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Investigation of the Anti-Inflammatory, Anti-Diabetic, and Antioxidant Potential of Ethanolic Extract of *Xanthium strumarium* Leaves: A Comprehensive In Vivo and In Vitro Evaluation

Md. Mahbubol Alam[1,2,*], Md. Jewel Islam[3], Shourav Nandi[3], Mahbuba Alam[3], Anjuman Ara[3], Lamia Akther Enni[3] & Fahamida Binta Anower[1,3]

[1]Department of Pharmacy, Faculty of Life and Earth Sciences, Jagannath University (JnU), Dhaka-1100, Bangladesh. [2]Department of Pharmacy, Faculty of Science and Engineering, University of Information Technology and Sciences (UITS), Dhaka-1212, Bangladesh. [3]Department of Pharmacy, Faculty of Science, Engineering and Technology, Bangladesh University (BU), Dhaka-1207, Bangladesh.
Corresponding Author (Md. Mahbubol Alam) Email: mahbubhasan089@gmail.com[*]

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ABSTRACT

Background: *Xanthium strumarium* L. is a medicinal herb extensively used in traditional medicine as a remedy for different diseases. In order to evaluate the anti-diabetic, anti-inflammatory, and antioxidant effects of the ethanolic leaf extract of *Xanthium strumarium*, this study was conducted.

Methods: For the assessment of the anti-inflammatory effect, carrageenan-induced paw edema in mice model was used. The anti-diabetic effect was tested in an oral glucose tolerance test (OGTT) model. Anti-oxidant effect was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Ethanolic extract of *Xanthium strumarium* was used in a dose of 500 mg/kg body weight and was compared with standard drugs such as diclofenac sodium (10 mg/kg) and metformin (100 mg/kg).

Results: In the study on anti-inflammatory activity, the ethanolic extract of *Xanthium strumarium* resulted in significant reduction in paw edema in comparison with diclofenac sodium (68.85 % and 82.24 % respectively). In the OGTT model, the extract significantly decreased plasma glucose levels during the experiment with 58.14 % inhibition compared to 84.41 % of metformin. In the DPPH assay, the extract possessed concentration dependent anti-oxidative activity with percentage inhibition rising from 4.38 % at 1 mg/ml to 56.75 % at 100 mg/ml and ascorbic acid with 8.76 % to 85.72 % inhibition over the same concentration range.

Conclusion: As a result of this study, it could be concluded that ethanolic leaf extract of *Xanthium strumarium* possesses significant anti-diabetic, anti-inflammatory and anti-oxidant activity. Pharmacological effects found in the study confirm the use of this plant in traditional medicine and make it promising in terms of finding a natural bioactive compound for the treatment of diabetes, inflammation, and oxidation associated diseases.

Keywords: Anti-Diabetic Activity; Anti-Inflammatory Activity; Antioxidant Activity; DPPH Assay; Ethanolic Extract; Medicinal Plants; Phytochemicals; *Xanthium strumarium*.

1.0. Introduction

Plants have been used as a source of medicinal products since ages and still are playing an essential part in drug discoveries. According to the World Health Organization (WHO), many people depend on herbal remedies to treat different diseases. The rising number of chronic diseases and side effects of artificial medications have caused scientists to look into the natural sources to discover biologically active compounds [1].

Inflammation is a biological process occurring in response to tissue damage, infections, and other adverse effects. This is the natural body reaction to defend its physiological homeostasis. Nevertheless, chronic inflammation participates in the development of many diseases like arthritis, diabetes, cardiovascular diseases, neurodegenerative diseases, and cancers. Anti-inflammatory drugs are often used in the management of inflammatory processes but may cause adverse effects on gastrointestinal, renal and cardiovascular system in case of prolonged usage [2]. Therefore, the search for new sources of anti-inflammatory drugs among medicinal plants has become topical nowadays.

Diabetes Mellitus is a chronic disease of metabolism, in which the person suffers from constant hyperglycemia either because of problems with insulin secretion or the action of insulin in the body or because of both these

factors. Some complications that arise due to diabetes include heart diseases, kidney diseases, nerve disorders, and eye disorders. The global prevalence of diabetes has increased alarmingly in the past few years and has become a big health problem all around the world. Many antidiabetic drugs are present but their side effects are still being considered [3].

Oxidative stress is an imbalance between the level of free radicals and the body's own antioxidant mechanisms. An excessive production of free radicals damages macromolecules (lipids, proteins, nucleic acids) and leads to many pathological states like diabetes mellitus, inflammation, aging, and cancers. Plant natural antioxidants help to neutralize free radicals and protect cells from oxidative damage [4].

Xanthium strumarium L. (family: Asteraceae) is an annual herbaceous plant that is widely distributed in the tropics and subtropics of the world [5]. This plant is widely used in folk medicine in the treatment of fever, headache, sinusitis, rheumatism, dermatological diseases, and inflammation [6]. Numerous phytochemical studies showed the presence of various active chemical substances like flavonoids, phenolic substances, sesquiterpene lactones, glycosides, alkaloids, tannins, and steroids [7]. These phytochemicals have various biological activity including antioxidative, anti-inflammatory, antimicrobial, antidiabetic and anticancer [8-9].

Many researches described pharmacological actions of *Xanthium strumarium*. Nevertheless, there is no comprehensive research which includes the evaluation of anti-diabetic, anti-inflammatory, and antioxidant activity of this plant by means of in vivo and in vitro experiments. Moreover, scientific validation of the traditional medical uses of *Xanthium strumarium* is necessary [10]. It is against this background that the current study aimed at evaluating the anti-diabetic, anti-inflammatory, and antioxidant properties of the ethanolic leaf extract of *Xanthium strumarium*. Anti-inflammatory property was measured based on the carrageenan-induced paw edema test, while the anti-diabetic and antioxidant activities were measured using OGTT and DPPH free radicals scavenging test respectively. The outcome of the current study will serve to generate scientific evidence in support of the traditional uses of *Xanthium strumarium* and help identify new plant-based drugs for managing diabetes, inflammation, and oxidative stress disorders.

1.1. Study Objectives

The broad objective was to assess the anti-diabetic, anti-inflammatory, and antioxidant properties of the ethanolic leaf extract of *Xanthium strumarium* in vivo and in vitro models. While the specific objectives included the following:

1) Preparation of ethanolic extract from the leaves of *Xanthium strumarium.*

2) Anti-inflammatory activity of the ethanolic leaf extract in the carrageenan-induced paw edema test in mice.

3) Anti-diabetic activity of the extract in the oral glucose tolerance test (OGTT) in experimental animals.

4) Antioxidant activity of the extract through DPPH free radical scavenging assay.

5) Comparison of the anti-inflammatory effect of the extract to that of the standard anti-inflammatory drug, diclofenac sodium.

6) Comparison of the anti-diabetic activity of the extract to the standard anti-diabetic drug, metformin.

7) Evaluation of the dose-related free radical scavenging ability of the extract compared to ascorbic acid.

8) Provision of scientific validation of the traditional use of *Xanthium strumarium* as a medicine.

9) Identification of the potential of *Xanthium strumarium* as a natural resource of bioactive compounds for the preparation of therapeutic drugs against diabetes, inflammation, and oxidative stress-associated diseases.

10) Generation of baseline information for future phytochemical, toxicological, and pharmacological studies on *Xanthium strumarium*.

2.0. Literature Review

The anti-inflammatory property of *Xanthium strumarium* has been widely studied in various in vivo and in vitro systems. It has been found that the plant extract effectively inhibits edema and suppresses the formation of pro-inflammatory mediators. The presence of sesquiterpene lactones, flavonoids, and phenolic compounds in the plant is thought to be responsible for these actions through the regulation of inflammatory signaling pathways and reduction of oxidative stress-mediated injury [11]. Based on the obtained results, it can be assumed that the anti-inflammatory effect of *Xanthium strumarium* is promising and comparable to conventional anti-inflammatory medications in particular experimental settings [2].

Various studies have shown the ability of *Xanthium strumarium* to have hypoglycemic effects. Various experiments conducted have shown that extracts from the plant can be effective in reducing the levels of blood glucose due to the increased sensitivity of insulin and protection of pancreatic β-cells against oxidative stress. This antidiabetic effect is mainly because of the presence of flavonoids and phenols which are antioxidants and have glucose regulating ability [3].

Various animal experiments have shown the effectiveness of *Xanthium strumarium* extracts in lowering blood glucose levels, making it a potential antidiabetic natural product. However, more research is needed to find out how this is achieved [8-9].

The antioxidant effects of *Xanthium strumarium* have been studied extensively. In numerous experiments, the plant showed a good free radical scavenging activity in DPPH and ABTS assays and other antioxidant assays. The high activity is caused by the presence of the plant phenolic and flavonoid compounds capable of donating electron and hydrogen atom and neutralizing reactive oxygen species [4]. It is important to mention that the plant antioxidant effect is very important since the process of oxidation plays a key role in the pathogenesis of diabetes and inflammatory diseases [11].

It should be noted that there were some studies focused on the investigation of pharmacological properties of *Xanthium strumarium*. However, despite the large number of researches, there was only little information about simultaneous anti-diabetic, anti-inflammatory, and antioxidant effects of the plant [12-15]. Moreover, due to different plant parts used, various methods of extraction, and different experimental systems, it has become obvious that further investigations are needed [16-17]. For this reason, the current study was designed to examine the

anti-diabetic, anti-inflammatory, and antioxidant properties of the ethanolic leaf extract of *Xanthium strumarium* in oral glucose tolerance test, carrageenan-induced paw edema test, and DPPH free radical scavenging assay.

3.0. Materials and Methods

3.1. Chemicals and Drugs

Analytical grade ethanol was used to extract the leaves of *Xanthium strumarium*. The carrageenan was used to induce paw edema in the anti-inflammatory activity experiment. The drug used as standard in the anti-inflammatory activity was diclofenac sodium, whereas metformin hydrochloride was the standard drug used in the OGTT antidiabetic experiment. The chemical D-glucose was used in inducing hyperglycemia in the OGTT experiment. DPPH and ascorbic acid were used as the free radicals and the reference antioxidants, respectively, in the antioxidant experiment. All distilled water and analytical-grade chemicals used in the experiments were bought from reputable commercial companies.

3.2. Experimental animals

Swiss albino mice of age eight weeks having a body weight range of 27-30 g were used in this experiment, collected from Jahangirnagar University, Dhaka, Bangladesh. The mice were provided with a proper environmental setup of 12 hours alternating light-darkness and temperature between 22-25°C along with 60-70% humidity level [18]. Pellets served as a food for maintaining the mice, which were collected from Jahangirnagar University, Dhaka. The animals used in this study strictly adhere to the institute rules regarding their purpose.

3.3. Collection of plants and preparation of extracts

The leaves of mature *Xanthium strumarium* plants belonging to the family Asteraceae were collected from several regions of Noakhali, Bangladesh. The collected leaves were thoroughly cleaned to remove dust, soil, and other extraneous materials. The leaves were then cut into small pieces and dried under sunlight for one week. After complete drying, the plant material was ground into a fine powder using a suitable grinder. For the preparation of the methanolic extract, 500 g of the dried leaf powder was soaked in 1800 mL of methanol in a clean glass container and kept for 15 days with occasional shaking to ensure maximum extraction of phytoconstituents. The resulting mixture was first filtered through cotton and subsequently through Whatman filter paper to obtain a clear filtrate [19].

3.4. Anti-inflammatory Activity

Anti-inflammatory effect of ethanolic leaf extract of *Xanthium strumarium* was done on Swiss albino mice weighing between 25 – 30 g of either sex. The animals were randomly grouped into three, having five animals per group. Group I acted as control and was orally administered with normal saline (10 mL/kg). Group II was the standard group receiving diclofenac sodium (10 mg/kg), while Group III received ethanolic extract of *Xanthium strumarium* leaves (500 mg/kg). Thirty minutes after administration of the drugs, acute inflammation was induced by injecting 0.05 mL of 1% carrageenan suspension intraplantarly to the right hind paw of all the animals. Paw volumes were recorded immediately before carrageenan injection (0 h) and also at 1, 2, 3, and 4 hours using

plethysmometer. Anti-inflammatory effect was estimated through comparison of paw edema volume of treated group and standard group with those of the control group.

$$\text{Inhibition of Edema (\%)} = \frac{(A - B)}{A} \times 100 \qquad (1)$$

Where:

A = Mean paw edema volume of control group,

B = Mean paw edema volume of treated/standard group [18].

3.5. Anti-diabetic Activity by Oral Glucose Tolerance Test (OGTT)

The anti-diabetic effect of the ethanolic leaf extract of *Xanthium strumarium* was determined through the use of Oral Glucose Tolerance Test (OGTT) in Swiss albino mice. Each of the three groups had five mice. In Group I (control) treatment was with distilled water (10 mL/kg), in Group II (standard) treatment was with metformin hydrochloride (100 mg/kg) and in Group III treatment was with the ethanolic leaf extract of *Xanthium strumarium* (500 mg/kg). The mice were fasted for about 16 hours overnight but with free access to water prior to the experiment. Fasting blood glucose levels (0 min) was determined by collecting blood samples from the tail veins of the animals using a glucometer. Following this procedure, all animals were orally administered with a glucose load of 2 g/kg body weight. Immediately after administration of glucose, treatments were orally administered to the different groups of animals. Blood glucose levels were then obtained after 60, 120, 180 and 240 minutes of glucose loading using a glucometer [18].

3.6. Antioxidant Activity by DPPH Free Radical Scavenging Assay

To assess the antioxidant activity of ethanolic extract of leaves of *Xanthium strumarium*, DPPH free radical scavenging assay was employed. Ascorbic acid served as the standard in this experiment. Various concentrations (1, 5, 10, 50, and 100 mg/mL) of both plant extract and ascorbic acid were prepared in methanol. Freshly prepared DPPH solution was prepared by dissolving DPPH in methanol. Then 3 mL of DPPH solution was added to 2 mL of the test sample or standard solution at their respective concentrations. The reaction mixtures were incubated in dark at room temperature for 30 min [20]. The absorbance of each reaction mixture was then determined at 517 nm wavelength using a UV–Visible spectrophotometer against methanol blank. The free radical scavenging activity was expressed as percentage inhibition of DPPH free radicals and calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{(Ao - At)}{Ao} \times 100 \qquad (2)$$

Where:

Ao = Absorbance of the control (DPPH solution without sample),

At = Absorbance of the test sample or standard solution,

The antioxidant activity of the plant extract was compared with ascorbic acid in terms of percentage inhibition of DPPH free radicals at various concentrations [21].

4.0. Result and Discussion

This current study determined the anti-inflammatory, antidiabetic, and antioxidant effects of *Xanthium strumarium* leaf extract via various in vivo and in vitro bioassay models. It was found that the extract contained significant pharmacological effects, hence validating the uses of the herb in the treatment of many ailments.

4.1. Anti-Inflammatory Activity

Inflammation is a protective biological process against infection and injuries; nevertheless, prolonged inflammation leads to the development of several chronic conditions. In the current study, inflammation of paw induced by carrageenan was utilized for evaluation of the anti-inflammatory activity of the *Xanthium strumarium* leaf extract. Inflammation induced by carrageenan is an experimental model where there is production of several inflammatory factors like histamine, serotonin, bradykinin, and prostaglandins.

It was found that the 500 mg/kg ethanolic leaf extract of *Xanthium strumarium* had profound reduction of paw edema with 68.85% inhibition in comparison with 82.24% inhibition caused by diclofenac sodium. The results were obtained using equation 1 as well as illustrated in Table 1 and Figure 1. This anti-inflammatory effect implies that the extract might have inhibited production or release of inflammatory mediators involved in carrageenan induced inflammation. The considerable decrease in volume of paws throughout the experimental period showed that the extract could prevent swelling.

Table 1. Effect of ethanolic leaf extract of *Xanthium strumarium* on carrageenan-induced paw edema in mice for anti-inflammatory activity.

Group	0 min	60 min	120 min	180 min	240 min	% Decrease in paw volume
Control	4.40	4.28	4.18	4.10	4.02	0
Diclofenac-Na (10mg/kg)	4.35	3.88	3.50	2.82	2.21	82.24
Xanthium stramarium (500mg/kg)	4.85	4.62	4.10	3.87	3.63	68.85

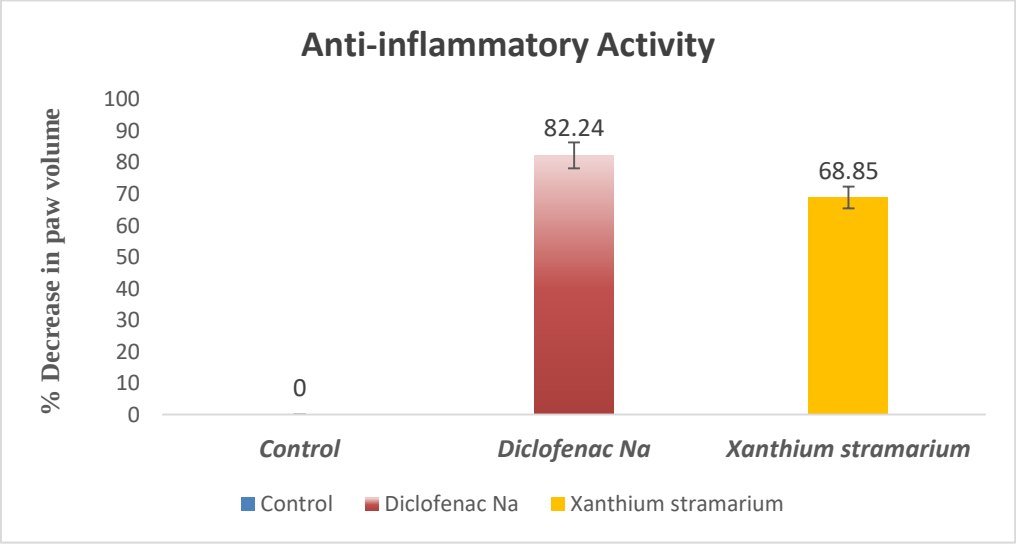

Figure 1. Anti-inflammatory activity of ethanolic leaf extract of *Xanthium strumarium* in carrageenan-induced paw edema model showing percentage inhibition of paw edema.

4.2. Anti-Diabetic Activity

The OGTT test is a valuable tool used for evaluation of the efficacy of regulation of glucose concentration in the blood after glucose challenge. In the current study, treatment with the ethanolic leaf extract resulted in a significant reduction of blood glucose concentration in glucose-loaded mice during the 240-minute period of the experiment, which described in Table 2 and Figure 2.

Table 2. Effect of ethanolic leaf extract of *Xanthium strumarium* on plasma glucose levels in glucose-loaded mice during the Oral Glucose Tolerance Test (OGTT).

Animal group	Dose	Plasma glucose level (mmol/L)									
		0 Min	Avg.	60 Min	Avg.	120 min	Avg.	180 min	Avg.	240 min	Avg.
Control (Water)	10 mL/kg	21.6	21.32	19.1	20.02	18.7	19.34	17.9	18.58	17.5	18.08
		20.3		19.1		18.8		17.3		17.1	
		20.6		19.8		19.6		18.8		18.5	
		21.7		20.8		19.5		19.1		18.9	
		22.4		21.3		20.1		19.8		18.4	
Standard (Metformin)	100 mg/kg	27.8	26.32	22.3	21.68	18.2	17.82	14.1	14.58	9.2	8.74
		25.9		21.6		19.4		16.5		9.1	
		25.8		22.5		17.7		14.3		8.8	
		26.8		20.2		16.6		13.7		7.7	
		25.3		21.8		17.2		14.3		8.9	
Xanthium stramarium	500 mg/kg	29.5	23.82	27.8	21.52	25.5	19.92	23.3	17.94	21.8	16.08
		22.0		19.0		17.2		16.2		14.6	
		18.3		17.7		16.2		14.7		12.3	
		21.6		19.1		17.8		16.3		14.3	
		27.7		24.0		22.9		19.2		17.4	

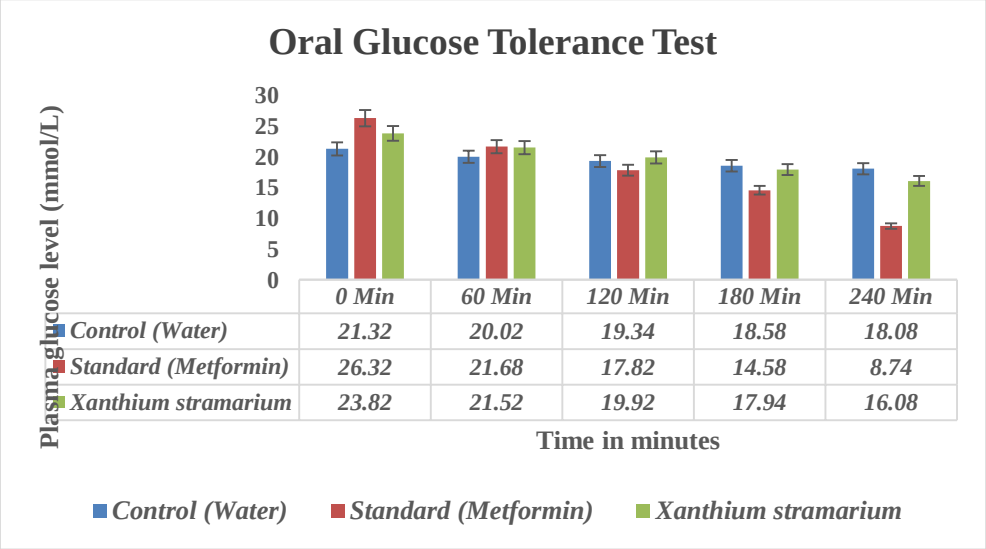

Figure 2. Effect of ethanolic leaf extract of *Xanthium strumarium* on plasma glucose levels during the Oral Glucose Tolerance Test (OGTT) in mice.

The percentage of reduction in the blood glucose concentration in the extract-treated group was 58.14%, while that in the group receiving metformin was 84.41%, which shown in Table 3 and Figure 3. Though the reduction of blood glucose levels caused by the extract was less potent than that induced by metformin, it was still impressive. It might

be attributed to the anti-hyperglycemic activity of the extract. Gradual reduction of blood glucose levels in the treated group might indicate improvement in peripheral glucose metabolism, increased sensitivity to insulin, insulin secretion stimulation, or inhibition of glucose uptake from intestines.

It could be supposed that the antidiabetic activity of *Xanthium strumarium* is caused by its phytochemicals, such as flavonoids, phenolics, alkaloids, and terpenoids. These compounds possess glucose-lowering activity due to their different mechanisms of action including antioxidant protection of pancreatic cells and changes in carbohydrate metabolism. As oxidative stress is involved in the pathogenesis of diabetes, the antioxidant properties of the extract might be responsible for its antidiabetic effect as well [22-23].

Table 3. Percentage reduction in blood glucose levels produced by ethanolic leaf extract of *Xanthium strumarium* and metformin in glucose-loaded mice.

Animal group	Dose	% of decrease glucose level
Control (Water)	10 ml/kg	0
Standard (Metformin)	100 mg/kg	84.41
Xanthium stramarium	500 mg/kg	58.14

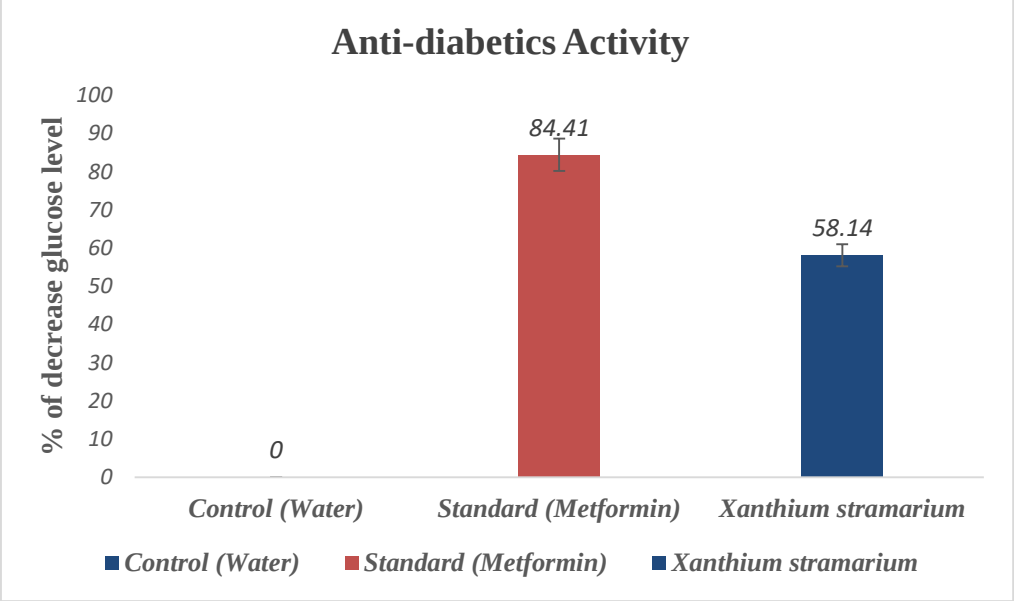

Figure 3. Comparative percentage reduction in blood glucose levels by ethanolic leaf extract of *Xanthium strumarium* and metformin.

4.3. Antioxidant Activity

Oxidative stress has been reported to be involved in the development of various chronic illnesses such as diabetes and inflammatory conditions. It, therefore, becomes a critical parameter in determining the efficacy of medicinal plants. In this study, the antioxidant activity of the ethanolic extract of leaves was analyzed using DPPH free radical scavenging method.

The ethanolic extract displayed concentration-dependent scavenging activity, showing an increase in percentage inhibition from 4.38% at 1 mg/mL to 56.75% at 100 mg/mL. Despite the extract having a relatively low antioxidant

activity compared to ascorbic acid, the standard antioxidant with an 85.72% inhibition at 100 mg/mL, the extract had notable free radical scavenging capability. All calculations were done using equation 2 as well as mentioned in Table 4 and illustrated in Figure 4. The antioxidants may work through the phenolic compounds and flavonoids which are known to donate hydrogen atoms or electrons to neutralize the radicals. Logarithmic regression analysis further supported the presence of dose-dependent antioxidant activity, where there is a positive relationship between concentration and percentage inhibition. These results agree with existing literature about the antioxidant activity of *Xanthium strumarium*.

Table 4. DPPH free radical scavenging activity of ethanolic leaf extract of *Xanthium strumarium* and ascorbic acid at different concentrations.

Concentration (mg/mL)	% inhibition	
	Ascorbic Acid	***Xanthium strumarium***
1	8.76	4.38
5	23.46	25.81
10	30.75	31.71
50	73.54	50.72
100	85.72	56.75

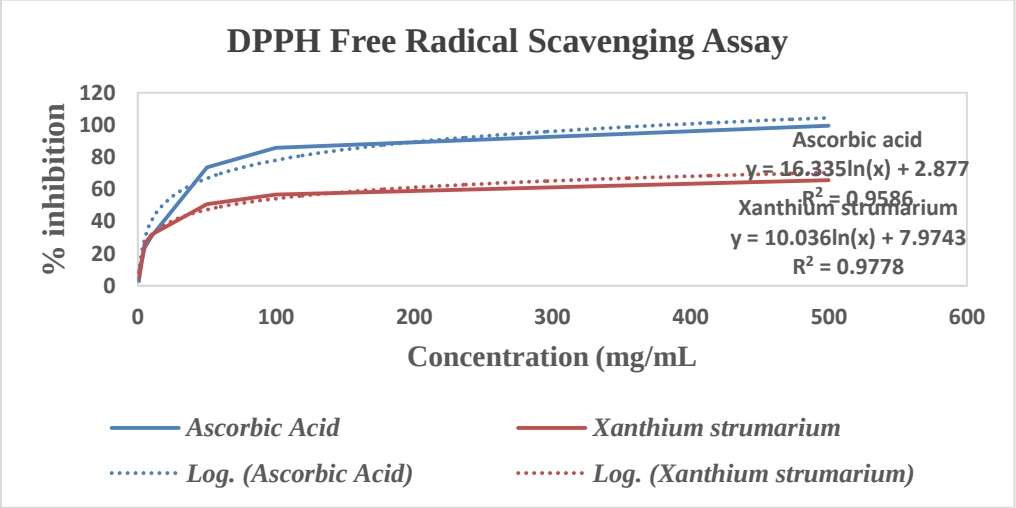

Figure 4. DPPH free radical scavenging activity of ethanolic leaf extract of *Xanthium strumarium* and ascorbic acid at different concentrations.

4.4. General Significance of the Experiment

As seen from the results of this experiment, the ethanolic leaf extract of *Xanthium strumarium* demonstrates high levels of anti-inflammatory, anti-diabetic, and antioxidant activity. All these pharmacological effects might be due to the synergetic effect of various bioactive compounds in the extract of the plant. At the same time, the fact that all three types of activity were demonstrated is especially important, as all these physiological conditions are interrelated.

The anti-inflammatory and antioxidant properties of the plant extract might affect its ability to treat diabetes through reducing inflammatory processes related to the disorder in glucose metabolism. Thus, these results can

provide scientific rationale for the traditional medicinal uses of the plant under study. However, additional research on phytochemical composition, toxicology, and isolation of active components is necessary.

5.0. Conclusion and Further Studies

5.1. Conclusion

The current study showed that ethanolic leaf extract of *Xanthium strumarium* has significant anti-inflammatory, antidiabetic, and antioxidant properties. In carrageenan-induced paw edema test, the plant extract was effective in showing a great reduction of inflammation with 68.85% inhibition as compared to 82.24% inhibition by standard drug diclofenac sodium. In OGTT, the extract caused significant reduction in the level of plasma glucose leading to 58.14% decrease in blood glucose as compared to 84.41% in metformin. Moreover, the extract exhibited significant antioxidant properties in DPPH free radical scavenging test causing inhibition of free radicals in concentration dependent manner.

The above pharmacological activities can be due to the presence of biologically active phytochemicals like flavonoids, phenolic compounds, tannins, alkaloids, terpenoids and sesquiterpene lactones found in *Xanthium strumarium*. The results proved the medicinal value of this plant and suggested that ethanolic leaf extract of *Xanthium strumarium* can be a good natural source for therapeutic agents to treat inflammation, hyperglycemia, and oxidative stress disorders.

5.2. Further Studies

For the further study on the therapeutic properties of *Xanthium strumarium*, it is suggested to conduct the following researches:

1) Combined qualitative and quantitative analysis of phytochemical substances, which cause the observed biological activity.

2) Purification and identification of the bioactive compounds, which are responsible for the anti-inflammatory, anti-diabetic and antioxidant effects of the extract.

3) Research on the mechanisms of the pharmacological effect of the extract.

4) Study of different doses of the extract in order to identify the optimum dose of treatment and the dose-dependence relationship.

5) Toxicity testing of long-term administration of the extract.

6) Study on the changes at the tissue level in order to identify the effects of protection in target organs caused by the use of the extract.

7) Research on the antidiabetic effect in chemically-induced diabetic animals in order to prove the glucose lowering effect of the extract.

8) Study of pharmacokinetic parameters of the active substances of the extract.

9) Clinical studies for the assessment of the efficiency and safety of *Xanthium strumarium* in humans.

Thus, the results obtained in the current study show that *Xanthium strumarium* possesses great pharmacological potential and requires further research for development of the new plant medicines.

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, Md. Jewel Islam and Shourav Nandi took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Fahamida Binta Anower, Mahbuba Alam, Anjuman Ara, and Lamia Akther Enni analyzed data. Md. Mahbubol Alam and Fahamida Binta Anower were 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

No Artificial Intelligence tools were used for this study.

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