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Rudimentary phytochemical screening and in vivo exploration of brawny hypoglycemic potency of *Aphanamixis Polystachya* (Wall.) parker seed extractives

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ABSTRACT

Aphanamixis polystachya (Wall.) Parker is a sanative herb with colossal corny convenience. This plant contains a wide range of secondary metabolites, which have been linked to numerous medicinal uses. The current investigation aimed to find the phytochemical components and assess the hypoglycemic impact of n-hexane, chloroform, and methanol extracts of *Aphanamixis polystachya* seeds. Phytochemical screening was carried out using qualitative analysis. To measure hypoglycemic activity, hyperglycemic lab rats with glucose and STZ were used. All of the extractives contained the phytochemicals flavonoids, terpenoids, and steroids. After 30 minutes of administration, the n-hexane extract showed a drop of 41.44% in blood glucose levels, while the chloroform extract exhibited the most significant reduction, 47.76%, at a dose of 150 mg/g BW. The fascinating discovery is that the methanolic extract, which contained carbohydrates absent in the other extracts, resulted in a 14.92% increase in blood glucose levels within 30 minutes of treatment. Organic seed extracts can prevent hyperglycemia, which will be a significant argument in favor of the *Aphanamixis polystachya* plant's common uses in traditional medicine to treat diabetes. More research is required to determine the exact mechanism of action, pinpoint the active ingredients, and clarify their structures.

Keywords: *Aphanamixis polystachya*, Phytochemical screening, Hypoglycemic, Hyperglycemia, Oral glucose tolerance test.

1. Introduction

Hyperglycemia, often known as high blood sugar, is an eminent feature of DM, which is a long-standing and immedicable metabolic malady bringing on either superfluous begetting of insulin hormone, waned responsiveness to insulin, or both (Al-Rasheed et al., 2018). A study has found that it significantly increases the chance of developing both communicable and non-communicable illnesses, such as tuberculosis, DM, and cancer (Qiu et al., 2023). Additionally, the coronavirus illness pandemic of 2019 may have raised the likelihood of insulin resistance and hyperglycemia, exacerbating the universal burden of DM (Mirzaei et al., 2021). A recent study reveals that, by 2030, the worldwide economic cost of DM is expected to reach over 2 trillion USD (Bommer et al., 2018). When taken as a whole, hyperglycemia seriously threatens human health, and greater awareness is needed.

Unworthy treatment of DM resulting from persistent hyperglycemia leads to herculean and occasionally internal impressions after a few days. Crucially, it increases the likelihood of retinopathy, nephropathy, nerve damage, and cardiovascular diseases, among other ailments (Mukherjee et al., 2006). The first-line treatment for T2DM is the oral antidiabetic medication MET (Bravard et al., 2021). In patients with type 2 diabetes, MET monotherapy reduces the fasting plasma glucose concentration by around 60–70 mg/dl (Cusi & DeFronzo, 1998). However, according to Inzucchi et al. (Inzucchi, 2002), up to 50% of individuals undergoing MET therapy experience gastrointestinal side effects. The use of natural items as alternative therapies is quickly gaining acceptability and appeal because of the negative impact and expense associated with current anti-T2DM drugs (Dhanabal et al., 2004).

Currently, there is an increased focus on the efficacy of medicinal plants in the traditional medical system to counteract both on-target and off-target untoward effects. Of the estimated 250,000 higher plants, less than 1% have been screened pharmacologically, and even fewer have been screened for DM (Arumugam et al., 2013). Lamentably, the traditional medicinal herbs used to treat DM have not been studied in toto (Fei et al., 2024).

Of great noteworthiness is a vital plant affiliated with the Meliaceae family, *Aphanamixis polystachya* (wall.) Parker is provincially familiar with "Pithraj," "Royna," "Tiktaraj," or "Baddiraj," which is profusely found in the Indo-Bangladesh subcontinent (Basu, 1999; Chopra & Nayar, 1956; Gupta et al., 2010). The literature search has indicated significant biological activity of this plant, including antioxidant, laxative, anti-cancer, insecticidal, antifeedant, thrombolytic, anti-bacterial, anti-rheumatic, anthelmintic, anti-fungal and analgesic properties (Haque et al., 2020; Mishra et al., 2014; H. Snigdha et al., 2016). Despite its strong pharmacological properties, little research has been found on this plant's hypoglycemic effects. Research on hypoglycemic activity is confined to *Aphanamixis polystachya* leaf essences only (Tiwari et al., 2019). Moreover, studies on seed extracts are restricted to conventional applications, including the assessment of insecticidal and antifeedant efficacy (Talukder & Howse, 1993). In the Indo-Bangladesh subcontinent, farmers have historically used *Aphanamixis polystachya* seed oil to protect their skin from the irritants found in contaminated water when harvesting jute. Assiduousness has been taken through this study to explore the therapeutic benefits against hyperglycemia and phytochemical screening of several organic extracts from *Aphanamixis polystachya* seeds.

2. Materials and method

2. 1. Chemicals and reagents

The following reagents were provided by the Department of Pharmacy, University of Rajshahi-6205: n-hexane, methanol, chloroform, Tween-80, Benedict's reagent, Fehling's Solutions A and B, Dragendroff's reagent, and Molich reagents. Glucose was bought from the neighborhood market under the Unilever brand Galaxose-D. Opsonin Pharma Limited, Barishal, Bangladesh, provided MET hydrochloride under the Met brand. STZ was culled from SimSon Pharma Limited, Mumbai, India.

2. 2. Collection and identification of the plant

Spick and span seeds of *Aphanamixis polystachya* were culled from Rajshahi, Bangladesh. They were in good condition and showed no signs of sickness. At a subsequent time, they were housed in the Phytochemistry Laboratory, Department of Pharmacy, University of Rajshahi, Bangladesh, following verification by the Botany department at the same university.

2. 3. Preparation of plant extracts

After rinsing the seeds with fresh water, they were subsequently exposed to sunlight for a few days to facilitate drying. The desiccated embryos were pulverized into a coarse powder using suitable laboratory grinding equipment. A mixture of 300 grams of powdered seeds and 600 milliliters of n-hexane was placed in a reagent container and subjected to maceration for ten days, with remittent jolting. Afterward, the combination was percolated using a Whatman filter paper, followed by an initial coarse filtration through a clean, white cotton material. After drying, the liquid (n-hexane) was removed by evaporation using a rotary evaporator operating at 120 rpm for 15 minutes at a temperature of 50°C. The result was an n-hexane extract (APHE) with a yellow hue, semisolid consistency, pleasant smell, and bitter taste, with an 8.67% yield value of the extractive. The relic segment was subjugated to successive solvent schemes using chloroform and methanol to prepare posterior essences. The identical procedure was employed to consecutively generate the semisolid, fragrant, acrid, brown-colored chloroform, and methanol extractive, APCE (5.33%) and APME (7.33%). Subsequently, the extracts were employed for phytochemical screening and in vivo hypoglycemic efficacy. The extractives were then kept in reserve in a lucid container, which was counterbalanced with an unequivocal aluminum foil in an arid area.

2. 4. Phytochemical screening

Using the procedures outlined in Evans (Evans, 2009) and Ghani (Ghani, 2003), the organic extractives of *Aphanamixis polystachya* seeds underwent several phytochemical group resolution studies in the Phytochemistry Laboratory, Department of Pharmacy, and University of Rajshahi.

2. 5. Hypoglycemic study design

2. 5. 1. Experimental animals

A total of 66 adult male Wistar rats (*Rattus norvegicus*), with a body weight ranging from 150 to 180 grams and an age of two months, were procured from the Animal House of Khulna University, Bangladesh and were perpetuated at the Pharmacology Laboratory of Bangabandhu Sheikh Mujibur Rahman Science and Technology University (BSMRSTU), Gopalganj, for evaluating hypoglycemic efficacy. Every individual rat was given seven days to acclimate to the unfamiliar environment before commencing the experiment. The rats were brought up during the trial with potable water and regular salubrious pellets. Over and above, they underwent natural lighting and darkness cycles for twelve hours each day and were facilitated with boundless ventilation at room temperature. The Department of Pharmacy, BSMRSTU, approved the experimental design and procedures (#bsmrstu16-11/22).

2. 5. 2. Grouping of experimental rats

Out of sixty-six, eleven groups of six male Wistar rats were randomly allocated (Andrade-Cetto et al., 2019). **Table 1** illustrates the number of treatment groups for hypoglycemic activity.

Table 1. Study groups and treatments for *in vivo* hypoglycemic activity.

Study groups	Treatments
TG1	N. Control (Distilled water)
TG2	H. Control
TG3	H.+ Metformin
TG4	H.+ APME
TG5	H.+ APCE
TG6	H.+ APHE
TG7	N. Control (Glucose)
TG8	G.+ Metformin
TG9	G.+ APME
TG10	G.+ APCE
TG11	G.+ APHE

2. 5. 3. Experimental induction of hyperglycemia

Rats who fasted the previous night were given an intraperitoneal injection of 60 mg/kg of the freshly dissolved STZ solution in 100 mm citrate buffer (pH 4.5). The blood glucose concentration was measured 48 hours later using an acceptable glucometer, Bioland G-423 (Bioland, Germany), collecting blood samples attained from piercing tail veins of speculative rats. Any animals with blood glucose magnitudes higher than 13.9 millimoles per liter were classified as hyperglycemic and allowed to participate in the trials (Tiwari et al., 2019).

2. 5. 4. Readiness of reactive medicaments and plant extractives

Guidelines 423 of the Organization for Economic Cooperation and Development dictated that extract containing 150 mg/kg BW should be used to screen hypoglycemic activity after an acute toxicity study (Tiwari et al., 2019). Samples were measured to suspend test samples at 150 mg/kg BW. The extracts were triturated in a single direction by mixing distilled water with a tiny Tween-80 (H. S. H. Snigdha et al., 2016). On the other hand, MET showed high solubility in water and was present as microcrystals. Due to its high efficacy, Metformin was formulated as a solution using distilled water. Each 0.1 ml solution contains 150 mg/kg BW of MET (Islam et al., 2009).

2. 5. 5. Hypoglycemic upshot of the plant extractives

With minor modifications, the hypoglycemic properties of plant extracts were evaluated in TG 1-6 using the methodology outlined by Andrade-Cetto and his colleagues (Andrade-Cetto et al., 2019). Following the induction of STZ, TG2–TG6 were prepared to assess the hypoglycemic effect at rest. The rats were deprived of water for sixteen hours. Glucose levels at the starting point were monitored in every individual rat. To compare the blood glucose levels of the control group with those of other groups receiving STZ, TG1 that was given distilled water was chosen. TG2 was chosen as the hyperglycemic control group; neither MET nor extracts were given to this group. TG3 represents the MET control group, which receives 150 mg/kg BW doses of MET intraperitoneally. The extractives of *Aphanamixis polystachya* seeds- APME, APCE, and APHE were given to TG4-TG6. The subsequent procedure involved extracting blood and quantifying the blood glucose concentration using an advisable glucometer. In this case, the tail vein was pierced at 0, 15, 30, 60, and 120 minutes, succeeding germane administration of the medication and plant extractives.

2. 5. 6. Oral glucose tolerance test (OGTT)

OGTT was conducted using the protocol with some adjustments followed by Islam and his colleagues (Islam et al., 2009). After 16 hours of abstaining from consuming food or drink except for water, TG7–TG11 was selected to participate in the OGTT evaluation. The starting point of blood glucose intensity was obtained by using a convenient glucometer. TG7 was acted to embody the control group, which received glucose solution only. TG8 was dealt with an intraperitoneal dose of MET (150 mg/kg BW). Afterward, a dose of 150 mg/kg BW of the three extracts - APME, APCE, and APHE derived from *Aphanamixis polystachya* seeds was administered intraperitoneally. A pristinely prepared glucose solution (2 ml) was administered to experimental rats of groups TG7-TG11 using an intragastric tube (2 gm/kg BW) following one hour of MET and plant extract treatments. Subsequently, the blood glucose level was assessed using a glucometer. Blood was culled by pricking the tail vein at intervals of 0, 30, 60, 90, 150, and 270 minutes.

2. 6. Statistical analysis

The consequences are reported as the mean ± standard error of the mean or as a percentage collated with the control groups. The statistical analyses were conducted with the aid of Graph Pad Prism software (Version 9.5.1, San Diego, CA, USA). Interrelationships between the means were thoroughly evaluated using one-way ANOVA and t-Student Newman–Keuls post hoc test. Correlations for hypoglycemic tests were calculated using blood glucose level values. Significance was attributed to p values ($p < 0.05$) at a 95% confidence level, while a precise code of behavior of p ($p < 0.0001$) was contemplated as very noteworthy.

3. Results

3. 1. Phytochemical analysis

Plants under investigation included flavonoids, terpenoids, steroids, alkaloids, and carbohydrates, according to a phytochemical screening **Table 2**. Except for tannins, the APME showed flavonoids, terpenoids, steroids, alkaloids, and carbohydrates. Besides carbohydrates and tannins, the APCE exhibited flavonoids, terpenoids, steroids, and alkaloids. Conversely, APHE displayed steroids, terpenoids, and flavonoids.

Table 2. Phytoconstituents of *Aphanamixis polystachya* seed extracts.

Constituent	Name of test	APME	APCE	APHE
Steroid and terpenoid	Libermann-Burchard's scrutiny	-	+	-
	Salkowski's scrutiny	+	+	+
Alkaloid	Mayer's scrutiny	-	+	-
	Hager's scrutiny	+	+	-
	Dragendroff's scrutiny	-	-	-
	Wagner's scrutiny	+	+	-
Phenolic and flavonoid	Zinc-Hydrochloride reduction scrutiny	+	+	+
Tannin	Ferric chloride scrutiny	-	-	-
Carbohydrate	Reducing sugars scrutiny	+	-	-

3. 2. In vivo hypoglycemic activity

3. 2. 1. Effect on STZ-induced investigational rats

Figure 1 presents the effects of MET and three distinct extracts of *Aphanamixis polystachya* seeds, namely APME, APCE, and APHE, on the blood glucose intensity in accustomed and STZ-instigated hyperglycemic probing rats. The data is presented as the mean ± standard error of the mean. Upon comparing the blood glucose levels of the hyperglycemic control group with those of the normal control group, it was seen that the animals of the hyperglycemic control group had consistently high glucose levels over 120 minutes. The standard medication, MET, lowered the glucose by 61.53% after 30 minutes and had a sustained, substantial hypoglycemic impact from 15 to 120 minutes.

The evaluation's outcome shows that two extractives, APCE and APHE, injected intraperitoneally had a hypoglycemic impact that was statistically significant ($p < 0.05$) and comparable to MET. In this case, both extracts, APCE and APHE, are almost equally effective, but APCE is the more potent. Blood glucose levels were lowered by APCE to 22.50%, 47.76%, 38.46%, and 6.97% in 15, 30, 60, and 120 minutes, respectively. Thus, after 30 minutes, APCE resulted in a maximum drop of 47.76% in blood glucose levels. In 15, 30, 60, and 120 minutes, APHE decreased blood glucose levels to 20.66%, 41.44%, 31.53%, and 5.03%, respectively. So, after 30 minutes, APHE reduced blood glucose levels to 41.44%. However, it's important to note that in 5, 30, 60, and 120 minutes, APME raised blood glucose levels to 6.55%, 14.92%, 2.62 %, and 1.55%, respectively.

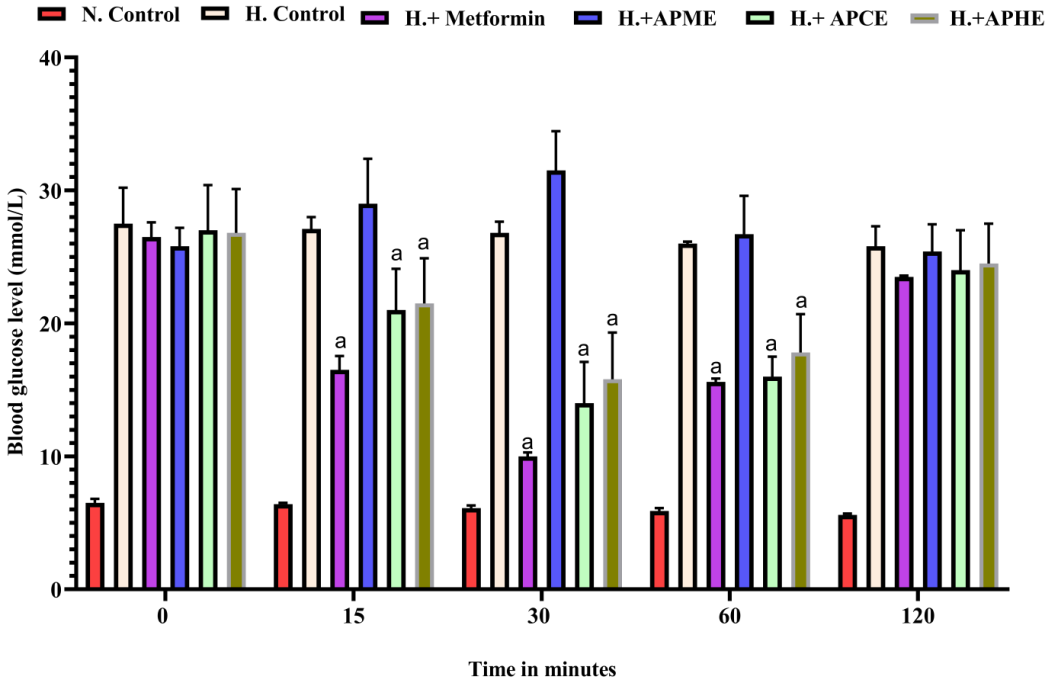

Figure 1. The effect of various fractions of *Aphanamixis polystachya* seeds on STZ-induced hyperglycemia in normal rats. The result is shown as (means ± standard error of the mean (SEM); one-way ANOVA and t-Student Newman–Keuls post hoc test with multiple comparisons at 95% confidence intervals; A significant difference in blood glucose level (mmol/L) is indicated by the letter 'a' when compared to the H. Control (hyperglycemic control) group ($p<0.05$).

3. 2. 2. Effect on glucose-induced rats

In an experiment to induce hyperglycemia, plasma glucose levels increased 1.5–2 times when glucose was consumed intragastrically. **Figure 2** shows the effects of three different extracts (APME, APCE, and APHE) of *Aphanamixis polystachya* seeds on blood glucose levels in normal and glucose-instigated hyperglycemia in customary investigational animals. The data is displayed as mean ± standard error of the mean.

The aforementioned experiment clearly shows that the usual medication MET lowered blood glucose levels to 29.41%, 26.66 %, 22.72%, 2.85%, and 1.5% in 30, 60, 90, 150, and 270 minutes, respectively, under the outcomes of OGTT. MET resulted in the most significant reduction of blood glucose levels by 29.41% after 30 minutes.

Blood glucose concentration was lowered by APCE to 23.52%, 20%, and 18% in 30, 60, and 90 minutes, respectively. The blood's APCE concentration falls with time, and after 150 minutes, the blood glucose level diminishes by 2.85 %. In 30 and 60 minutes, respectively, APHE lowered blood glucose levels to 17.64 % and 13.33%. After 90 minutes, the blood glucose level declines by 9.09% while the blood concentration of APHE falls. However, it's mentionable that within 30, 60, 90, 150, and 270 minutes, respectively, APME raised blood glucose levels to 22.72 %, 20%, 9.09%, 6.66%, and 2.94%.

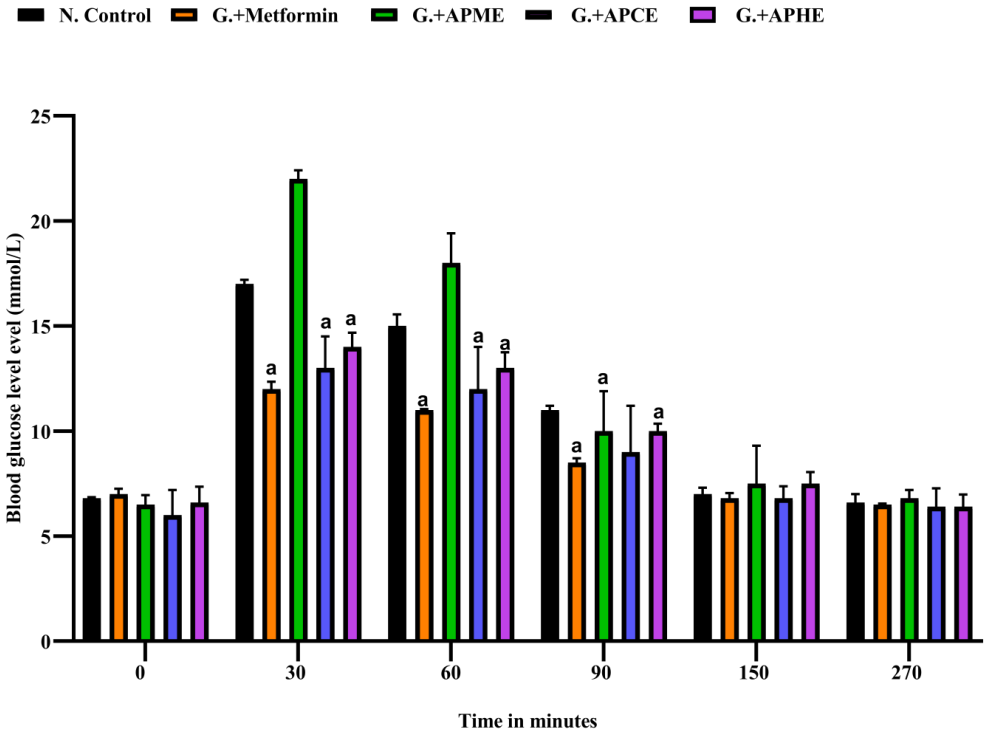

Figure 2. The effect of various fractions of *Aphanamixis polystachya* (Wall.) on glucose-induced hyperglycemia in normal rats. The result is shown as (means ± standard error of the mean (SEM); one-way ANOVA and t-Student Newman–Keuls post hoc test with multiple comparisons at 95% confidence intervals; A significant difference in blood glucose level (mmol/L) is indicated by the letter 'a' when compared to the N. Control (Glucose) group ($p<0.05$).

4. Discussion

A cytotoxin called STZ selectively destroys the pancreatic β-cells, causing an outstanding drop in blood insulin intensities. In the absence of insulin, the liver's ability to absorb glucose will be hindered, leading to a drop in the liver's glycogen levels (Patel et al., 2012). The assessment's repercussions demonstrate that intraperitoneal injection of two extractives, APCE and APHE, at the administered doses of 150 mg/kg BW can resolve this issue, aid in glucose absorption by cells, and facilitate the production of liver glycogen compared to hyperglycemic control animals (Tiwari et al., 2019). Furthermore, these two extracts have a similar pattern of blood glucose decrease to that of the conventional medication MET.

The aforementioned result is consistent with a prior study in which extracts were administered intraperitoneally to hyperglycemic rats, resulting in a considerable reduction in blood glucose levels. Methanolic extract from *Aphanamixis polystachya* leaves has been shown in a previous study to have possible hypoglycemia effects in rats induced by STZ (Tiwari et al., 2019). Furthermore, not much study has been discovered when *Aphanamixis polystachya*'s other parts—aside from its leaves—are used to test for the hypoglycemic effects. In this investigation, it was found that n-hexane and chloroform extracts

of *Aphanamixis polystachya* seeds are powerful hypoglycemic agents when compared to mice treated with MET and hyperglycemic control animals.

Another method by which plant extracts are tested for acute hypoglycemia is by an increase in oral glucose tolerance. This widely used OGTT was initially created to categorize carbohydrate tolerance because the test's results for plasma glucose and insulin represent the capacity of pancreatic cells to release insulin and the tissue's susceptibility to insulin (Stumvoll et al., 2000). The degree of hyperglycemia caused by glucose loading in treated rats appears to have been significantly decreased by the chloroform and n-hexane extracts of *Aphanamixis polystachya* seeds in this investigation compared to control animals. This suggests that chloroform and n-hexane extracts could somewhat postpone the development of hyperglycemia. It is believed that improved glucose tolerance is related to plasma insulin's availability and functionality (Tiwari et al., 2019).

Compared to the standard medication MET at a dose of 150 mg/kg BW, this may have been a significant factor in the extracts' hypoglycemic ability to reduce glucose-induced hyperglycemia in the treated mice, which enhances OGTT outcomes by lowering blood glucose levels and increasing insulin action. In general, MET accelerates glucose transport and glycogen synthesis, raises insulin receptor tyrosine kinase activity, increases the number and affinity of insulin receptors in muscle and adipocytes, and decreases hepatic gluconeogenesis and glycogenolysis through AMPK-dependent and AMPK-independent mechanisms to exert a powerful and complex effect on enhancing blood glucose homeostasis (Rena et al., 2017).

The hypoglycemic activity of *Aphanamixis polystachya* may be due to the presence of tannins, flavonoids, saponins, and sterols in the methanolic leaf extract. These compounds perform through pancreatic β-cell regeneration and decrease blood sugar levels (Tiwari et al., 2019). According to a different study, the primary mechanism by which the phenolic compounds of *Aphanamixis polystachya* produce their hypoglycemic effects. These substances regulate the enzymes linked to glucose metabolism, augment the activity of β-cells and insulin, and encourage insulin release (Hotamisligil, 2006; Hotamisligil et al., 1993).

A phytochemical screening in this work revealed the presence of flavonoids, terpenoids, steroids, and alkaloids in the chloroform and n-hexane extracts of *Aphanamixis polystachya* seeds. Numerous studies showed that in STZ-induced hyperglycemic rats, steroids exhibited intense hypoglycemic action by diminishing tumid blood glucose magnitudes and flourishing insulin intensity (Daisy et al., 2009). The scientific community has taken notice of Proença et al.'s (Proença et al., 2022) study on the hypoglycemic efficacy of flavonoids, copiously present in *Aphanamixis polystachya* (wall.) parker seeds that fire at DPP-4 and PTP1B.

Alkaloids, basic nitrogenous compounds that plentifully exist in *Aphanamixis polystachya* seeds, function similarly to other natural products in regulating glucose metabolism. They do this by either inducing or inhibiting a diversity of aspirant proteins, for instance, glucose transporters, AMPK, sterol regulatory element-binding proteins 1, glucokinase, glucose-6-phosphatase, and acetyl-CoA carboxylase (Muhammad et al., 2021). Thus, phytochemicals have overwhelmed APCE and APHE as potent hypoglycemic agents. It's interesting to note that APME contains carbohydrates. In contrast, the other two extracts did not, and this causes APME to elevate blood glucose levels in both STZ-induced and glucose-induced rats.

5. Conclusion

Aphanamixis polystachya is notable for several naturally occurring bioactive metabolites with remarkable therapeutic potential. Based on the above discussion, we can conclude that *Aphanamixis polystachya* seed extracts are an excellent source of hypoglycemic properties. This is only a preliminary study; additional research is inevitable to examine other physiological, pharmacological, and toxicological parameters along with the isolation of bioactive components that guarantee the genuine causes of the extractives' pursuit and their undistorted demeanor with the least negative impact. With any luck, more research will identify the active phytoconstituent and reveal the structure of the novel compound.

Conflict of Interest

The authors declare that there is no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All necessary data generated or analyzed during this study are included in this article. Any additional data could be available from the corresponding author upon request.

Author Contributions

H. M. Shadid Hossain Snigdha conceived the idea, outlined the content, and edited the manuscript; Dr. Md. Ekramul Haque supervised the research work; Md. Tahajul Islam collected information, performed the experiments, and developed the manuscript; MD. Shadin Mostakim analyzed and interpreted the data and wrote the manuscript.

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Abbreviation

AMPK: AMP-activated protein kinase.
ANOVA: Analysis of Variance.
APCE: Aphanamixis polystachya chloroform extract.
APHE: *Aphanamixis polystachya* n- hexane extract.
APME: *Aphanamixis polystachya* methanol extract.
BW: Body Weight.
DPP-4: Dipeptidyl peptidase-4.
DM: Diabetes Mellitus.
G: Glucose.
MET: Metformin.
N: Normal.
OGTT: Oral Glucose Tolerance Test.
PTP1B: Protein Tyrosine Phosphatase 1B.
STZ: Streptozotocin.
T2DM: Type 2 Diabetes Mellitus.
TG: Treatment Group.
USD: United States Dollar

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