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Research article

Pharmacological Investigation of Anti-coagulant, Anti-hyperglycaemic, and Anti-hyperlipidemic Potential of Ethanol Extract of *Amomum subulatum* Seeds

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ABSTRACT

Amomum subulatum, often known as black cardamom (Zingiberaceae), is used in traditional medicine to reduce the risk of diabetes, stroke, heart disease, and other conditions. The present work was undertaken to evaluate the effects of ethanol extract of Amomum subulatum seeds on blood plasma clotting time, glucose concentration levels, percentage of glucose diffusion, and lipid profile such as serum concentrations of bad cholesterol e.g. low-density lipoprotein (LDL), triglycerides (TG), and cholesterol parameters. Herein, the in vitro anticoagulant activity of the extract was assessed through a prothrombin time (PT) test on blood plasma, antihyperlipidemic activity in vivo was assessed using the high-fat diet overload test on Swiss-albino mice, and antihyperglycemic potential was evaluated through in-vivo oral glucose tolerance test (OGTT) and in-vitro glucose diffusion assay. In comparison to hyperlipidemic mice, the results demonstrated that 4 % ethanol extract combined with a dietary supplement decreased the levels of low-density lipoprotein, triglycerides, and cholesterol. When compared to the standard activity of warfarin (5 mg/ml), a prothrombin time (PT) of roughly 7 minutes suggested that Amomum subulatum (15 mg/ml) was acting as an anticoagulant agent. The extract at 500 mg/ml significantly inhibited the diffusion of glucose through a semi-permeable barrier and, at 500 mg/kg dose significantly decreased blood glucose levels in diabetic mice (7.63±0.15, 7.5 ±0.12, and 5.03 ±0.08 mM/L at 30, 90, and 150 minutes, respectively). Phytochemical screening revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, etc. in the ethanol extract. Considering the results of this investigation, Amomum subulatum has potent anticoagulant, antihyperglycemic, and antihyperlipidemic effects that make it useful for treating cardiac problems and diabetes.

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Introduction

Worldwide, medicinal plants have been widely used and regarded as an essential source of new pharmaceuticals since the beginning of human civilization [Balunas, 2005; Grover, 2002]. This is because plants naturally contain compounds referred to as phytonutrients, such as steroids, fatty acids, alkaloids, terpenoids, flavonoids, and fatty acids [Leitzmann, 2016]. *Amomum subulatum*, commonly known as big cardamom, black cardamom, or Bengal cardamom, is an herbaceous perennial plant that belongs to the Zingiberaceae (ginger) family and is referred to as the "Queen of Spices".

The big and clustered black cardamom tree with red stems has many thin, glossy, and tropical leaves. The fruit-bearing cardamom seeds are borne at the base of yellowish-white blossoms. The cardamom seed is used as a spice due to its strong, Smokey flavor, which is like that of camphor. This plant is inherent to the Eastern Himalayas and has become widespread in Bhutan, Nepal, and India [Bisht, 2011]. It has historically been used as a prophylactic and therapeutic drug for several illnesses, such as oral infections, lung congestion, digestive problems, and

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throat disorders. *Amomum subulatum*'s essential oil contents, which are mostly composed of 1,8-cineole followed by α-terpineol, and limonenes, are thought to play a preventative role [Dhakal, 2022; Bhandari, 2013]. Research both in-vivo and in-vitro has shown the pharmacological benefit of *Amomum subulatum* against a range of medical diseases, such as tumor inhibition, inflammation suppression, renal and hepatoprotective actions, and free radical scavenging [Gautam, 2016].

Hyperglycemia, a metabolic disorder linked to changes in the metabolism of fat, protein, and carbohydrates, is a hallmark of diabetes mellitus. Type 1, type 2, and gestational diabetes are the three main forms of the illness [Dilworth, 2021]. The health-related quality of life (HRQOL) of individuals with type 2 diabetes may be significantly impacted by diabetes complications [Lloyd, 2001]. Additionally, type 1 diabetes is linked to a worse quality of life and an increased risk of sadness, anxiety, and discomfort throughout a lifetime [Duinkerken, 2020]. However, one more metabolic abnormality linked to higher risk factors for the onset of diabetes is hyperlipidemia. In addition to their relationship with diabetes, high serum levels of cholesterol, triglycerides, and LDL are important risk factors for the early onset of cardiovascular disorders such as hypertension, atherosclerosis, and coronary heart disease [Ansarullah, 2009]. Moreover, blood coagulation plays a crucial role in thrombosis and cardiovascular diseases (CVD) due to blood's high triglyceride and cholesterol levels [Adhyapak, 2016]. A blood clot that limits blood flow at the site of injury is formed by the hemostasis mechanism [Orfao, 2008]. Plant-based herbal remedies have become increasingly popular in recent years and bioactive components of these plants can be investigated in experimental and clinical investigations for their potential to have antihyperglycemic, antihyperlipidemic, and cardio/reno-protective effects [Fatehi-Hassanabad, 2005; Sotoudeh, 2019]. Some herbs are commonly sold in the market as blood thinners and their anticoagulant activity has been shown by previous studies [Al-Saadi, 2011; Zhu, 2018]. The World Health Organization (WHO) also supports the consumption of herbal drugs to treat ailments.

However, due to a lack of comprehensive chemical analysis and pharmacological research, *Amomum subulatum* is not receiving much attention for the innovation of new medicines. The purpose of this study is to investigate the possible therapeutic effects of *Amomum subulatum* seeds ethanol extract on hyperlipidemia, hyperglycemia, and coagulation of blood conditions.

Materials and Method

Chemicals

Amomum subulatum dried seeds were collected from Khulna, Bangladesh's local market. The standard drugs glibenclamide, atorvastatin, acarbose, and warfarin were acquired from Sigma Aldrich, USA. A 95 % ethanol was purchased from BDH Ltd., England for use in the extraction. To measure the blood glucose level, a glucometer and strips were bought from Roche Diagnostics India Pvt. Ltd., India. We bought d-glucose and tween-80 from Sigma Aldrich, USA. The supplier of beef tallow was Essential Depot, Inc., Sebring, Florida, USA. The following materials were utilized for the phytochemical identification test: hydrochloric acid, sodium hydroxide, ferric chloride, nitric acid, sodium bicarbonate, and different chemical reagents bought from Labmerk Chemicals LTD, India. We bought commercial reagent kits from Biolabo S.A., Paris, France to measure LDL, triglycerides, and cholesterol. Every used chemical was analytically graded.

Experimental Animals

The experiment was conducted using widely used Swiss-albino mice (*Mus musculus*) strain that were collected from the Pharmacology Laboratory, Jahangirnagar University, Savar, Dhaka, and housed in the animal house of Pharmacy Discipline Khulna University, Bangladesh. The mice were two months old, with weights ranging from 20 to 35 gm. The animals were housed in cages made of stainless steel, with six mice per cage at random, and were kept at 25 ± 2 °C with a 12-hour light/dark cycle. Throughout the trial, the animals were given standard pellets (white flour 22.2 gm, roasted Bengal gram flour 60 gm, skim milk powder 5 gm, casein 4 gm, refined ground nut oil 4 gm, salt mixture 4 gm, and vitamin mixture 0.5 gm in 100 gm) and distilled water, except for the fasting phase. The Department of Pharmacy Ethics Committee of Khulna University (Ethical Approval No.: KU/PHARM/AEC/15/006/36) in Bangladesh approved the study methodology, and the care and handling of the animals complied with recognized ethical standards for laboratory animals which comply with OECD guidelines [OECD, 2001].

Qualitative Phytochemical Screening

Plant Collection and Crude Extraction Preparation

The *Amomum subulatum* dry seeds were purchased from Khulna, Bangladesh's local market on March 7, 2017. Adulteration of any kind was forbidden during collection. After removing unwanted elements, the collected seeds were further shed and dried for a week. After that, the seeds were ground into a fine powder using a grinder, sealed in a container, and placed in cool, dry, and dark until the analytical process started. An 1100 ml of 95 % ethanol was used to soak 550 gm of finely ground material in a clean and flat bottom glass container. After that, the container was sealed and stored for the next 14 days with sporadic shaking and stirring. Then, the contents were filtered using a piece of clean cotton and Wattman filter paper (0.22 µm). Following that, water bath temperature and ceiling fan air were used to dry the filtrate (ethanol extract), resulting in a gummy and oily bulk crude extract of 1.60 percent. Yield was determined by using the following formula [David, 2017].

$$\%\ Yield = \frac{Weight\ of\ the\ crude\ extract}{Weight\ of\ dried\ powdered\ sample} \times 100$$

Chemical Group Test

The chemicals and reagents like, Molisch’s, Benedict, Mayer’s, Dragendroff’s, Hager’s, Libermann-

Burchard reagents, and Fehling's solution were used to qualitatively analyze the crude extract for the presence of different chemical groups. To determine the common group of chemical constituents found in *Amomum subulatum* seeds, this investigation utilized turbidity and precipitation reactions and examined under ultraviolet spectroscopy (UV-Vis) light, or a particular colour change. In each test, 0.1 gm of dry extract was dissolved in 100 ml 95 % ethanol (0.1 % (w/v) solution). These tests were conducted according to the reference protocols [Zerkani, 2022; Koffi, 2009].

In-vitro Anti-coagulant Activity Assay
Blood Sample Collection
Blood samples were taken from healthy adults between the ages of 20 and 25. The volunteers were not suffering from diabetes, dyslipidemia, coagulation disorders such as hemophilia A or B, or any cardiovascular disease like hypertension, or congestive heart failure. Moreover, they were not smokers and did not take non-steroidal anti-inflammatory medicine recently. To extract blood from the right arm vein, sterile syringes were used, and the samples were then placed individually in a test tube containing trisodium citrate to stop the scavenging process. The blood cells were then separated from the plasma using a centrifuge for 15 minutes at a speed of 3000 rpm, yielding Pure Platelet Plasma (PPP) for the prothrombin time test. Each person's collected plasma sample was placed separately into sterile, clean, and flat surface glass containers, and kept at room temperature [Alarcon-Aguilara, 1998; Loew, 2002].

Prothrombin Time Test
For the prothrombin time test, 0.2 ml of plasma, 0.1 ml of different concentrations (200 to 6.25 mg/ml) of Amomum subulatum seed extract, and 0.3 ml of $CaCl_2$ (25 mM) were blended in a sterile fussion test tube. For the control, 0.2 ml of plasma, 0.3 ml of $CaCl_2$ (25 mM), and 0.1 ml of 0.9% saline were added to the second fussion test tube. For the standard, 0.2 ml of plasma, 0.3 ml of $CaCl_2$ (25 mM), and 0.1 ml of warfarin (5 mg/ml) were put into the third fussion test tube. Every test tube was kept in a water bath at 37 °C for incubation. Every five seconds, the test tubes were tilted to record the beginning and final clotting times using a stopwatch. The prothrombin time is the duration of coagulation of plasma. Every test was run three times, and the mean scavenging time was recorded [Azwanida, 2015; Sofowara, 1982].

Anti-hyperglycaemic Assay
In-vivo Oral Glucose Tolerance Test (OGTT)
For the Oral glucose tolerance tests (OGTT), young Swiss albino mice, 4-5 weeks old, weighing between 20 and 28 gm were used. The OGT tests were conducted by the previously published protocol with a few minor modifications [Shewamene, 2015]. The test mice were kept in a fasting condition for at least 10 to 16 hours, during which they were only given water to drink. The mice were divided into four groups, identified as groups I, II, III, and IV, with four mice in each group, after being chosen at random. Every group was given a certain treatment, such as the test sample, the standard, and the control. The doses of the test sample, reference, and control drugs were modified per the initial precise weights of each mouse. At the zero-hour mark, feeding needles were used for administering 10 mg/kg body weight of glibencamide ((0.01×body weight) ml solution), 100, 250, and 500 mg/kg body weight of test samples ((0.01×body weight) ml solution), and 10 mg/kg body weight of a 2 % tween-80 control ((0.01×body weight) ml solution) as a reference. After 30 minutes, all groups were given a glucose solution containing 2 gm/kg body weight ((0.01×body weight) ml solution). Following the administration of glucose solution, blood samples were taken from the tail vein at 30, 90, and 150 minutes later to determine the blood glucose level using a glucometer and strips.

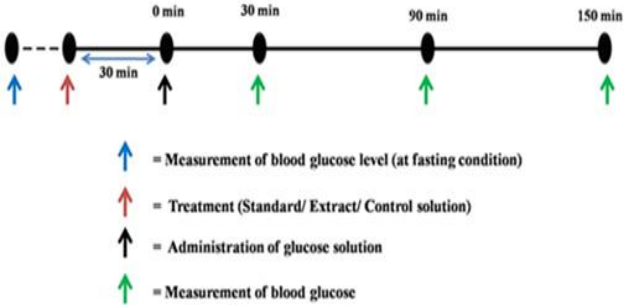

Figure 1. Oral glucose tolerance test experimental protocol briefly.

In-vitro Glucose Diffusion Inhibition Assay
For the glucose diffusion inhibition assay, 1.0 ml of 0.15 mM NaCl, and 7.5 ml of 0.22 mM D-glucose solution were taken into three separate 8 cm × 6.4 mm dialysis tubes. Then, 0.5 ml of ethanol extract solution (400 µg/ml), 0.5 ml of acarbose (25 µg/ml) standard solution, and only water was added into separate dialysis membranes and sealed on both ends. Then, sealed tubes were placed into a 50 ml breaker containing 22.5 ml of 0.15 M NaCl solution. The beaker is kept at room temperature with the sonicator in place. After that, the 2.5 ml solution was taken from outside the dialysis membrane in the beaker, and a new 0.15 M NaCl solution was added at 0, 30, 90, 120, 150, and 180-minute time intervals. Then, the absorbance of glucose in the external solution is measured using a UV-Vis spectrophotometer with a 381 nm wavelength and compared to control for determining the glucose concentration [Colman, 1994; Joy, 1999].

$$\%\ of\ Inhibition = \frac{Abs381nm\ of\ control\ -\ Abs381nm\ of\ sample}{Abs381nm\ of\ control}\ x\ 100$$

In-vivo Anti-hyperlipidemic Activity Assay
Diet-induced Hyperlipidemic Model
For the experiment, young Swiss albino mice weighing between 25 and 35 gm, aged 6-7 weeks, were selected. Mice were given an oral atherogenic diet (High Fat Diet (HFD) consisting of 35 % coconut, 10 % peanut, 1 % vitamin B complex, and 20.0 % beef tallow w/w) for 30 days while still receiving a regular oral meal supplement to become

hyperlipidemic. The mice were randomly selected into five groups of four animals each: I (standard diet mice), II (HFD induced mice), III (HFD and standard drug induced mice), IV (HFD and 2 % extracted mice), and V (HFD and 4 % extracted mice). The diet was provided to the mice without restriction. Following 14 days of stomach intubation, the mice received plant extracts suspended in 0.2 % tween 80 at doses of 2 % and 4 % once daily in the morning. Every group was given the same amount of atherogenic food during these days. The vehicle and the hyperlipidemic diet were given to the control animals as well. All animals were weighed and killed with chloroform after 30 days to measure different biochemical markers. The animals were fasted for 12 hours before their blood was drawn. A lancet was used to puncture the face vein to obtain blood. The blood samples were taken in plain, left to clot at room temperature for 20 minutes, and then centrifuged for 20 minutes at 3000 rpm. The collected serum was stored until analysis at -18 °C. Serum was used to estimate the mice's serum lipid profile, including total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), using a biochemical auto-analyzer and the appropriate commercial test kits by the manual's recommendations [Asgharpour, 2012].

Results and Discussion

The seed of *Amomum subulatum* has great therapeutic value in treating a variety of illnesses, including tumor inhibition, inflammatory suppression, hepatoprotective and renal effects, and free radical scavenging [Gautam, 2016]. Our study's main goal was to determine how effective *Amomum subulatum* seed extract is as an anticoagulant, antihyperglycemic, and antihyperlipidemic agent.

Phytochemical Screening

As seen in Table 1, the ethanol extract of *Amomum subulatum* seed exhibits a variety of phytochemicals. It has been demonstrated that the findings nevertheless point to the possibility that one or more phytochemical groups, including flavonoids, alkaloids, phenolic compounds, and tannins, may be linked to the regulation of diabetes, the lowering of lipid profiles, and the thinned blood.

Table 1: Phytochemical screening of *Amomum subulatum* seeds ethanol extract.

Group Test	Observations	Result
Carbohydrate test	A bluish-violet ring at the junction	+
Alkaloid test	Yellow precipitate	+
Glycoside test	Brick-red precipitate	+
Phenolic compound test	Dark-green precipitate	+
Flavonoids test	Showed red colour	+
Tannin test	Blue-green precipitate	+
Steroid test	Showed reddish-purple colour	+
Protein and amino acid test	Yellow and blue colour was not found	-
Acidic compound test	Effervescence was not found	-
Saponin test	No persistent frothing	-

+ indicates present and - indicates absent

In-vitro Anti-coagulant Activity Test

Herbaceous plants include a variety of bioactive substances, including vitamins and phenolic and polyphenolic chemicals, which can influence coagulation processes. According to studies, these antiplatelet medicinal herbals are beneficial for coronary atherothrombotic disorders. According to studies, these medicinal herbals with antiplatelet properties benefit coronary atherothrombotic diseases [Mohd, 2016]. This work assessed anticoagulant activity directly on human blood samples by measuring the PT. The PT is one of the most crucial tests for tracking coagulation, detecting blood clotting abnormalities, and monitoring anticoagulant medication [Yazdanparast, 2008]. Bioactive substances such as polyphenols, flavonoids, terpenoids, and others are thought to have an antiplatelet effect. These substances interfere with various platelet signalization pathways, such as the ADP pathway, intracellular Ca^{2+} mobilization reduction, TXA2 formation, and phosphoinositide breakdown to eliminate platelet disorders [El-Haouari, 2016]. *Amomum subulatum* seed extract was tested in-vitro on isolated platelets. The results indicated a considerable suppression of platelet aggregation when compared to the warfarin standard medicine. The average coagulation time for 5.00 mg/ml of standard (warfarin) was 8.32±0.25 minutes, and for 12.5 mg/ml of *Amomum subulatum* seed extract it was 7.33±0.35 minutes. Our findings provide scientific credence to the traditional usage of *Amomum subulatum* as an anticoagulant by demonstrating longer coagulation times at high doses, which is consistent with evidence (Figure 2) on the plant's anticoagulant properties.

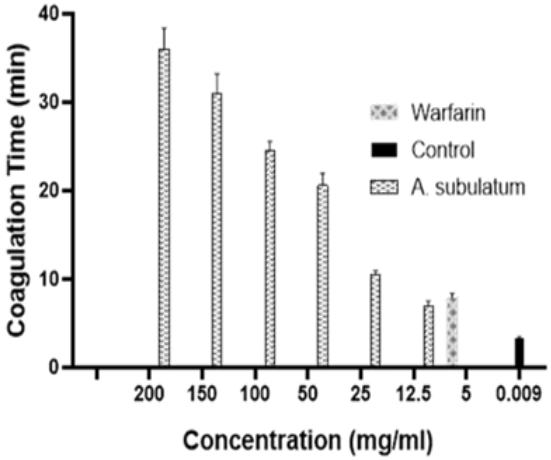

Figure 2. In-vitro plasma clotting time effect of *Amomum subulatum* seeds ethanol extract and warfarin as standard for evaluating anti-coagulant activity.

Antihyperglycemic Activity Test

In-vivo Oral Glucose Tolerance Test (OGTT)

Diabetes mellitus is a disorder characterized by insufficient regulation of blood glucose levels. Nowadays, phytomedicine has recently been proposed as a treatment for diabetes mellitus in addition to conventional medicine. These phytomedicine contain biological macromolecules including glycosides,

flavonoids, alkaloids, and terpenoids with a variety of anti-diabetic potentials. The fundamental mechanisms underpinning their anti-diabetic actions through improved insulin secretion, reduced insulin resistance, and increased hepatic glycogen synthesis, by showing antioxidant and anti-inflammatory activities. Generally regarded as harmless, these naturally occurring substances may play a part in the prevention or management of diabetes mellitus [Singh, 2022]. *Amomum subulatum* seeds contain flavonoids, tannin, and phenolic compounds, the results showed a dose-dependent reduction in glucose when administered the ethanol extract orally. In comparison to the normal control group at the same time intervals, the group that received conventional glibenclamide (10 mg/kg) showed significantly less hyperglycemia (glucose-induced) at 30, 90, and 150 minutes (8±0.15, 4.13±0.15, and 3.2±0.15, mM/L, respectively). The 250 mg/kg dose of *Amomum subulatum* resulted in blood glucose levels of 8.57 ±0.23, 7.8 ±0.067, and 5.5 ± 0.23 mM/L at 30, 90, and 150 minutes, respectively. Furthermore, the ethanol extract of *Amomum subulatum* seeds at a dose of 500 mg/kg significantly decreased hyperglycemia at 30, 90, and 150 minutes by 7.63±0.15, 7.5 ±0.12, and 5.03 ±0.08 mM/L, respectively compared to the control group. The OGTT was used to determine how well the body utilized a particular kind of sugar. The observed results indicate that phenolic compounds with potential anti-diabetic effects can be produced from natural sources [Deka, 2022].

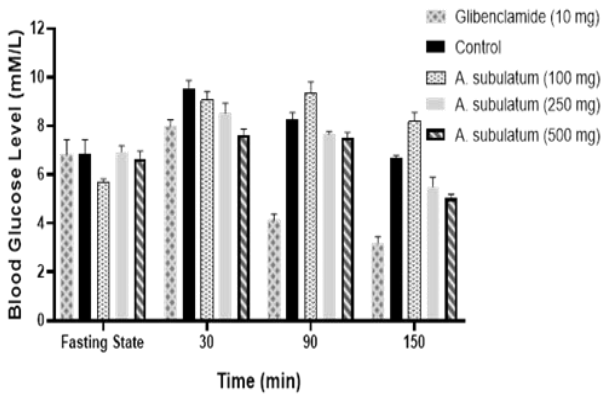

Figure 3. In-vivo anti-hyperglycaemic effect of *Amomum subulatum* seeds ethanol extract at different concentrations and glibenclamide as standard at different time intervals.

In-vitro Glucose Diffusion Activity Test

Minimizing postprandial hyperglycemia is one strategy for managing diabetes mellitus [Mathew, 2020]. Insulin-mediated glucose absorption occurs mostly in skeletal muscle during the postprandial phase. A balanced relationship between tissue (muscle, liver, and fat) sensitivity to insulin and insulin production is necessary for maintaining normal glucose homeostasis [Wickramaratne, 2016]. Plants with a variety of phytochemicals, including tannins (punicalagin), phenolic compounds (6-gingerol), terpenes (thymoquinone), alkaloids (berberine), and flavonoids (quercetin). These phytochemicals possess hypoglycaemic activity [Alam, 2022]. According to earlier research, phytochemicals can have strong anti-diabetic benefits by boosting insulin secretion and sensitivity, boosting muscle and adipose tissue's absorption of glucose, and supporting the beta cells in the pancreas [Singh, 2022; Alam, 2022]. Additionally, phytochemicals can prevent diabetes mostly by inhibiting the diffusion of glucose and, to a lesser extent, by lowering glycation [Sattar, 2012]. This study shows that *Amomum subulatum* seed extracts can lessen the symptoms of diabetes mellitus by increasing the percentage of glucose uptake to levels comparable to those of the standard medication acarbose. To enable the development of these compounds as drugs or chemical leads, more investigation is necessary to ascertain the precise mechanisms of action of these compounds. It was found that the percentage of inhibition rose over time in the presence of *Amomum subulatum*. Acarbose, the standard drug, showed a minimum of 92.53 % and a maximum of 95.22 % at 180 minutes of glucose diffusion inhibition, while the ethanolic seed extract of *Amomum subulatum*, at a concentration of 400 µg/ml, showed a minimum of 70.65 % and a maximum of 74.54 % inhibition at 180 minutes. The percentage inhibition of glucose diffusion assay was done at various time intervals such as 30, 60, 90, 120, 150, and 180 minutes through an in-vitro model. Significant inhibition of the migration of glucose across a 0.22 µm dialysis membrane was demonstrated by the standard drug and extract as compared to the control. This impact increased throughout time, from 30 to 180 minutes. The *Amomum subulatum* extract inhibits the hypoglycaemic effect by either boosting glucose absorption or lowering the rate at which glucose diffuses. Research indicates that the concentration and molecular mass of soluble fibers, as well as the viscosity of the soluble polysaccharides extracted from *Amomum subulatum* seeds, are related to the suppression of the glucose diffusion effect [Gallagher, 2003]. The seed extract is being compared with acarbose, so the antidiabetic effect of the extract was supposed to be due to the delay or reduction of glucose absorption, thus managing post-prandial hyperglycemia. Figure 4 tabulates the values.

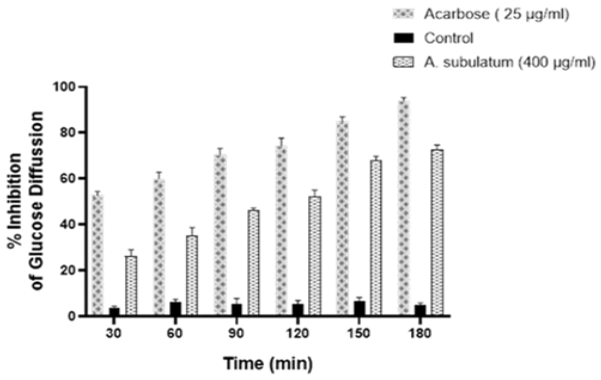

Figure 4. In-vitro assay for evaluating the glucose diffusion effect of *Amomum subulatum* seeds ethanol extract and acarbose as standard at different time intervals.

In-vivo Anti-hyperlipidemic Activity Test

The serum concentrations of LDL, triglycerides, and cholesterol increased significantly in the HFD-treated rats. High blood cholesterol, particularly low-density lipoprotein (LDL), was the primary risk factor for coronary heart disease. Plant-derived rich flavonoids and phenolic compounds are widely known to possess a variety of pharmacological qualities, most notably anti-hyperlipidemic actions [Kim, 2021]. In comparison to HFD-induced hyperlipidemic mice, treatment with *Amomum subulatum* seed extract of HFD with 2 % and HFD with 4 % significantly reduced serum lipid levels in the mice. Triglycerides were 275±1.20 and 152±1.20, LDL was 58.06±0.61 and 43.9±1.01 mg/dl, and cholesterol was 231.33±0.88 and 183±0.58, respectively. Mice on a high-fat diet given Atorvastatin (10 mg/kg) revealed levels of LDL 49.08±0.89 mg/dl, triglycerides 160±1.16, and cholesterol 221.6±1.20 mg/dl (Table 2). This finding suggests that in mice fed a high-fat diet, our seed extract might help to restore the catabolism of triglycerides. This effect might result from enhanced absorption of triglycerides carried in VLDL by peripheral organs and enhanced clearance of triglyceride-rich lipoproteins via increased lipase lipoprotein activity [Jawed, 2019]. Furthermore, the extract exhibited hypocholesterolemia activity because these receptors at the hepatocyte level redistributed plasma LDL-cholesterol to the liver and extra-hepatic tissues, where it was supposed to be eliminated as bile acids. This outcome is consistent with other research assessing the hypolipidemic impact of plant extracts [Sidorova, 2017]. Over four weeks of treatment, the daily administration of HFD with 4% seed extract helped to moderate the non-significant increase in body weight. The total mass was 37.8±0.90 mg after three weeks. Compared to mice on a high-fat diet and given 10 mg/kg of atorvastatin, the weight of the mice on the high-fat diet was 36.9±0.82 mg (Table 3). These findings are in line with several earlier research investigations that evaluated the potential of plant extracts to lower hyperlipidemia [Bouhrim, 2020]. After two weeks of a high-fat diet (10 mg/kg), atorvastatin-treated mice showed 7.8±0.23 mM/L blood glucose, which was significantly different from HFD-induced animals (Table 4). After two weeks of HFD, the blood glucose level with 4 % extracted mice was 10.73±0.20 mM/L. In fat-overloaded mice, the *Amomum subulatum* seed extract markedly reduced the levels of cholesterol, triglycerides, and LDL while also causing a decrease in body weight. These findings support the extract's favorable effects on lipid profile control and its potential to prevent cardiovascular disease. This is in line with earlier research looking at plant extracts' ability to lower hyperlipidemia [Bekkouch, 2019; Surya, 2017].

Table 2: Effect of *Amomum subulatum* seeds ethanol extract on serum markers.

Treated Animal Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)
Controlled Mice	101.1±1.76	90.06±1.11	31.07±0.64
High Fat Diet (HFD) Mice	309.3 ±1.20	415.02±1.27	78.04±0.82
HFD with 10 mg/kg Atorvastatin Treated Mice	221.6±1.20	160± 1.16	49.08±0.89
HFD with 2% Extracted Mice	231.33±0.88	275±1.20	58.06±0.61
HFD with 4% Extracted Mice	183 ±0.58	152± 1.20	43.9 ±1.01

Values are expressed as mean ± standard error of the mean. (n = 3)

Table 3: Effect of *Amomum subulatum* seeds ethanol extract on average mice body weight.

Treated Animal Group	Average Body Weight (mg)			
	1st week	2nd week	3rd week	4th week
Controlled Mice	36.3±0.79	37.6±0.57	38.8±0.74	39.6±1.48
High Fat Diet (HFD) Mice	36.9±0.79	39.4±0.90	41.7±1.29	44.6±1.20
HFD with 10 mg/kg Atorvastatin Treated Mice	36.0±0.74	36.5±0.82	36.9±0.82	37.6±1.14
HFD with 2 % Extracted Mice	36.4±0.65	38.6±1.30	40±1.11	41.4±1.15
HFD with 4 % Extracted Mice	36.1±0.65	36.5±1.06	37.8±0.90	39.6±1.20

Values are expressed as mean ± standard error of the mean. (n = 3)

Table 4: Effect of *Amomum subulatum* seeds ethanol extract on blood glucose level in mice.

Treated Animal Group	Blood Glucose Level (mM/L)	
	At first Day	At 15 Days
Controlled Mice	6.0±0.23	8.2±0.09
High Fat Diet (HFD) Mice	6.2±0.20	18.5±0.18
HFD with 10 mg/kg Atorvastatin Treated Mice	6.1±0.29	7.8±0.23
HFD with 2 % Extracted Mice	6.3±0.20	14.23±0.23
HFD with 4 % Extracted Mice	6.2±0.23	10.73±0.20

Values are expressed as mean ± standard error of the mean. (n = 3)

Conclusion

Medicinal plants are considered the "backbone" of conventional medicine, and a valuable source (mainly from plants, herbs, and shrubs) of life for all people because of their many therapeutic properties and total naturalness. Traditional medicine has always played a significant role in the global health system. The oral administration of the ethanol extract of *Amomum subulatum* seeds demonstrated remarkable and strong anticoagulant activity, antihyperglycemic activity, and antihyperlipidemic activity that might aid in preventing heart and diabetic complications, according to the results and discussion above. The phytochemical substances included in the ethanol extract may be the cause of these actions. Further, a study to support the use of *Amomum subulatum* seeds.

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Conflict of Interest

None of the authors present any conflicts of interest.

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