



# INTERNATIONAL JOURNAL OF PHARMA PROFESSIONAL'S RESEARCH



## In-vitro Antidiabetic Activity of *Xanthium strumarium* L. Leaves

Shipra\*, Vineet Kumar, Anil Kumar, Bhanoo Pratap Singh, Akanksha Sharma

School of Pharmacy, Monad University, Hapur, U.P., India

**Keywords:** Diabetes mellitus, antidiabetic, antioxidant, Xanthium strumarium, acarbose

### Corresponding Author-

Shipra

[shipratyagi3696@gmail.com](mailto:shipratyagi3696@gmail.com)

School of Pharmacy, Monad University, Hapur, U.P., India

**ABSTRACT:** Diabetes mellitus has become a major public health and economic problem across the globe. The inadequacies, as well as serious adverse effects associated with conventional medicines, led to a determined search for alternative natural therapeutic agents. This study aimed to evaluate the *in-vitro* antidiabetic effects of *Xanthium Strumarium* L. Leaves Extracts. The % yield of acetone and ethyl acetate extract was found to be 1.9% and 2.5% respectively. Qualitative phytochemical tests confirmed the presence of alkaloids, glycosides, saponins, fats and oils, and phenols in acetone extract while alkaloids, glycosides, glycosides, saponins, steroids and phenols were present in ethyl acetate extract. The *in-vitro* antidiabetic and antioxidant studies of the extract revealed that the ethyl acetate extract of *Xanthium strumarium* L. leaves showed good antidiabetic and antioxidant potential when compared with acetone extract and significant with standard compound Acarbose.

**Introduction:** Diabetes is one of the major causes of premature death worldwide. Every ten second a person dies from diabetes related causes mainly from cardiovascular complications. In 2007, diabetes caused 3.5 million deaths globally. Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the

global population, and this report projected to increase to 366 million by 2030.<sup>1,2</sup>

### 1.1 Causes:

The causes of diabetes can be broadly categorized into two main types: Type 1 diabetes and Type 2 diabetes.

**1.1.1 Type 1 Diabetes:** Autoimmune Response: In Type 1 diabetes, the immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas. The exact cause of this autoimmune response is not fully understood, but genetics and environmental

factors may play a role. It is often diagnosed in children and young adults and is not typically associated with lifestyle or obesity.<sup>3</sup>

### 1.1.2 Type 2 Diabetes:

**1.1.2.1 Genetics:** Genetics can play a significant role in Type 2 diabetes. Having a family history of diabetes increases the risk of developing the condition.

**1.1.2.2 Insulin Resistance:** In Type 2 diabetes, the body's cells become resistant to the effects of insulin, and the pancreas may not produce enough insulin to maintain normal blood sugar levels. Insulin resistance is often associated with obesity and physical inactivity.

**1.1.2.3 Lifestyle Factors:** Several lifestyle factors can contribute to Type 2 diabetes, including:

**1.1.2.3.1 Obesity:** Excess body weight, particularly around the abdomen, is a major risk factor for Type 2 diabetes.

**1.1.2.3.2 Physical Inactivity:** A sedentary lifestyle can increase the risk of developing diabetes.

**1.1.2.3.3 Unhealthy Diet:** Diets high in sugary foods, refined carbohydrates, and saturated fats can contribute to the development of Type 2 diabetes.

**1.1.2.3.4 Smoking:** Smoking is associated with an increased risk of diabetes.

**1.1.2.3.5 Stress:** Chronic stress may affect blood sugar levels and contribute to the development of Type 2 diabetes.<sup>4,5</sup>

**1.1.3 Gestational Diabetes:** This type of diabetes occurs during pregnancy and is believed to be influenced by hormonal changes and genetics. It typically resolves after pregnancy but can increase the risk of Type 2 diabetes later in life for both the mother and child.

**1.1.4 Other Causes:** Some rare forms of diabetes are caused by specific genetic mutations or medical conditions, such as monogenic diabetes

or secondary diabetes due to certain medications or medical conditions.<sup>6</sup>

**1.2 Symptoms:** People with diabetes often urinate more frequently than usual because excess sugar in the blood gets eliminated through urine. Frequent urination can lead to dehydration, causing increased thirst. Despite eating, individuals with diabetes may feel extremely hungry because their cells are not getting the necessary energy due to insulin-related issues. People with diabetes, especially Type 1, may lose weight even when they eat more because the body cannot properly use glucose for energy. Diabetes can lead to a lack of energy and persistent tiredness, often due to the body's inability to efficiently use glucose. High blood sugar levels can cause changes in the shape of the eye's lens, affecting vision temporarily.

Diabetes can impair the body's ability to heal wounds and injuries, increasing the risk of infections. High blood sugar can weaken the immune system, making individuals more susceptible to infections, especially urinary tract and skin infections. Elevated blood sugar levels can damage nerves, leading to tingling sensations or numbness, particularly in the hands and feet (diabetic neuropathy). Mood changes, including irritability, can be a symptom of fluctuating blood sugar levels.<sup>7</sup>

Type 1 diabetes often develops rapidly, with symptoms appearing over a few weeks or even days. Some individuals with uncontrolled Type 1 diabetes may develop a condition called diabetic ketoacidosis (DKA), which can lead to a fruity-smelling breath, rapid breathing, and confusion. Type 2 diabetes symptoms may develop slowly, and some people may not even notice any symptoms initially. Dark, thickened patches of skin, often found on the neck, armpits, or groin, can be a sign of insulin resistance associated with Type 2 diabetes.<sup>8,9</sup>

## 2. Materials and Method:

### 2.1 Collection of Raw Material:

*Xanthium strumarium* L. leaves were collected in December 2019 from Hapur Uttar Pradesh, India

and authenticated by Dr. Sunita Garg, Emeritus Scientist, CSIRNISCAIR and Mr. RS Jayasomu, Senior Principal Scientist, Head RHMD, Reference number NISCAIR/RHMD/Consult/2020/3672-73. A voucher specimen of the plant was preserved at the Raw Material Herbarium and Museum, Delhi (RHMD) for future reference.

**2.2 Preparation of Extract:** The fresh leaves were subjected to size reduction with the help of a stainless steel grinder and collect the fine power of the leaves. Extraction was done with a hot Soxhlet extraction process using acetone and ethyl acetate as solvent then the extracts were concentrated to dryness with the help of a water bath and finally air dried. The obtained dried extracts of *Xanthium strumarium* L. leaves were weighed and extractive value was calculated. It was kept in an air-tight container and stored in a desiccator and used for investigation of their potential.

### 2.3 Extraction Yield (%) and Physical Characteristics of Extracts of Leaves of *Xanthium strumarium* L.:

Results of % extraction yield and physical characteristics (Appearance and Consistency) of extracts are presented in Table 1.

**Table 1.** Extraction yield (%) and physical characteristics of extracts of leaves of *Xanthium strumarium* L.

Extracts	Extraction Yield (%)	Appearance	Consistency
Acetone extract	1.9	Green	Oily
Ethyl acetate extract	2.5	Greenish yellow	Greasy

### 2.4 Extraction Yield (%) of Acetone Extract and Ethyl Acetate Extract:

**2.4.1 Chemicals and instruments used:** Acetone, ethyl acetate, other chemicals used for extraction purposes and phytochemical tests were

of laboratory grade (LR) reagents. Sonicator, soxhlet distillation unit.

## 3. Experimental Methodologies:

### 3.1 Preliminary Phytochemical Screening:

Leaves extracts of the plant *Xanthium strumarium* L. were subjected to chemical tests for the identification of their active constituents.

**3.2 Tests for Carbohydrates:** A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

**3.2.1 Molisch's Test:** Filtrate was treated with 2-3 drops of 1% alcoholic  $\alpha$ -naphthol solution and 2 ml of con. H<sub>2</sub>SO<sub>4</sub> was added along the sides of the test tube. The appearance of violet colored ring at the junction of two liquids shows the presence of carbohydrates. Another portion of the extract was hydrolyzed with HCl for a few hours in a water bath and the hydrolysate was subjected to Legal and Bortrager's test to detect the presence of different glycosides.

**3.2.2 Bortrager's Test:** Little quantity of extracts was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. The ammonia layer acquires a pink color showing the presence of glycosides.

**3.3 Tests For Proteins:** A small quantity of the extract was dissolved in a few ml of water and treated with the following reagents.

**3.3.1 Millon's Reagent:** The appearance of red color shows the presence of protein and free amino acids.

**3.3.2 Biuret Test:** Equal volumes of 5% NaOH solution and 1% copper sulphate solution were added. The appearance of pink or purple color shows the presence of proteins and free amino acids.

### 3.4 Tests for Amino Acids:

**3.4.1 Ninhydrin Reagent:** The appearance of purple color shows the presence of proteins and free amino acids.

### **3.5 Tests For Fat, Oils and Gums:**

**3.5.1 Spot Test:** A small quantity of extract was separately pressed between two filter papers. The appearance of oil stain on the paper indicates the presence of fixed oil. A few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

**3.5.2 Solubility Test:** Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water.

**3.5.3 Filter Paper Test:** Filter paper gets permanently stained with oils.

**3.5.4 Tests for Gums:** Fehling's or Benedict's solution shows a red color with gums.

**3.6 Tests for Steroid:** The extract was refluxed with a solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

**3.6.1 Libermann Burchard Test:** The residue was dissolved in a few drops of acetic acid, and 3 drops of acetic anhydride were added followed by a few drops of con.  $H_2SO_4$ . The appearance of a bluish-green color shows the presence of phytosterol.

### **3.7 Test for Glycosides:**

**3.7.1 Legal Test:** To the extract, 1ml of pyridine and a few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. The appearance of pink to red color shows the presence of glycosides.

### **3.8 Test for Alkaloids:**

**3.8.1 Wagner's Test:** To 1 ml of the extract, 1 ml of Wagner's reagent was added. A reddish-brown precipitate indicates the presence of alkaloids.

**3.8.2 Hager's Test:** To 1 ml of the extract, 1 ml of Hager's reagent (Picric acid solution) was added. Yellow-colored precipitate indicates the presence of alkaloids.

**3.8.3 Dragendorff's test:** To 2 ml of the extract add 1 ml of Dragendorff's reagent along the side of the test tube. The formation of orange or orange-reddish brown precipitate indicated the presence of alkaloids.

**3.9 Tests for Saponins:** The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

**3.10 Tests for Phenolic Compounds and Tannins:** A small quantity of the extract was taken separately in water and tested for the presence of phenolic compounds and tannins using the following reagents.

- Dil.  $FeCl_3$  solution (5%) -violet color
- 1% solution of gelatin containing 10% NaCl -white precipitate
- 10% lead acetate solution -white precipitate.

### **3.11 Tests for Flavonoids:**

**3.11.1 With Aqueous NaOH Solution:** Blue to violet colour (anthocyanins), yellow colour (flavones), yellow to orange (Flavonones).

**3.11.2 With Con.  $H_2SO_4$ :** Yellow-orange colour (anthocyanins), yellow to orange color (flavones), orange to crimson (Flavonones)

**3.11.3 Shinoda Test:** A small quantity of the extract was dissolved in alcohol and a piece of magnesium was followed by Con. HCl dropwise was added and heated. The appearance of the magenta color shows the presence of flavonoids.

### **3.12 Preliminary Phytochemical Screening of Acetone Extract and Ethyl Acetate Extract**

Preliminary phytochemical screening of acetone extract and ethyl acetate extract revealed the presence of alkaloids, glycosides, saponins, fats and oils, phenols, and Steroids.

**Table 2:** Preliminary phytochemical screening of acetone extract and ethyl acetate extract

S. No.	Phytochemicals	Acetone Extract	Ethyl-acetate Extract
1.	Carbohydrates	-	-
2.	Proteins	-	-
3.	Alkaloids	+	+
4.	Glycosides	+	+
5.	Saponins	+	+
6.	Amino acids	-	-
7.	Steroids	-	+
8.	Flavonoids	-	-
9.	Tannins	-	-
10.	Phenols	+	+
11.	Fixed oil and Fats	+	-

(-) Absent (+) Present

**3.13 Assessment of *In-Vitro* Antidiabetic Activity:** The antidiabetic effect of plants and their active principles can be assessed *in-vitro* using a variety of biological test systems. They play a major role in the evaluation of antidiabetic properties as an initial screening tool prior to *in-vivo* studies. Cases of diabetes mellitus around the world are increasing day by day. It has been predicted that India, China and the United States will have the largest number of diabetic patients by 2030. Therefore, the anti-diabetic study has attracted a huge number of scientists and various *in-vitro* and *in-vivo* models have been developed for anti-diabetic study. In drug development, the *in-vitro* techniques are an important method of confirming a particular biological activity of a compound before animal experiments *In-vitro* cell lines are an alternative to animal models to study the particular activity of drugs. To produce more reproducible results, the *in-vitro* cell lines from colonal cells, cell lines are used.<sup>10</sup>

**3.14 Inhibition of  $\alpha$ -Amylase Activity:** Starch solution (0.5% w/v) was prepared by stirring potato starch (0.125 g) in 20 mM sodium phosphate buffer with 6.7 mM sodium chloride (pH 6.9; 25 ml) in a boiling water bath for 15 min. The  $\alpha$ -amylase solution was prepared by mixing 1 U/ml of  $\alpha$ -amylase in the same buffer. The colorimetric reagent was prepared by mixing an equal volume of sodium potassium tartrate tetrahydrate solution and 96 mM 3, 5-dinitro salicylic acid (DNS) solution. Starch solution (1000  $\mu$ l) was mixed with increasing concentrations of Acetone and Ethyl acetate extract (25, 50, 100, 200, 400, and 800  $\mu$ g/ml), acarbose (10– 320  $\mu$ g/ml), and to this 1000  $\mu$ l of the  $\alpha$ -amylase solution was added and incubated at 25°C for 3 min to react with the starch solution. A 1000  $\mu$ l of 96 mM DNS reagent was added to the above solution, and the contents were heated for 15 min on a boiling water bath. The final volume was made up with distilled water, and the absorbance was measured at 540 nm using UV-1800, Shimadzu Corporation, Kyoto, Japan. The percentage inhibition and 50% inhibitory concentration (IC<sub>50</sub>) value was calculated.

**3.15 Inhibition of  $\alpha$ -Glucosidase Activity:** The  $\alpha$ -glucosidase enzyme inhibition activity was determined by incubating 100  $\mu$ l of  $\alpha$ -glucosidase enzyme (1 U/ml) solution with 100  $\mu$ l of phosphate buffer (pH 7.0) which contains 100  $\mu$ l of Acetone and Ethyl acetate extract (25–800  $\mu$ g/ml), acarbose (0.1–3.2  $\mu$ g/ml) at 37°C for 60 min in maltose solution. To stop the  $\alpha$ -glucosidase action on maltose, the above reaction mixture was kept in boiling water for 2 min and cooled. To this, 2 ml of glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose by the action of  $\alpha$ -glucosidase enzyme. The percentage inhibition and 50% inhibitory concentration (IC<sub>50</sub>) value was calculated.<sup>11</sup>

## 4. Result

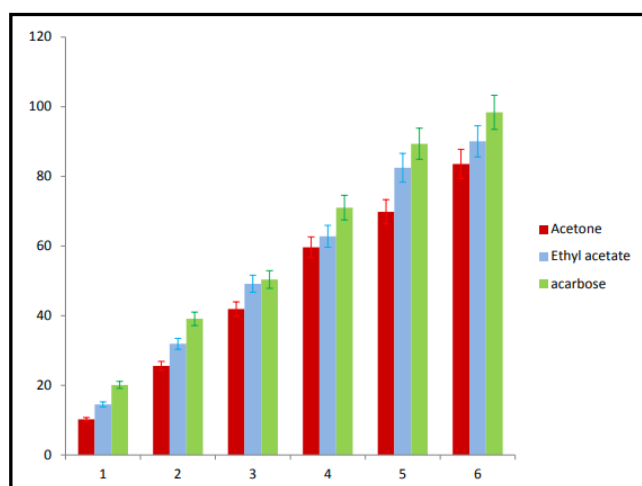
### 4.1 *In-Vitro* Antidiabetic Activity:

**4.1.1 Inhibition of  $\alpha$ -Amylase Activity:** Acetone extract produced 10.23 $\pm$ 0.12% inhibition of  $\alpha$ -amylase activity at 25 $\mu$ g/ml and 83.56 $\pm$ 0.34% at

800 $\mu\text{g/ml}$  concentrations, respectively, and its IC<sub>50</sub> was found to be 281.42 $\pm$ 0.02 $\mu\text{g/ml}$ . Ethyl acetate extract produced 14.52 $\pm$ 0.12% inhibition of  $\alpha$ -amylase activity at 25 $\mu\text{g/ml}$  and 92.06 $\pm$ 0.34% at 800 $\mu\text{g/ml}$  concentrations, respectively, and its IC<sub>50</sub> was found to be 212.15 $\pm$ 0.41 $\mu\text{g/ml}$ . The standard drug acarbose exhibited 20.12 $\pm$ 0.3% inhibition of  $\alpha$ -amylase activity at 10 $\mu\text{g/ml}$  and 98.39 $\pm$ 0.71% at 320 $\mu\text{g/ml}$  concentrations, respectively, and its IC<sub>50</sub> for acarbose was found to be 141.13 $\pm$ 0.89 $\mu\text{g/ml}$ .

**Table 3.** Extract inhibition of  $\alpha$ -amylase activity

Extracts % inhibition				
Concentration ( $\mu\text{g/ml}$ )	Acetone	Ethyl acetate	Concentration ( $\mu\text{g/ml}$ )	Acarbose
25	10.23 $\pm$ 0.12	14.52 $\pm$ 0.12	10	20.12 $\pm$ 0.3
50	25.59 $\pm$ 0.45	31.91 $\pm$ 0.45	20	39.09 $\pm$ 0.78
100	41.89 $\pm$ 0.98	49.16 $\pm$ 0.19	40	50.39 $\pm$ 0.9
200	59.63 $\pm$ 0.54	62.78 $\pm$ 0.68	80	71.01 $\pm$ 0.54
400	69.83 $\pm$ 0.45	75.45 $\pm$ 0.1	160	86.34 $\pm$ 0.62
800	83.56 $\pm$ 0.34	92.06 $\pm$ 0.34	320	98.39 $\pm$ 0.71
IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	281.4 2 $\pm$ 0.0	212.1 5 $\pm$ 0.4	IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	141.1 3 $\pm$ 0.8



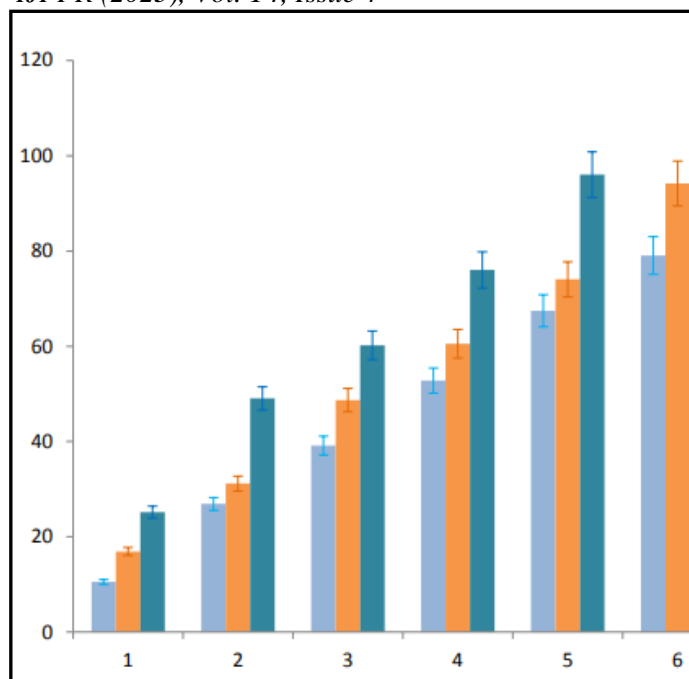
**Figure 1.** Graphical representation of  $\alpha$ -Amylase inhibitory Activity of *Xanthium strumarium* L. leaves extracts

#### 4.1.2 Inhibition of $\alpha$ -Glucosidase Activity:

Acetone extract produced 10.52 $\pm$ 0.15% inhibition of  $\alpha$ -glucosidase activity at 25  $\mu\text{g/ml}$  and 79.06 $\pm$ 0.88% at 800 $\mu\text{g/ml}$  concentrations, respectively, and its IC<sub>50</sub> was found to be 314.56 $\pm$ 0.74 $\mu\text{g/ml}$ . Ethyl acetate extract produced 16.93 $\pm$ 0.44% inhibition of  $\alpha$ -glucosidase activity at 25 $\mu\text{g/ml}$  and 94.16 $\pm$ 0.74% at 800  $\mu\text{g/ml}$  concentrations, respectively, and its IC<sub>50</sub> was found to be 219.97 $\pm$ 0.65 $\mu\text{g/ml}$ . The standard drug acarbose exhibited 25.18 $\pm$ 0.15% inhibition of  $\alpha$ -glucosidase activity at 0.1 $\mu\text{g/ml}$  and 98.19 $\pm$ 0.32% at 3.2 $\mu\text{g/ml}$  concentrations, respectively, and its IC<sub>50</sub> for acarbose was found to be 0.17 $\pm$ 0.73 $\mu\text{g/ml}$ .

**Table 4.** Extract inhibition of  $\alpha$ -glucosidase activity

Extracts/% inhibition				
Concentration ( $\mu\text{g/ml}$ )	Acetone	Ethyl acetate	Concentration ( $\mu\text{g/ml}$ )	Acarbose
25	10.52 $\pm$ 0.15	16.93 $\pm$ 0.44	0.1	25.18 $\pm$ 0.15
50	26.91 $\pm$ 0.62	31.19 $\pm$ 0.1	0.2	49.05 $\pm$ 0.17
100	39.16 $\pm$ 0.11	48.69 $\pm$ 0.9	0.4	60.19 $\pm$ 0.23
200	52.78 $\pm$ 0.1	60.53 $\pm$ 0.84	0.8	76.01 $\pm$ 0.61
400	67.45 $\pm$ 0.49	74.03 $\pm$ 0.34	1.6	96.02 $\pm$ 0.10
800	79.06 $\pm$ 0.88	94.16 $\pm$ 0.74	3.2	98.19 $\pm$ 0.32
IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	314.56 $\pm$ 0.74	219.97 $\pm$ 0.65	IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	0.17 $\pm$ 0.73



**Figure 2.** Graphical representation of  $\alpha$ -Glucosidase inhibitory Activity of *Xanthium strumarium* L. leaves extracts

**5. Conclusion:** Both the extracts showed good *in-vitro* antidiabetic potential against the  $\alpha$ -amylase and  $\alpha$ -Glucosidase. Acetone extract produced  $10.52 \pm 0.15\%$  inhibition of  $\alpha$ -glucosidase activity at  $25 \mu\text{g/ml}$  and  $79.06 \pm 0.88\%$  at  $800 \mu\text{g/ml}$  concentrations, respectively, and its  $\text{IC}_{50}$  was found to be  $314.56 \pm 0.74 \mu\text{g/ml}$ . Ethyl acetate extract produced  $16.93 \pm 0.44\%$  inhibition of  $\alpha$ -glucosidase activity at  $25 \mu\text{g/ml}$  and  $94.16 \pm 0.74\%$  at  $800 \mu\text{g/ml}$  concentrations, respectively, and its  $\text{IC}_{50}$  was found to be  $219.97 \pm 0.65 \mu\text{g/ml}$ . The standard drug acarbose exhibited  $25.18 \pm 0.15\%$  inhibition of  $\alpha$ -glucosidase activity at  $0.1 \mu\text{g/ml}$  and  $98.19 \pm 0.32\%$  at  $3.2 \mu\text{g/ml}$  concentrations, respectively, and its  $\text{IC}_{50}$  for acarbose was found to be  $0.17 \pm 0.73 \mu\text{g/ml}$ . Acetone extract produced  $10.23 \pm 0.12\%$  inhibition of  $\alpha$ -amylase activity at  $25 \mu\text{g/ml}$  and  $83.56 \pm 0.34\%$  at  $800 \mu\text{g/ml}$  concentrations, respectively, and its  $\text{IC}_{50}$  was found to be  $281.42 \pm 0.02 \mu\text{g/ml}$ . Ethyl acetate extract produced  $14.52 \pm 0.12\%$  inhibition of  $\alpha$ -amylase activity at  $25 \mu\text{g/ml}$  and  $92.06 \pm 0.34\%$  at  $800 \mu\text{g/ml}$  concentrations, respectively, and its  $\text{IC}_{50}$  was found to be  $212.15 \pm 0.41 \mu\text{g/ml}$ . The standard drug acarbose exhibited  $20.12 \pm 0.3\%$  inhibition of  $\alpha$ -amylase activity at  $10 \mu\text{g/ml}$  and  $98.39 \pm 0.71\%$  at  $320 \mu\text{g/ml}$  concentrations,

respectively, and its  $\text{IC}_{50}$  for acarbose was found to be  $141.13 \pm 0.89 \mu\text{g/ml}$ . Finally it was concluded that ethyl acetate extract of *Xanthium strumarium* L. leaves showed good antidiabetic potential when compared with acetone extract and significant with standard compound Acarbose.

## 6. References

1. Das AK, Rai M, A world without diabetes and its complications: A preventive program, Type. 2008; 2: 1.
2. Wild S, Roglic G, Green A, Sicree R, King H, Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27(5): 1047-53.
3. Sharma AK, Kumar A, Yadav SK, Rahal A, Studies on antimicrobial and immunomodulatory effects of hot aqueous extract of *Acacia nilotica* L. leaves against common veterinary pathogens, Veterinary Medicine International. 2014; 2014.
4. Geetha TS, Geetha N, Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogon citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu, International Journal of Pharmtech Research. 2014; 6(2): 521-9.
5. Gotama TL, Husni A, Antidiabetic activity of *Sargassum hystrix* extracts in streptozotocin-induced diabetic rats, Preventive Nutrition and Food Science. 2018; 23(3): 189.
6. Deshpande SN, Preliminary Phytochemical Analysis and In Vitro Investigation of Antibacterial Activity Of *Acacia Nilotica* Against Clinical Isolates, Journal of Pharmacognosy and Phytochemistry. 2013; 1(5): 23-7.
7. Kifle ZD, Yesuf JS, Atnafie SA, Evaluation of in vitro and in vivo anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of *hagenia abyssinica* (rosaceae), Journal of Experimental Pharmacology. 2020: 151-67.

*IJPPR (2023), Vol. 14, Issue 4*

8. Ouassou H, Bouhrim M, Bencheikh N, Addi M, Hano C, Mekhfi H, Ziyat A, Legssyer A, Aziz M, Bnouham M, In vitro antioxidant properties, glucose-diffusion effects,  $\alpha$ -amylase inhibitory activity, and antidiabetogenic effects of *C. Europaea* extracts in experimental animals, *Antioxidants*. 2021; 10(11): 1747.
9. Narkhede MB, Ajimire PV, Wagh AE, Mohan M, Shivashanmugam AT, In vitro antidiabetic activity of *Caesalpina digyna* (R.) methanol root extract, *Asian Journal of Plant Science and Research*. 2011; 1(2): 101-6.
10. Antony J, Debroy S, Manisha C, Thomas P, Jeyarani V, Choephel T, In-vitro cell line Models and Assay methods to study the Anti-diabetic Activity, *Research Journal of Pharmacy and Technology*. 2019;12(5): 2200-6.

Nguyen QV, Wang SL, Nguyen AD, In vitro  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition, and in vivo anti-hyperglycemic effects of *Psidium littorale* Raddi leaf extract, *Research on Chemical Intermediates*. 2018; 44: 1745-53.