening has been showed in the table 1

Table 1: Results of phytochemical screening of DAH:

Chemical Groups	Test Methods	Result
Alkaloids	Mayer's Method	–
	Wagner's Method	–
	Hager's Method	–
Glycosides	Keller-Killiani Method	++
	Concentrate H_2SO_4 Method	+
	Salkowski's Method	++
Carbohydrates	Fehling's Experiment	++
	Molisch's Experiment	+
	Benedicts Experiment	+
Flavonoids	Ammonium Test	++
	Aluminium Chloride method	++
	Shinoda Experiment	–
	Ferric Chloride Experiment	++

Tannins	Lead Acetate Experiment	++
	Potassium Dichromate Method	++
Saponin	Foam Experiment	++
	Haemolysis Test	+
Phenols	Ellagic Acid Test	+
Terpenoids	Salkowski Method	++
Sterols	Salkowski Test	+
	Liebermann-Burchard Test	–
Proteins and Amino Acids	Biuret's Method	–
	Ninhydrin Experiment	–
Resins	Mohler's test	+
Fats and Fixed Oils	Stain Test	–

(++)→High quantity; (+)→Low quantity; and (-)→Absent

Data presented in Table 1, the results indicate that glycosides, carbohydrates, flavonoids, tannins, saponins, and terpenoids are prominently present in the DAH extract. Resins, sterols, and phenols are detected in lesser amounts, while alkaloids, proteins, amino acids, fats, and fixed oils are absent.

3.2 α-amylase inhibition assay

Figure 1 illustrates the percentage of α-amylase inhibition observed at concentrations such as 250, 500, 1000, and 2000 µg/mL of the DAH, and the standard, acarbose (STD). A clear dose-dependent increase in inhibitory activity was noted for DAH, with inhibition rates of 22.29%, 32.74%, 44.18%, and 68.25% at the respective concentrations. In comparison, the standard acarbose exhibited inhibition rates of 28.99%, 40.23%, 55.23%, and 85.29% at the same concentrations. Since higher percentages of inhibition correlate with stronger enzymatic activity suppression, these findings indicate that the DAH extract possesses notable anti-diabetic potential.

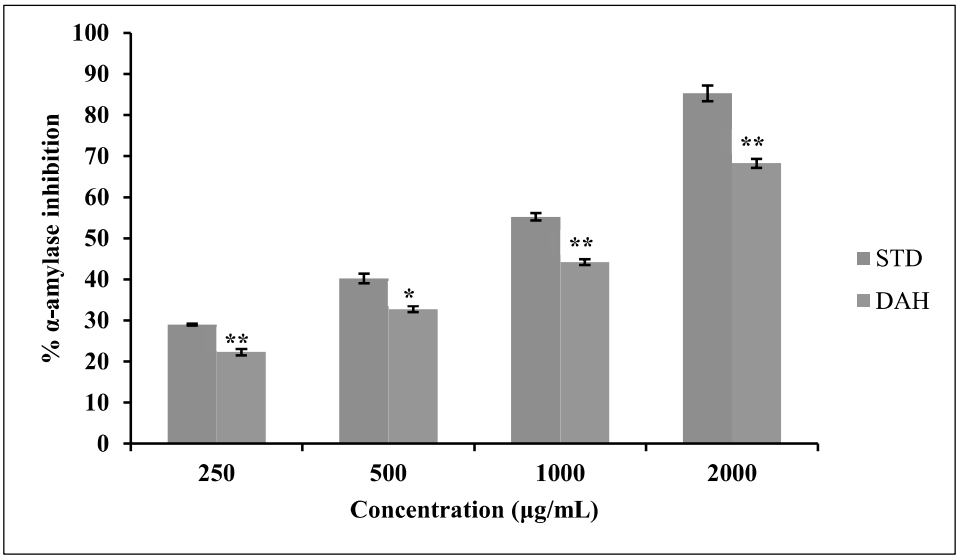

Figure 1: The α-amylase inhibitory capacity of DAH mentioned as the mean ± SD (n = 3). Statistical significance is indicated as *P<0.01, and P<0.001 when compared to the standard acarbose (independent samples t-test). STD = Standard acarbose, and DAH = Dichloromethane extract of *A. heterophylla*.

The IC_{50} of test and standard samples was calculated using linear regression analysis between % α-amylase inhibition, and concentration. The observed value is shown in table 2.

Table 2: IC_{50} value of DAH, and STD

Sample	IC_{50} (mg/mL)
DAH	1.26 ± 0.32*
STD	0.85 ± 0.09

Each value is expressed as mean ± SD (n = 3). DAH = Dichloromethane extract of *A. heterophylla*. *P < 0.001 against standard acarbose (independent samples t-test).

3.3 Glucose adsorption capacity assay

Glucose adsorption capacity of DAH at various concentrations of glucose (10, 20, 50, 100, and 200 mM/mL) are shown in figure 2. The DAH showed effective glucose adsorption of 8.13 %, 13.20 %, 17.40 %, 20 %, and 30 % at 10, 20, 50, 100, and 200

mM/mL concentration of glucose, respectively. These results indicate that DAH had significant glucose adsorption ability.

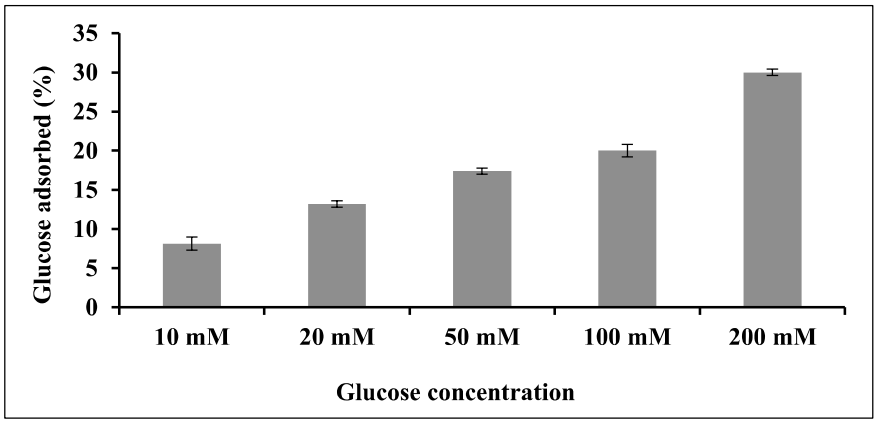

Figure 2: Glucose adsorption capacity of dichloromethane extract of *A. heterophylla*.

Results are shown as mean ± SD (n = 3).

4. Discussions

Diabetes is a metabolic ailment manifested by persistent elevated level of blood glucose. Various plant-derived compounds have shown promise in managing diabetes by regulating blood glucose, and enhancing carbohydrate metabolism (Jugran *et. al.,* 2021). Key bioactive compounds contributing to diabetes management include simple phenolics, catechins, berberine, ginsenosides, curcumin, stevioside, gingerols, capsaicin, anthocyanins, resveratrol, genistein, and hesperidin. Additionally, several phytochemicals such as ascorbyl palmitate, berberine, caffeic acid, cassiaside, catalpol, ellagic acid, ferulic acid, galangin, mangiferin, and papaverine have demonstrated significant anti-diabetic effects, and potential in alleviating diabetes-related complications (Parveen *et. al.,* 2021).

Plant derived chlorogenic acid, ellagic acid, caffeic acid, gallic acid, and other phenolic or polyphenolic compounds; quercetin, rutin, kaempferol, genistein, chrysin like flavonoids; stilbenes, and lignans, have demonstrated anti-diabetic properties. These compounds help reduce glucose levels, and mitigate chronic conditions associated with hyperglycemia by offering antioxidant defense, and hindering starch digestion (Deka *et. al.,* 2022). In the management of elevated blood sugar, delayed starch digestion based on digestive enzymes restriction may offer substantial benefits for diabetic patients, complementing traditional diabetes treatments (Li *et. al.,* 2022). Precisely, the impeding of α-amylase has emerged as a substitute strategy to manage

type 2 diabetes, as it reduces glucose making from carbohydrate-rich foods, which is a primary contributor to postprandial hyperglycemia. This enzyme blocking strategy using inhibitors offers a noteworthy role in diabetes management (Jiang *et. al.,* 2021). Phenolic compounds are known for their notable hypoglycemic effects, because of their capacity to obstruct α-glucosidase, and stimulate cellular glucose uptake (San *et. al.,* 2021). Phytochemical analysis of DAH revealed the presence of several groups of bioactive compounds, including glycosides, carbohydrates, flavonoids, tannins, saponins, terpenoids, resins, sterols, phenols, alkaloids, proteins, amino acids, fats, and fixed oils (Table 1). *In-vitro* α-amylase inhibition assay demonstrated that all concentrations of DAH exhibited α-amylase blocking properties in a concentration dependent manner (Table 2, and Figure 1). The maximum α-amylase inhibition was observed at of 2000 µg/mL concentration with approximately 68.25% inhibition, while the standard acarbose showed 85.29% inhibition at the same concentration. This α-amylase inhibition capacity of DAH might be beneficial in lowering blood glucose in type 2 diabetes by limiting glucose availability for absorption or by slowing the rate of glucose absorption at the small intestine mucosal border. Several studies indicate that traditional α-amylase inhibitors slow the conversion of starch to glucose, thereby reducing blood glucose levels (Gupta *et. al.,* 2020). The phenolic, and flavonoid components found in DAH are likely linked to its α-amylase inhibitory activity, as supported by other research (San *et. al.,* 2021; & Xiao, 2022). The glucose adsorption capacity study demonstrated that DAH could effectively bind glucose, with its binding capacity showing a dependency on glucose concentration (Figure 2). Interestingly, DAH showed efficient glucose adsorption ability at both low (10 mM), and high (200 mM) concentrations. Therefore, the strong α-amylase inhibitory activity, coupled with the glucose adsorption ability of DAH, highlights its remarkable similarity, and suggests as a potential anti-diabetic candidate.

5. Conclusion

In conclusion, our *in-vitro* study showed that DAH has potent hypoglycemic properties which may be due to the bioactive phytochemicals like flavonoids, sterols, terpenoids, and poly-phenols. *In-vitro* hypoglycemic study opens the gate for further *in-vivo* study, and may thereby lead to explore the medicinal values of all components of *Araucaria heterophylla* plant.

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