

Evaluation of the Glucose-Lowering, Thrombolytic, and Antioxidant Activities of the Methanolic Extract of *Commelina communis*: An In Vivo and In Vitro Investigation

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ABSTRACT

Background: *Commelina communis* is one of the medicinal plants that are traditionally used in many therapeutic applications. This study was conducted to determine the glucose lowering, thrombolytic and antioxidant activities of the methanolic extract of *Commelina communis* using in vivo and in vitro experimental models.

Methods: The antidiabetic activity of the methanolic extract was evaluated by conducting Oral Glucose Tolerance Test (OGTT) on glucose loaded mice with doses of 250 and 500 mg/kg, where metformin (100 mg/kg) was the standard drug. Thrombolytic activity was determined by performing in vitro clot lysis experiment with streptokinase and distilled water as the positive and negative control, respectively. Antioxidant activity was assessed using the DPPH free radical scavenging method in which ascorbic acid served as the reference standard.

Results: In the OGTT experiment, there was dose dependent decrease in plasma glucose level due to the methanolic extract. The 500 mg/kg dosage showed 64.32% decrease in plasma glucose level while the 250 mg/kg dosage showed 46.41% decrease compared to 75.70% for metformin. For thrombolytic experiment, there was $36.18 \pm 1.26\%$ clot lysis activity with the methanolic extract; $87.87 \pm 1.64\%$ clot lysis activity with streptokinase and $3.4 \pm 0.48\%$ clot lysis activity with the negative control. There was concentration dependent free radical scavenging activity in the DPPH experiment with IC₅₀ value of 69.68 µg/mL in the methanolic extract, 24.27 µg/mL in ascorbic acid.

Conclusion: It can be concluded that the methanolic extract of *Commelina communis* exhibits remarkable glucose lowering, moderate thrombolytic and considerable antioxidant activities. Therefore, *Commelina communis* can be considered as one of the potential natural sources for bioactive molecules for treatment of diabetes and oxidative stress related conditions.

Keywords: Antidiabetic Activity; Antioxidant Activity; Clot Lysis; *Commelina communis*; DPPH Assay; Glucose-Lowering Activity; Methanolic Extract; Oral Glucose Tolerance Test (OGTT); Thrombolytic Activity.

1.0. Introduction

Various plants have provided numerous therapeutically active agents since ancient times, and now medicinal plants still represent an important resource for new drug discovery against many illnesses. Growing interest in natural compounds with biological activity has led to scientific exploration of ethnopharmacological plants as a source of novel drugs [1]. One of such ethnopharmacological plants which are widely studied nowadays is *Commelina communis* L. (Commelinaceae family), called Asiatic dayflower. The genus of *Commelina* includes more than 200 species located in tropical and subtropical areas of the world. These plants are usually applied to treat fever, inflammation, infections, diabetes, and some other diseases. Several studies described a variety of bioactive compounds, including flavonoids, phenolics, alkaloids, glycosides, tannins, saponins, and others present in plants from the genus of *Commelina* [2].

Diabetes mellitus represents a chronic disease, which is characterized by prolonged hyperglycemia due to dysfunction of insulin secretion and/or insulin action. Nowadays, there is a great need to develop new effective agents against this disease because of its high prevalence among population. Traditional medicines widely used plants from the genus *Commelina* for diabetes treatment [3]. According to the previous studies, extracts of

Commelina communis had strong antihyperglycemic activity as a consequence of α -glucosidase inhibitors, which decrease carbohydrate hydrolysis and glucose absorption. It was shown experimentally that the extract of *Commelina communis* reduces the level of blood glucose in diabetic animals [4].

Thrombotic disorders continue to be among the major causes of mortality and morbidity all over the world. Formation of blood clots can lead to such complications of cardiovascular system as myocardial infarction, stroke, and pulmonary embolism. However, though thrombolytics used nowadays are effective enough, their application is characterized by side effects, expensive price, and increased risk of hemorrhage. That is why nowadays, searching for more safe and less expensive antithrombotics from plant sources has become topical. Natural secondary metabolites such as flavonoids and phenols possess thrombolytic and cardio-protective properties which suggest their further investigation for medicinal purposes [5].

Oxidative stress is a common factor contributing to the development of many chronic illnesses such as diabetes mellitus, cardiovascular diseases, neurodegenerative disorders, and cancers. Antioxidants are responsible for neutralizing reactive oxygen species and preventing the oxidation of various cellular structures [6]. *Commelina communis* contains a variety of antioxidant flavonoids, namely, orientin, isoorientin, vitexin and other phenols. All these constituents possess significant free radical scavenging properties and might be responsible for pharmacological actions of the herbal material. Phytochemical studies performed previously detected numerous antioxidant substances in the plant that scavenged free radicals and inhibited activity of α -glucosidase [7].

However, despite the number of data concerning the pharmacological properties of *Commelina communis*, studies on its glucose-lowering, antioxidant and thrombolytic properties conducted concurrently are not sufficiently numerous yet. Thus, the current study was undertaken to evaluate glucose-lowering activity of the methanolic extract of *Commelina communis* based on the Oral Glucose Tolerance Test (OGTT) in mice as well as its throm

1.1. Objectives of the Study

In this study, the pharmacological effects of the methanolic extract of *Commelina communis* were studied via assessment of glucose-lowering, thrombolytic, and antioxidant properties. Specifically, the aims of the study include:

- 1) The aim of assessing the glucose-lowering activity of the methanolic extract of *Commelina communis* through the performance of the Oral Glucose Tolerance Test (OGTT) on glucose-loaded mice.
- 2) Comparison of the antihyperglycemic property of the extract with the standard antihyperglycemic drug metformin.
- 3) The aim of the evaluation of the in vitro thrombolytic property of the extract in terms of clot lysis activity.
- 4) Comparison of the thrombolytic activity of the extract with the standard thrombolytic agent streptokinase.
- 5) The objective of evaluating the antioxidant property of the methanolic extract by performing the DPPH free radical scavenging test.

6) Determination of the IC_{50} value of the extract and comparison of its free radical scavenging capability with the standard free radical scavenging compound ascorbic acid.

2.0. Literature Review

Diabetes mellitus is a chronic metabolic disorder defined as the presence of elevated blood glucose concentration due to either reduced insulin production or reduced insulin function. In line with the data released by the International Diabetes Federation, the number of people suffering from diabetes keeps increasing [8]. Natural products have been used as effective alternatives in the treatment and management of diabetes mellitus. It was shown that extracts of *Commelina communis* possess the ability to inhibit α -glucosidase enzyme. This enzyme participates in carbohydrate hydrolysis and therefore, inhibition of its action leads to delayed glucose absorption from the digestive system. In addition, it has been experimentally shown that *Commelina* plants possess the ability to decrease blood glucose concentration and increase glucose tolerance. This might be explained by the presence of certain substances, such as flavonoids and phenolic compounds, which can protect pancreatic β -cells by antioxidant effect and improve insulin sensitivity [9]. The OGTT (Oral Glucose Tolerance Test) is a method of investigation for determining glucose lowering ability of a particular substance. Extracts of plants possessing glucose lowering ability in this model are promising agents in diabetes treatment [10].

Despite medical advancements, cardiovascular diseases still account for most deaths globally. One of the main contributors to myocardial infarction, ischemic stroke, and pulmonary embolism is thrombosis, where clots form in the blood vessels. The thrombolytic agents currently in use such as streptokinase, urokinase, and tissue plasminogen activator, although effective in dissolving blood clots, are costly, have a risk of bleeding, and cause allergic reactions. In turn, much effort has been directed towards finding natural thrombolytics [11]. Flavonoids, tannins, and phenolics are the chemicals responsible for clot lysis seen in different medicinal plants due to their fibrinolytic, platelet aggregation inhibition, and vasculature properties. Although there is limited research regarding thrombolytic properties of *Commelina communis*, it has a diverse chemical profile which can be beneficial in terms of cardiovascular health [12].

Oxidative stress occurs as a result of the disparity between the amount of the formation of reactive oxygen species and the action of the antioxidant system of the organism. Increased oxidative stress leads to the formation of various diseases such as diabetes, cardiovascular disorders, cancer, neurodegeneration, and inflammation [13]. Antioxidants function by neutralizing free radicals and preventing the destruction of cells. The importance of antioxidants from plants is defined by their effectiveness and relative safety. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals scavenging test is the most widely used method of the assessment of the antioxidant activity. This method evaluates the ability of the compound to donate the hydrogen atoms or electrons to neutralize DPPH radicals. The lower IC_{50} value means the increased antioxidant activity [14]. Researches demonstrate that *Commelina communis* shows considerable antioxidant activity due to the great amount of flavonoids and phenolic compounds. Shibano et al. found out that some isolated substances of *Commelina communis* have great scavenging activity against free radicals. Also, the antioxidative substances can be responsible for the antidiabetic and cardiovascular protective properties of the plant [15].

Despite earlier reports regarding the pharmacological activities of *Commelina communis* individually, there is a lack of studies that examine the three biological activities (glucose-lowering, thrombolytic, and antioxidant properties) together. Since this herb is traditionally used in medicine, coupled with a wide range of its phytochemicals, it is necessary to scientifically verify these activities [1]. Hence, the present study was conducted to determine the ability of the methanolic extract of *Commelina communis* to lower the glucose level via oral glucose tolerance test, as well as its thrombolytic and antioxidant activities.

3.0. Materials and Methods

3.1. Chemicals and Reagents

Methanol was used as the solvent in the preparation of the plant extract. Metformin was used as the control drug for the testing of glucose reducing ability, while Streptokinase was used as the positive control in the thrombolytic test. Distilled water was used as the negative control. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was used as the source of the free radicals in the antioxidant test, and Ascorbic acid was used as the control antioxidant.

3.2. Experimental Animals

Swiss albino mice (*Mus musculus*) both male and female weighing 25–35 gm and 6-8 weeks old, were used in the analgesic activity test. The animals were procured from an authorized animal breeding laboratory. The animals were obtained from Jahangirnagar University, Savar, Dhaka, Bangladesh, and maintained in standard polypropylene cages under controlled environment conditions of temperature $25\pm 2^{\circ}\text{C}$, humidity 55-65%, and photoperiod of 12h light/12h dark. The animals were allowed food and water freely. For one week before beginning the experiments, the animals were acclimatized to the laboratory conditions. All experiments performed in this study adhere to all ethical protocols of laboratory animals [8].

3.3. Preparation of Crude Extract (Cold Extraction Method)

The entire plants of *Commelina communis* were collected from Kurigram district during spring season and were identified by an experienced botanist. The collected plants were thoroughly washed with distilled water to remove any dust particles and other impurities before being dried at room temperature in shade till constant weight was achieved. The dried plant materials were pulverized to make coarse powder using mechanical grinder. Approximately 250 g of powdered plant materials were extracted using 1000 ml of methanol (ratio of solvent to plant material is 4:1) in a clean and dry glass beaker. The beaker was properly covered and kept at room temperature for 12 days for extraction purpose and was periodically shaken. The resultant mixture was first filtered using a clean cotton cloth followed by filtration using Whatman No. 1 filter paper. The filtrate was further concentrated under reduced pressure using rotary evaporation technique to obtain crude methanolic extract. The extraction of 250 g of dried powder of whole plant using methanol gave 33 g of crude extract which corresponds to 13.2% (w/w) yield of the extract [11].

3.4. Oral Glucose Tolerance Test (OGTT)

The antidiabetic effect of the methanolic leaf extract of *Commelina communis* was studied using Oral Glucose Tolerance Test (OGTT) on glucose loaded mice. All animals were starved for 16 hours before conducting the

OGTT test by giving them only water. Three groups of mice (n=3) were used as follows: Group I (Control): distilled water (10 mL/kg); Group II (standard): metformin hydrochloride (100 mg/kg); Group III (test): Methanolic leaf extract of *Commelina communis* (500 mg/kg) [9]. The glucose solution was administered 30 minutes after the drugs administration. Five samples of blood were taken from the mice at various time intervals (0, 60, 120, 180, 240 minutes). Measurement of the plasma glucose concentration was carried out using the glucometer [10].

3.5. Thrombolytic Activity

Blood sample was taken from volunteers (number of volunteers = 5). The volunteers had no history of intake of anticoagulants and oral contraceptives on the basis of ethical considerations. Blood samples (1 mL) were allowed to clot through their incubation in pre-weighed sterilized microcentrifuge tubes for 45 minutes at 37 °C. Weight of the clot was measured after removal of the serum [5]. Extracts of the plant material were prepared by dissolving 100 mg of the material in 10 ml distilled water and then filtration. Concentrations of the extract varied from 2 to 10 mg/ml. 100 µL of the extract was added to each clot. Positive control included the use of Streptokinase while distilled water was used as negative control. Tubes were kept at 37°C for 90 minutes [6]. Fluid was removed afterwards and weight of the clot was measured again. Percentage clot lysis was calculated on the basis of weight of the clots before and after treatment [11]. The percentage of clot lysis was calculated as:

$$\text{Clot Lysis(\%)} = \frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}} \times 100 \quad (1)$$

3.6. Antioxidant Activity by DPPH Free Radical Scavenging Assay

In order to find out the antioxidant potential of methanolic extract of leaves of *Commelina communis*, DPPH scavenging assay was performed. In the present study, ascorbic acid was chosen as standard. Different concentrations (1, 5, 10, 50, and 100 mg/mL) of methanolic extract of *Commelina communis* as well as ascorbic acid were prepared. Freshly prepared solution of DPPH was made by dissolving DPPH in methanol. Then 3 mL of DPPH was added to 2 mL of test or standard solution at their different concentrations. Reaction mixtures were allowed to incubate in the dark for 30 minutes. Absorbance of each reaction mixture was taken at 517 nm using UV-Visible Spectrophotometer. The percentage of inhibition of DPPH free radicals by samples was determined by following equation:

$$\text{Inhibition of DPPH free radicals (\%)} = \frac{(A_o - A_t)}{A_o} \times 100 \quad (2)$$

Where A_o and A_t are the absorbance of control (DPPH solution without sample) and test sample or standard solution, respectively [16].

4.0. Result and Discussion

This current investigation has examined the glucose lowering, thrombolytic and antioxidative activities of the methanolic extract of *Commelina communis* via experimental animal and laboratory-based procedures. From the results obtained, it is evident that the plant has significant biological activities hence the basis for its traditional uses and a promising source of biologically active components.

4.1. Antihyperglycemic Activity

The antihyperglycemic activity of the methanolic extract was determined using OGTT in glucose loaded mice. The data revealed a dose-dependent decrease in plasma glucose concentrations, whereby, at 500 mg/kg, the extract showed 64.32% decrease in blood glucose concentration at 240 minutes while at 250 mg/kg it showed 46.41% decrease. In comparison to the standard drug, metformin which had shown a greater reduction of 75.70%, the methanolic extract still showed considerable antihyperglycemic activity. It can therefore be said that the extract of *Commelina communis* has the capacity of improving glucose tolerance and lowering postprandial hyperglycemia. This can be explained by the fact that flavonoids, phenolic compounds and many other phytochemicals are known to exist in *Commelina* species. They have been found to inhibit carbohydrate digesting enzymes, promote glucose utilization, increase insulin sensitivity and protect pancreatic beta cells from oxidative damage. The antihyperglycemic activity of the methanolic extract is summarized in Table 1 and shown in Figure 1 and Figure 2.

Table 1. Effect of Methanolic Extract of *Commelina Communis* on Plasma Glucose Levels in Glucose-Loaded Mice During the Oral Glucose Tolerance Test (OGTT).

Group	Dose	0 min	60 min	120 min	180 min	240 min	% lessening of Plasma glucose level
Control (Water)	10 mL/kg	24.75	24.03	22.6	21.68	20.94	-
Standard (Metformin)	100 mg/kg	26.35	21.88	18.8	14.65	10.67	75.70
<i>Commelina communis</i> Extract	250 mg/kg	27.53	26.21	24.55	22.17	20.42	46.41
	500 mg/kg	29.51	25.35	22.16	20.87	18.83	64.32

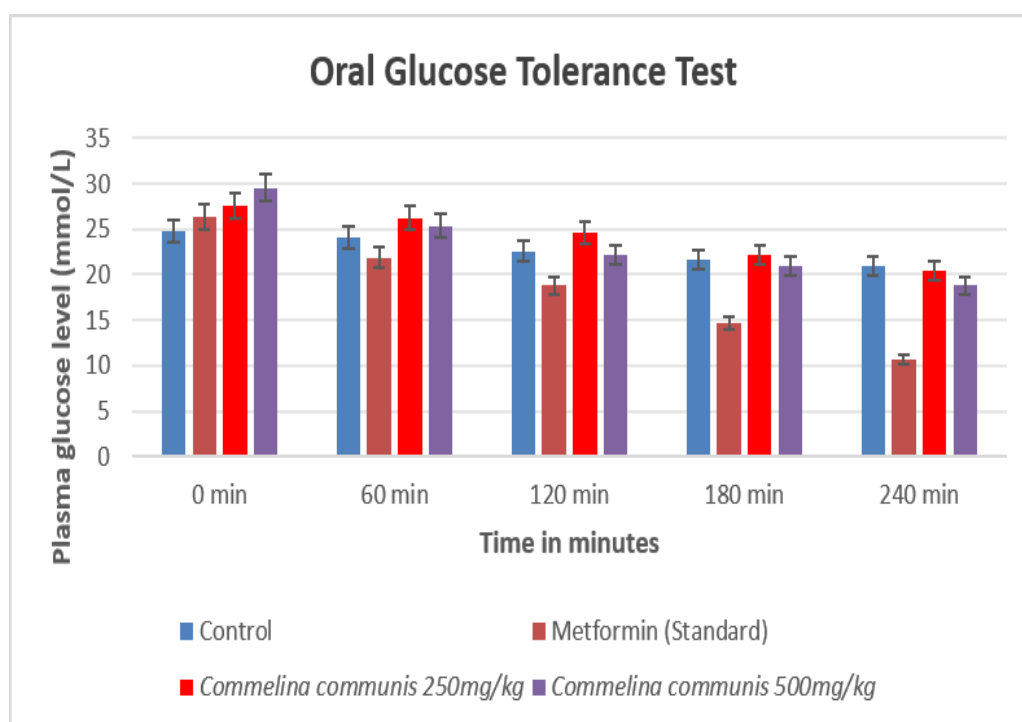


Figure 1. Effect of methanolic extract of *Commelina communis* on plasma glucose levels during the Oral Glucose Tolerance Test (OGTT) in mice.

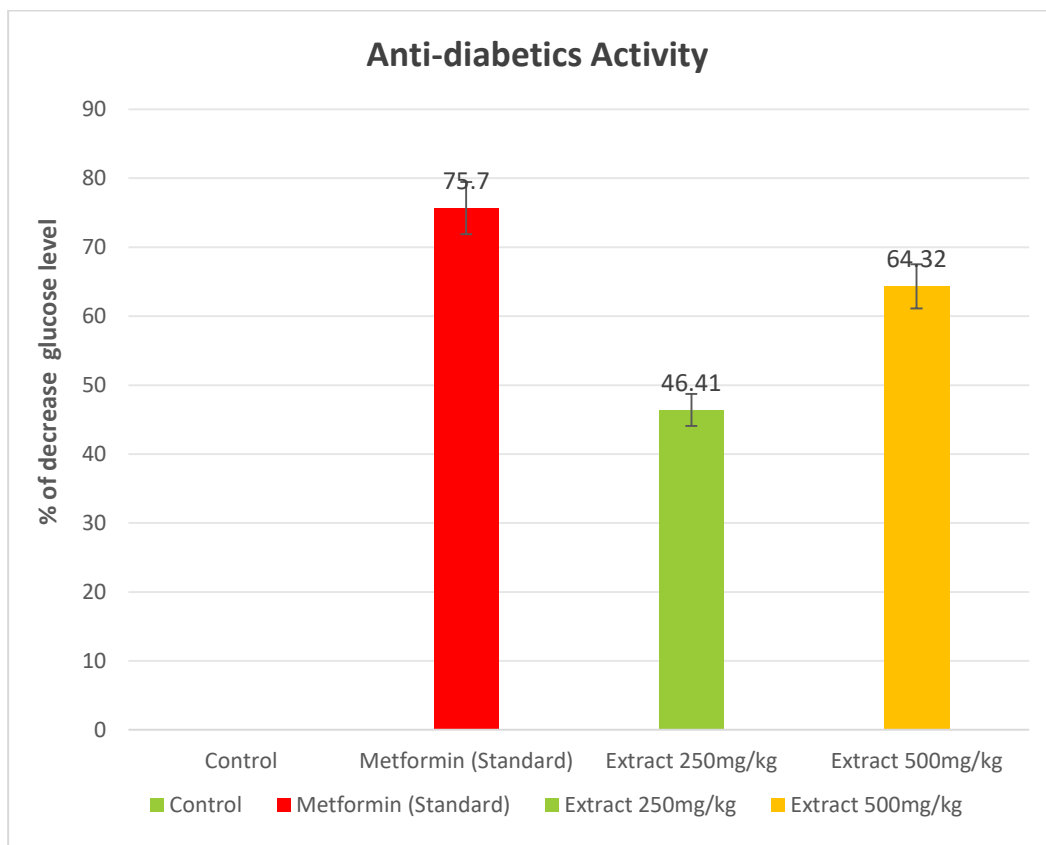


Figure 2. Comparative percentage reduction in blood glucose levels by methanolic extract of *Commelina communis* and metformin.

4.2. Thrombolytic Activity

From the results, calculated using Equation 1, shown in Table 2 and Figure 3, it was clear that the evaluation of the thrombolytic activity of the plant was done via an in vitro clot lysis test where $36.18 \pm 1.26\%$ clot lysis was recorded by the extract compared with $87.87 \pm 1.64\%$ by streptokinase and $3.4 \pm 0.48\%$ by the negative control. Despite being significantly less active than streptokinase, the extract had a significantly higher clot lysis activity than the negative control which implied the existence of active ingredients which can promote clot dissolution. Flavonoids, phenolics, and tannins from plants have previously been shown to display fibrinolytic and anti-platelet activities. The moderate clot lysis seen in this experiment may therefore be due to similar phytochemical ingredients in *Commelina communis*. Further studies need to be conducted to isolate the active ingredients responsible for such an effect.

Table 2. In Vitro Thrombolytic Activity of the Methanolic Extract of *Commelina Communis* determined by Clot Lysis Assay (N = 3).

Sample	% of Clot Lysis (Mean $\pm$ SD)
Negative control (Distilled water)	3.4 ± 0.48
Positive control (Streptokinase)	87.87 ± 1.64
<i>Commelina communis</i> extract	36.18 ± 1.26

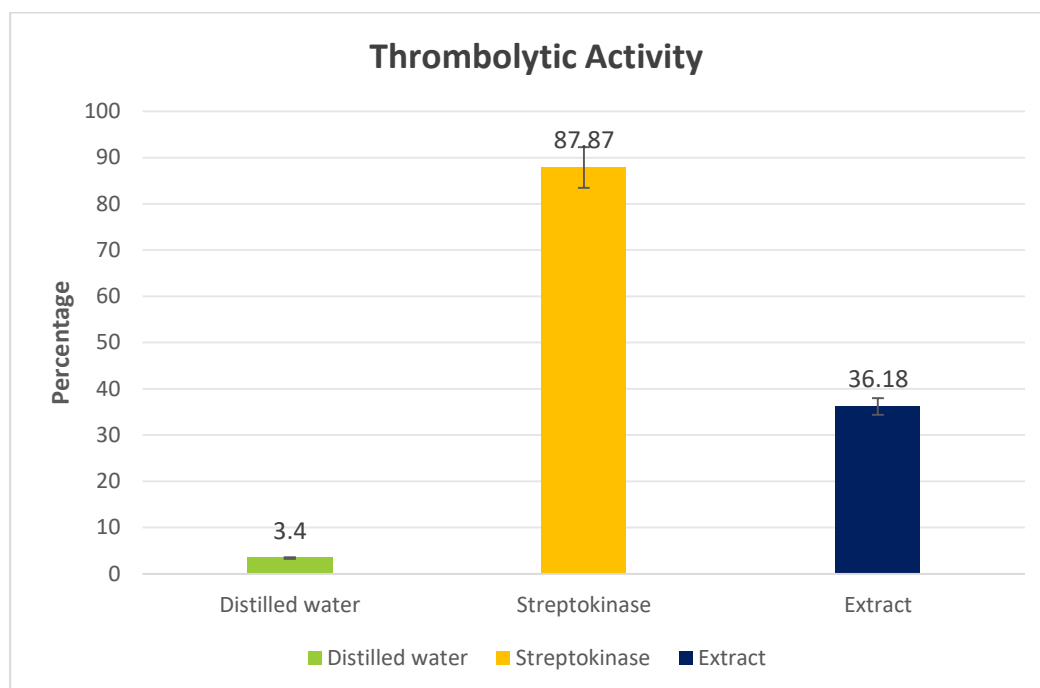


Figure 3. Comparative Clot Lysis Activity of the Methanolic Extract of *Commelina Communis*, Streptokinase, and Distilled Water.

4.3. Analgesic Activity

Antioxidant activity of the extract, calculated using Equation 2, shown in Table 3 and Figure 4, was determined by the DPPH free radicals scavenging capacity where the extract demonstrated concentration dependent free radicals scavenging capacity with an IC_{50} value of 69.68 $\mu\text{g/mL}$ while ascorbic acid showed an IC_{50} value of 24.27 $\mu\text{g/mL}$. The extract was slightly less potent than the standard compound but the significant amount of radicals scavenging capacity showed that there were effective antioxidant ingredients present. The primary activity of antioxidants is their hydrogen or electron donation capacity whereby they neutralize and prevent oxidative stress and damage. The antioxidant activity observed may therefore be due to flavonoids, phenolic acids and other secondary metabolites known from *Commelina communis*. Since oxidative stress plays an important role in the development of diabetes and cardiovascular complications, the antioxidant property observed may be partly responsible for its glucose lowering and possible cardioprotective activity.

Table 3. DPPH Free Radical Scavenging Activity of Methanolic Extract of *Commelina Communis* and Ascorbic Acid at Different Concentrations.

Concentration ($\mu\text{g/ml}$)	Control	Standard (Ascorbic acid)		<i>Commelina communis</i> Extract	
	Absorbance (nm)	Absorbance (nm)	% of Inhibition	Absorbance (nm)	% of Inhibition
1	0.802	0.687	14.34	0.78	2.74
5		0.55	31.42	0.67	16.46
10		0.447	44.26	0.56	30.17
50		0.318	60.35	0.449	44.01
100		0.209	73.94	0.327	59.23
IC_{50}			24.27		69.68

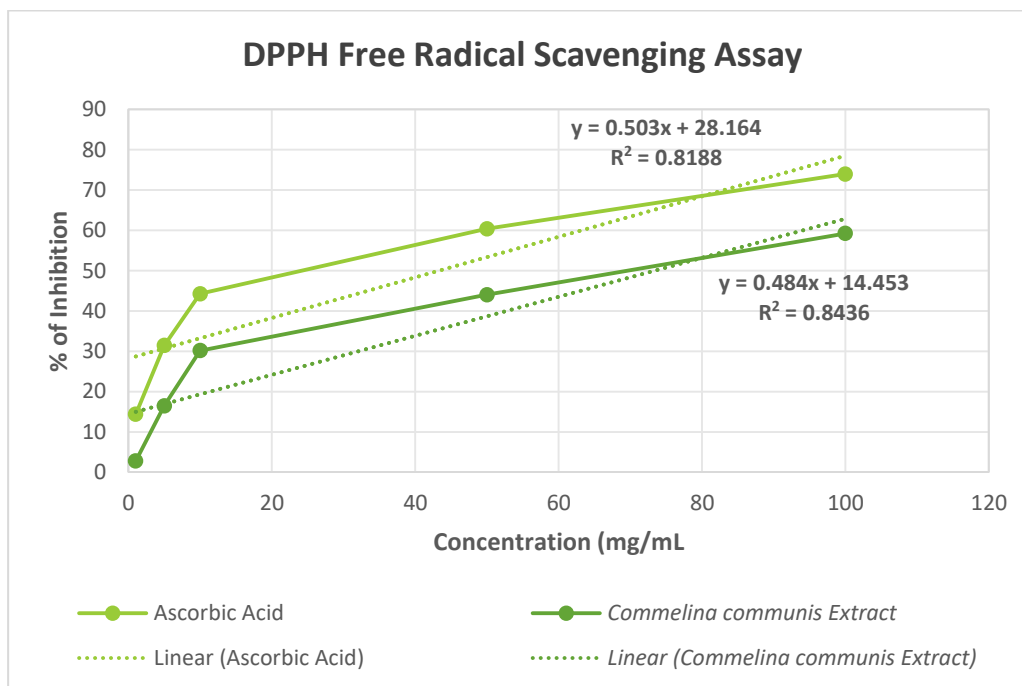


Figure 4. DPPH Free Radical Scavenging Activity of Methanolic Extract of *Commelina Communis* and Ascorbic Acid at Different Concentrations.

4.4. Overall Pharmacological Significance

Overall, the outcome of experiments presented in Tables 1-3 and Figures 1-4 clearly show that methanolic extract of *Commelina communis* is endowed with considerable biological activities described in this study may have correlations with one another. For example, oxidative stress has been shown to interfere with insulin signaling and cause hyperglycemia; at the same time, the antioxidant agents can restore glucose metabolism and enhance the body's defense against oxidative injury. On the other hand, the antioxidants can contribute to the maintenance of vascular health and prevent thrombus formation. Thus, the simultaneous antidiabetic, thrombolytic, and antioxidant activities of the methanolic extract of *Commelina communis* indicate the possibility of multifunctional pharmacology that might prove to be helpful in the treatment of metabolic and cardiovascular diseases.

To summarize, the results of this study demonstrate that the methanolic extract of *Commelina communis* demonstrates considerable antihyperglycemic activity, moderate thrombolytic activity, and appreciable antioxidant activity. It is interesting that the high concentration of the extract was more effective in lowering plasma glucose levels than the low dose, while clot lysis and free radical scavenging were also demonstrated by the plant extract. These results suggest that the use of *Commelina communis* from a scientific point of view is justified. Nevertheless, further research is necessary in order to identify the active compounds contained in the extract.

5.0. Conclusion and Further Studies

5.1. Conclusion

From the present study, it was evident that methanolic extract of *Commelina communis* exhibits good antidiabetic, fair thrombolytic and good antioxidant activities. In OGTT test, the extract revealed dose dependent decrease in

the levels of blood glucose in both 250 mg/kg and 500 mg/kg doses, but 500 mg/kg was found to be more effective than 250 mg/kg. Also, the extract possessed considerable ability for clot lysis in comparison to the negative control. Moreover, it demonstrated concentration dependent free radical scavenging effect in DPPH assay. It can thus be concluded that *Commelina communis* is a potent source of active natural compounds with promising therapeutic uses against hyperglycemia, thrombosis and oxidative stress related diseases.

5.2. Further Studies

In order to validate and develop the pharmacological potentials of *Commelina communis* even more, further studies need to be done on the isolation of bioactive compounds from *Commelina communis*, determination of their mode of actions and thorough evaluation of their safety and efficacy.

- a) Phytochemical studies for isolation and characterization of the bioactive compounds need to be done.
- b) Toxicity studies have to be done to determine the safety of the extract.
- c) Molecular mechanisms of glucose lowering, thrombolysis and antioxidative effects need to be determined.
- d) The efficacy of the fractions isolated and their bioactive compounds should also be tested in experimental animals.
- e) Other pharmacological effects like anti-inflammatory, cardioprotective and antimicrobial actions should also be studied.
- f) Preclinical and clinical trials should be conducted to evaluate its therapeutic potentials in human diseases.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contributions

Md. Mahbubol Alam, Fatema Akhter, Mosa. Tanha Aktar, Afrin Farzana, and Jakia Jahan Jui took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Ahsan Ullah and Fahamida Binta Anower analyzed data. Fahamida Binta Anower was involved in the design of the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials

Supplementary information is available from the authors upon reasonable request.

Institutional Review Board Statement

Not applicable.

Ethical Approval

All animal experiments were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Every effort was made to ensure the humane handling of animals and to minimize pain, discomfort, and stress throughout the experimental procedures.

Informed Consent

Not applicable for this study.

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Declaration of Artificial Intelligence

The authors declare that artificial intelligence was not used in the writing, analysis and preparation of this manuscript.

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