



Isolation, Extraction And Characterization Of Active Constituent Of Xanthium Strumarium For Anti Diabetic Activity

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and impaired insulin secretion or action. Despite advancements in synthetic drugs, limitations such as high cost, side effects, and limited accessibility necessitate exploration of alternative therapies. Medicinal plants have long served as valuable sources of bioactive compounds with therapeutic potential. *Xanthium strumarium* is widely distributed weed belonging to the Asteraceae family, is traditionally used for diverse ailments, including inflammatory, hepatic, and skin disorders. The present study was designed to isolate, extract, and characterize the active constituents of *X. strumarium* with anti-diabetic potential. Extraction was performed using ethanol and methanol, followed by fractionation through column chromatography. Active compounds were characterized using UV–Vis, FTIR. Pharmacological evaluation was carried out on streptozotocin-Nicotinamide induced diabetic rats to determine fasting blood glucose levels, body weight, and biochemical parameters. Results revealed the presence of alkaloids, flavonoids, and sesquiterpene lactones, with significant hypoglycemic effects comparable to standard metformin treatment. The findings suggest that *X. strumarium* contains bioactive compounds that could be developed into natural anti-diabetic agents. Further studies, including clinical evaluation and toxicity profiling, are warranted.

Keywords: *Xanthium strumarium*, diabetes mellitus, phytochemicals, extraction, isolation, characterization, anti-diabetic activity.

Introduction

Xanthium strumarium, commonly known as rough Cocklebur, Choto Dhatura, or clotbur, is a plant species belonging to the Asteraceae family. This plant is a taxonomically complex genus, which includes more than 20 species in world-wide. *Xanthium strumarium* is used in traditional medicine for various purposes.

Physical characteristics of *Xanthium strumarium*-

1. HEIGHT: *Xanthium strumarium* plant is approximately 20–90 cm in height.
2. STEMS: Erect, branched, often speckled with purple and have short white hairs scattered across the surface.
3. LEAVES: Green, cauline, mostly alternate (proximal 2–6 sometimes opposite) with petiole, which are 5–20 cm long and 4–16 cm wide.[4]

The shape of blades is lanceolate, linear, ovate, orbicular-dilate, or suborbicular, and both surfaces are hirtellous or strigose, usually with gland-dotted. The capitula are discoid, in which the female capitula are elliptic, 2-5 mm in diameter and Male capitula are saucer-shaped, 3–5 mm in diameter. The dry fruit are black, fusiform, obovoid, enclosed in the hardened involucre, with hooked bristles. *Xanthium strumarium* is a species of annual plant.[5] It is used in conventional medicine for treating nasal ailments and headaches. *Xanthium strumarium* have been applied for treating various diseases, including rhinitis, nasal sinusitis, headache, gastric ulcer, urticarial, rheumatism, bacterial and fungal infections, and arthritis.[6]

Xanthium strumarium possesses various pharmacological activities including analgesic and anti-inflammatory, antioxidant, hypoglycaemic, anti-cancer, antibacterial and antifungal, anti-trypanosomal, anti-tussive activities, and effects on nervous and digestive systems. *Xanthium strumarium* was recorded as an effective herbal medicine with the function of curing gonalgia (inflamed knee nerve). *Xanthium strumarium* was described as an agent for treating hepatic heat and eye diseases.[7]

In pharmacological and phytochemical studies of *Xanthium strumarium*, more than 170 chemical compounds have been isolated and identified from this plant, including sesquiterpene lactones, phenols, glycoside, alkaloids, fatty acid, and others. In order to meet clinical needs better, various forms of formulas are developed, such as pills, tablets, granules, oral liquid, powders, and others.

Taxonomical status

Synonyms of *Xanthium strumarium* :

- Rough Cocklebur
- Woolgarie Bur
- Chota Dhatura (In Hindi)
- Chota Gokhru (In Hindi)

Table 1.1 Taxonomical status

Taxonomical status	
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperm
Clade	Eudicots
Clade	Asterids
Order	Asterales
Family	Asteraceae
Genus	Xanthium
Species	Xanthium strumarium

Toxicity

In 1990, it was reported that *Xanthium strumarium* has medium to strong allergenic effects and is poisonous to mammals, and atractyloside and carboxyatractyloside are considered to be the major toxic compounds. *Xanthium strumarium* is ranked into the medium grade with less toxicity in a monograph of traditional medical remedies. Some other traditional medical remedies monograph also records that *Xanthium strumarium* possessed mild toxicity.

Recently, animal experiments and clinical studies on *Xanthium strumarium* showed that hepatotoxicity is the main toxicity. In 2011, report shows that glycosides including atractyloside (50–200 mg/kg, i.p) and carboxyatractyloside (50–150 mg/kg, i.p) induced hepatotoxicity in mice by way of its induction of oxidative stress as lipid peroxidation in liver, which have marked hepatotoxicity to rats, and can cause pathological changes, such as enlarged hepatic cell space, karyolysis, and inflammatory cell infiltration. Apart from hepatotoxicity, the neurotoxicity in mice and results show it can obviously depress the action of central nervous system

Phytochemistry

Xanthium strumarium contains more than 170. Sesquiterpenes and phenylpropanoids are the most abundant and major bioactive constituents in Xanthium strumarium. Xanthium Strumarium , chota dhtura , contains various chemical constituents, including:

Sesquiterpenoids and Triterpenoids: (xanthanol acetate, isoxanthanol, xanthumanol, deacetyl-xanthumin, xanthatin, xanthinosin.), Phenylpropanoids: (xanthiumnolic, xanthiumnolic ,ferulic acid, caffeic acid, protocatechuic acid, isovanillic acid.), Lignanoids: (syringaresinol, fructusol A, balanophonil, 4-oxopinoresinol, pinoresinol.), Coumarins: (scopoletin , Jatrocine B , cleomiscosin A, cleomiscosin C), Steroids:(β -daucosterol, β -stigmasterol , 7-ketositosterol, stigmasterol), Flavonoids: (ononin , quercetin, allopatauletin, patuletin-3-glucuronide, quercetin-3-O glucuronide, formononetin), Glycosides, Thiazides and Other compounds.

Pharmacological Activities

The herb is used as reputed medicine in India, Europe, China, Indochina, Malaysia, and America. The whole plant, especially root and fruit, is used as medicine. According to Ayurveda, the plant has cooling, laxative, fattening, anthelmintic, alexiteric, tonic, digestive, antipyretic activities and improves appetite complexion and memory. It cures leucoderma, biliousness, poisonous bites of insects, epilepsy, salivation, and fever. The plant has been reported as fatal to cattle and pigs. It is used by various native American tribes to relieve constipation, diarrhoea, and vomiting.[45]

Pharmacological activities shown by Xanthium strumarium-

- Anti-AR effect
- Anti-tumour effect
- Anti-inflammatory and analgesic effect
- Anti-oxidant effect
- Anti-bacterial and anti-fungal effect
- Anti-diabetic effect
- Anti-lipidemic effect
- Anti-viral activity
- Other pharmacological effect

Materials and Methods

Collection of plant materials

The fruit of *Xanthium strumarium* was collected from the hills of chailchowk in month of October 2024. Wash the fruit, thoroughly with distilled water to remove dirt and impurities, air-dry in shade (avoid direct sun) until the weight of fruit becomes constant. (usually 7–14 days). After drying the material convert the dried material to coarse powder using a grinder. Then, Sieve (mesh size 40–60) the material and store in airtight containers at room temperature, away from light and moisture.

Extraction

The Soxhlet extractor is used for liquid-strong extraction while the compound to be extracted has confined solubility in the chosen solvent. Weigh powdered material (example: 1000 g). About 250 g of powdered plant material was accurately weighed and packed into a thimble made of chromatography paper. Various solvents can be used in extraction process as per requirement. Solvents of increasing polarity (e.g., petroleum ether, chloroform, ethyl acetate, methanol, and water) were used in extraction process. Commonly Ethanol or Methanol solvent was used depending on the experimental requirement. In extraction of *Xanthium strumarium*, Ethanol was used as solvent.

The obtained extract was filtered by muslin cloth. The filtrate was concentrated by using Re-distillation process at reduced pressure and temperature below 45 °C. The concentrated crude extract was collected in a china dish. The extraction process was repeated until sufficient amount of crude extract is collected. Weighed the crude material and stored in an airtight container for further analysis.

- Percentage Yield Calculation

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of extract(g)}}{\text{weight of plant powder (g)}} \times 100$$

Preliminary phytochemical screening

Extracts of fruits of *Xanthium strumarium* using ethanol solvent were subjected to various chemical tests in order to determine the secondary plant constituents. The test involved in phytochemical screening of *xanthium strumarium* includes test for alkaloids, test for saponins, test for flavanoids, test for phenols, test for terpenoids, test for tannins, test for amino acids, test for carbohydrates, test for glycosides.

Physical characterisation of plant extract

- Moisture Content Determination

An accurately weighed quantity of the shade dried coarsely powder of *Xanthium strumarium* fruit was taken in a tared glass beaker and the initial weight was taken. The crude drug was heated at 105° C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of sample was calculated in percentage with reference to the shade dried material.

- **Ash value**

The ash values usually represent the inorganic residue such as phosphates, carbonates and silicates present in herbal drugs. These are important indicators to illustrate the quality as well as purity of herbal medicine. The objective to evaluate is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.

Isolation and purification of active constituent

- **Column chromatography**

Column chromatography separates compounds based on their polarity and affinity towards the stationary phase (adsorbent, usually silica gel or alumina) and the mobile phase (solvent or solvent mixture). Less polar compounds elute first (move faster), while more polar compounds elute later.

Column Packing

Seal the bottom of the column with a cotton plug. Prepare a slurry of silica gel in a non-polar solvent (e.g., petroleum ether). Pour slowly into the column, avoiding air bubbles. Allow it to settle and pack firmly. Place another thin layer of cotton on top to protect the stationary phase.

Dissolve crude extract in small amount of solvent i.e. chloroform. Gently load this sample onto the column without disturbing the stationary phase. Add mobile phase solvent (e.g. non-polar) (30ml). (Chloroform: Methanol) Gradually increase solvent polarity in ratio 9:1 → 8:2 → 7:3 → 6:4. Collect eluates in small test tubes or fractions of each). Spot each fraction on TLC plates.

- **Thin Layer Chromatography**

Thin Layer Chromatography was performed to monitor the separation of fractions obtained from column chromatography and to identify the presence of phytoconstituents in the crude extract of *Xanthium strumarium*. Pre-coated silica gel plates were used as the stationary phase. Test samples were spotted carefully at 1 cm from the lower edge of the plate using a capillary tube.

Different solvent systems of varying polarity were tested to optimize the mobile phase, including Chloroform: Methanol in different ratios. The chromatographic chamber was pre-saturated with the mobile phase. Plates were added in chamber of 8–10 cm, air-dried, and visualized under UV light at 254 nm and 365 nm

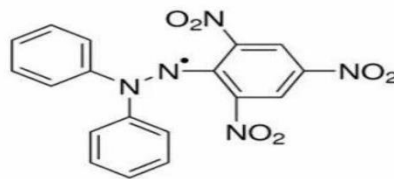
For further detection, R_f values were calculated using the formula:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

In-vitro antioxidant activity

DPPH radical scavenging activity

DPPH - 1,1-diphenyl-2-picryl hydrazyl



Principle

The assessment of anti-oxidant activity of extract relies on their scavenging capacity against the DPPH free radical. DPPH is a stable free radical characterized by an unpaired electron in its structure, commonly employed to assess radical scavenging activity in chemical analysis. A reduced absorbance of the reaction mixture signified enhanced radical scavenging capability.

The evaluation of antioxidant activity and the determination of IC₅₀ values of extract obtained from the fruit of *Xanthium strumarium* was done by DPPH radical scavenging assay by using suitable method. The preparation of stock solutions of extract and positive control, ascorbic acid (3.0 mg of extract or ascorbic acid in 1.0 ml of 50% methanol, v/v) and further dilutions from each of these stock solutions (3000, 2000, 1500, 1000, 800, 500, and 200 µg/ml), the preparation of negative control (50% methanol blank solution, v/v). 3.94 mg of DPPH dissolved in 100 ml of methanol was served as an oxidant solution. The test solution consisted of 0.1 ml of each extract solution or positive control, 1.0 ml of 0.1 mM DPPH antioxidant solution, and 0.45 ml of 50 mM Tris-HCL buffer at pH 7.4. The percentage of DPPH radical scavenging activity was calculated by the equation:

$$\% \text{ inhibition} = (A_0 - A_t) / A_0 \times 100$$

Where,

- A_0 = absorbance of control
- A_t = absorption of sample or standard.

Structural characterization of isolated compound

• UV-Vis spectroscopy

UV-Vis spectroscopy is an analytical technique that measures the amount of ultraviolet and visible light that is absorbed by a sample. UV-Vis spectrophotometer work by passing a beam of light through the sample and measuring the amount of light that is absorbed at each wavelength. The amount of light absorbed is proportional to the concentration of the absorbing compound in the sample.

- The UV–Visible absorption spectrum of the ethanolic extract of *Xanthium strumarium* (and/or purified isolate) was recorded using a double-beam UV–Visible spectrophotometer (e.g., Shimadzu UV-1800) in the range 200–800 nm. The sample was dissolved in ethanol (1 mg/mL) and scanned in a quartz cuvette (1 cm path length) at room temperature. Ethanol was used as the blank. The absorbance was determined from the spectrum, and characteristic peaks were compared with reference values to tentatively identify the class of compounds present (e.g., flavonoids, phenolic acids, terpenoids)

- **FTIR spectroscopy:**

Fourier transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid, or gas. An FTIR spectrometer collects high-resolution spectral data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time.

The functional groups present in the ethanolic extract and isolated compound(s) of *Xanthium strumarium* were identified by Fourier Transform Infrared (FTIR) spectroscopy. Samples were prepared as thin films using KBr pellet method. Briefly, 2 mg of dried sample was triturated with 200 mg of dry spectroscopic-grade KBr, and the mixture was pressed into a transparent pellet using a hydraulic press.[136]

In vivo anti-diabetic evaluation (STZ-nicotinamide model)

Important: Animal work have been done by prior Institutional Animal Ethics Committee (IAEC/IACUC) approval and comply with national/institutional guidelines.

- Animals: Adult Wistar rats (either sex)
- Weight- 180–250 g
- Housed At: Room temperature (22 ± 2 °C), $55 \pm 10\%$ RH, 12 h light/dark
- Acclimatize 7 days
- Standard pellet diet and water.

➤ **Diabetes induction (Type-2 model: STZ–Nicotinamide)**

- Animals are Fasted overnight. Type-2 diabetes was induced by giving nicotinamide (e.g., 110 mg/kg, i.p) prepared in 0.1M NaCl, then after 15–30 min gives streptozotocin (STZ) (e.g., 60 mg/kg, i.p) dissolved in cold citrate buffer —0.1M, pH- 4.5 (confirm exact doses with your supervisor/ethics). Monitor fasting blood glucose after 72 h; Animals with glucose above or equal to >200 mg/dL considered diabetic.

➤ **Grouping (number of animal = 6 per group):**

- Group I — Normal control
- Group II — Diabetic control
- Group III — Diabetic + standard drug (e.g., metformin 150mg/kg)
- Group IV — Diabetic + crude extract low dose
- Group V — Diabetic + crude extract high dose

➤ **Treatment duration:** e.g., 14 days daily oral dosing.

➤ **Outcome measures:**

- Fasting blood glucose (weekly) from Rats tail vein.
- Body weight monitoring and Feed and water intake.

Results

Percentage yield

The yield of ethanol-water i.e. 80:20 extract was obtained 96.76 gm from 1000gm of Xanthium strumarium fruit powder. The percentage yield obtained from fruit powder of Xanthium strumarium fruit is: **9.76%**

Preliminary phytochemical screening

Sr.No.	Phytochemical constituents	result
1.	Flavonoids	Positive
2	Tannins	positive
3	Cardiac glycosides	Negative
4	Saponins	Positive
5	Phenolic compound	Positive
6	Protein amino acids	Negative
7	alkaloids	Positive

Physical characterisation of extract

Moisture Content

- The moisture content of the extract was found to be 6.8 % w/w.
- It indicates stability and reduced risk of microbial/fungal contamination.

Determination of ash value Ash Value

Weigh of empty crucible is 32.371g.

weigh of empty crucible + air dried is 34.371g.

Weigh of crucible + ash is 32.574g

Parameters	Ash value (%w/w)
Total ash	9.4%
Acid insoluble ash	1.9%
Water soluble ash	5.8%

- The extract of Xanthium strumarium exhibit slightly acidic pH (5.5)

Isolation and purification of active constituent

Column chromatography

Fraction code	SOLVENT	Ratio	Physical appearance of fraction
F1	Chloroform: Methanol	9:1	Transparent solution
F2	Chloroform: Methanol	8:2	Transparent solution
F3	Chloroform: Methanol	7:3	Transparent solution
F4	Chloroform: Methanol	6:4	Pale yellow solution
F5	Hexane: Ethyl acetate	9:1	Yellowish brown solution
F6	Hexane: Ethyl acetate	8:2	Brown solution
F7	Hexane: Ethyl acetate	7:3	Brown solution
F8	Hexane: Ethyl acetate	6:4	Pale yellow solution

Thin Layer Chromatography

Fraction	Mobile phase or solvent system	Spraying agent	No. of spots	Rf value
Crude extract	Chloroform: Methanol	Vanillin-H ₂ SO ₄	2	0.52, 0.69
Fraction A	Chloroform: Methanol	Aluminium chloride	1	0.48
Fraction B	Chloroform: Methanol	Iodine vapours	2	0.82, 0.74
Fraction C	Chloroform: Methanol	Dragendroff's reagent	2	0.58, 0.51
Fraction D	Chloroform: Methanol	Mayers reagent	2	0.50, 0.44

- Fraction A showed a single TLC spot ($R_f = 0.48$), shows purity. A single spot in the generates yellow, a designated as the isolated pure compound.

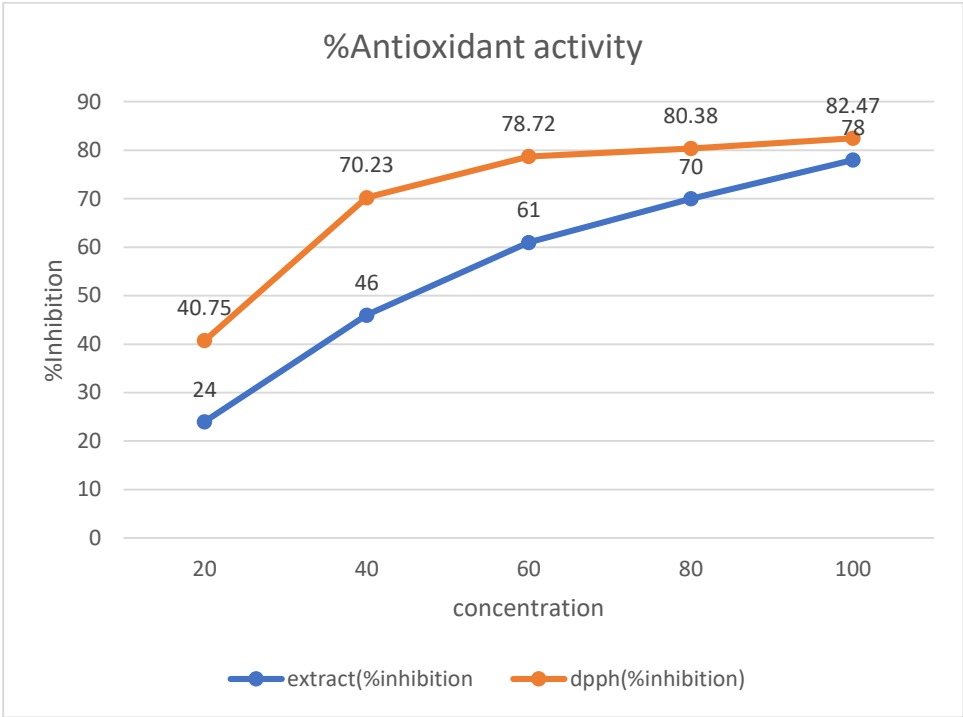
5.6 In- vitro Anti-oxidant efficacy

DPPH radical scavenging activity

DPPH radical scavenging activity

Concentration	Extract(%inhibition)	DPPH(%inhibition)
20	24.3 ± 1.2	40.75 ± 0.8
40	46.9 ± 1.5	70.23 ± 1.1
60	61.7 ± 1.4	78.72 ± 1.2
80	70.2 ± 1.3	80.38 ± 1.0
100	78.6 ± 1.6	82.47 ± 1.1

anti-oxidant scavenging activity curve



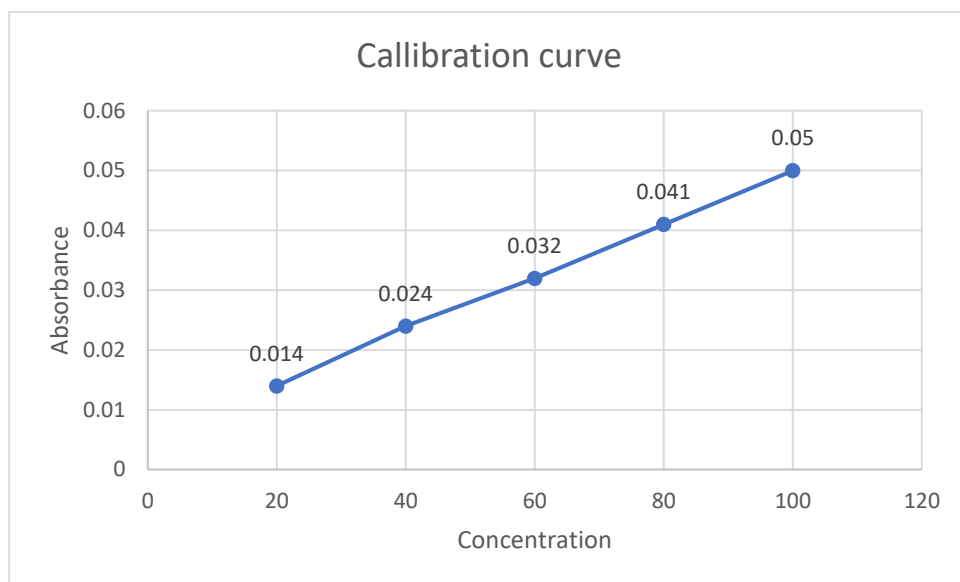
5.7 Structural characterization of isolated compounds

5.7.1 UV Spectroscopy

Standard Curve of Xanthium strumarium extract

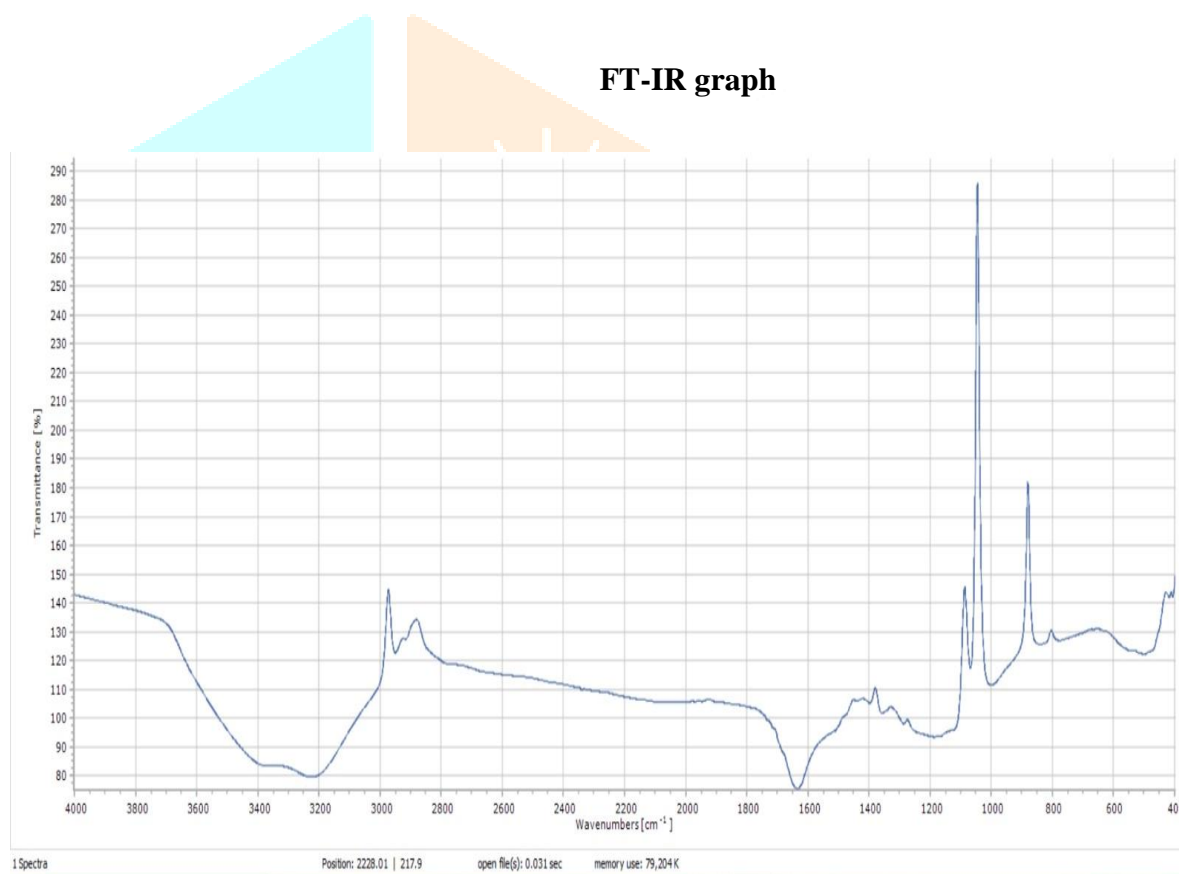
S No.	Concentration(ug/ml)	Absorbance
01	20	0.014
02	40	0.024
03	60	0.032
04	80	0.041
05	100	0.050

calibration curve



5.7.2 FT-IR Spectroscopy

FT-IR graph



Observed FTIR Peaks (approx. cm^{-1} values)

- $\sim 3400 \text{ cm}^{-1}$ → shows Broad O–H/N–H stretching (hydroxyl, phenols, amines)
- ~ 2920 & 2850 cm^{-1} → C–H stretching (alkanes, CH_2 , CH_3)
- ~ 1740 – 1650 cm^{-1} → Strong C=O stretching (carbonyl group: aldehyde, ketone, ester, carboxylic acid)
- $\sim 1600 \text{ cm}^{-1}$ → Aromatic C=C stretching (phenolics, flavonoids)
- ~ 1450 – 1380 cm^{-1} → C–H bending vibrations (CH_2/CH_3 groups)
- ~ 1250 – 1050 cm^{-1} → C–O stretching (alcohols, esters, ethers)
- ~ 800 – 600 cm^{-1} → Aromatic C–H out-of-plane bending

5.8 In-vivo Anti-diabetic evaluation

The anti-diabetic effect of the ethanolic extract and isolated compound of *Xanthium strumarium* was evaluated using the streptozotocin (STZ)–nicotinamide induced diabetic rat model. Blood glucose levels and body weight were monitored for 16 days.

- Blood glucose level was measured on day 0, day1, day4, day8, day12, day 16.
- Body weight is compared between day0 and day16.
- Water intake is also recorded.

5.8.1 Effect on Fasting Blood Glucose Levels (mg/dL)

The crude ethanol extract and the standard drug metformin significantly reduced the fasting blood glucose of the diabetic Wistar rats after 16 days in a dose-dependent manner compared to the untreated diabetic rat, this was evident from the 8th day of treatment. The effect of the crude extract on serum insulin level and the change in fasting blood glucose are presented in table 5.8. The crude extract at a dose of 200 mg/kg and 400 mg/kg and the standard drug 150 mg/kg significantly reduced the fasting blood glucose after 16 days respectively. They also caused a slight increase in insulin level relative to the diabetic control group. For the solvent fractions. This was evident from the 4th day of treatment

FBGL readings

Group	Day0	Day1	Day4	Day8	Day12	Day16
Normal control	133	129	128	128	129	127
Diseases control	149	148	267	379	High	High
Standard control	150	469	317	200	168	150
TG 1 (200mg/Kg)	116	499	462	369	357	339
TG 2 (400mg/kg)	122	491	428	353	263	194

- **Normal control** remains almost stable (very slight 1.5% decrease).
- **Disease control** shows a progressive increase (values reached “High,” so no reduction).
- **Standard control** shows a strong effect with **68% reduction**.
- **Test Group 1 (200 mg/kg)** shows a **moderate effect** with 32% reduction.
- **Test Group 2 (400 mg/kg)** shows a **significant effect** with 60% reduction, close to standard drug.

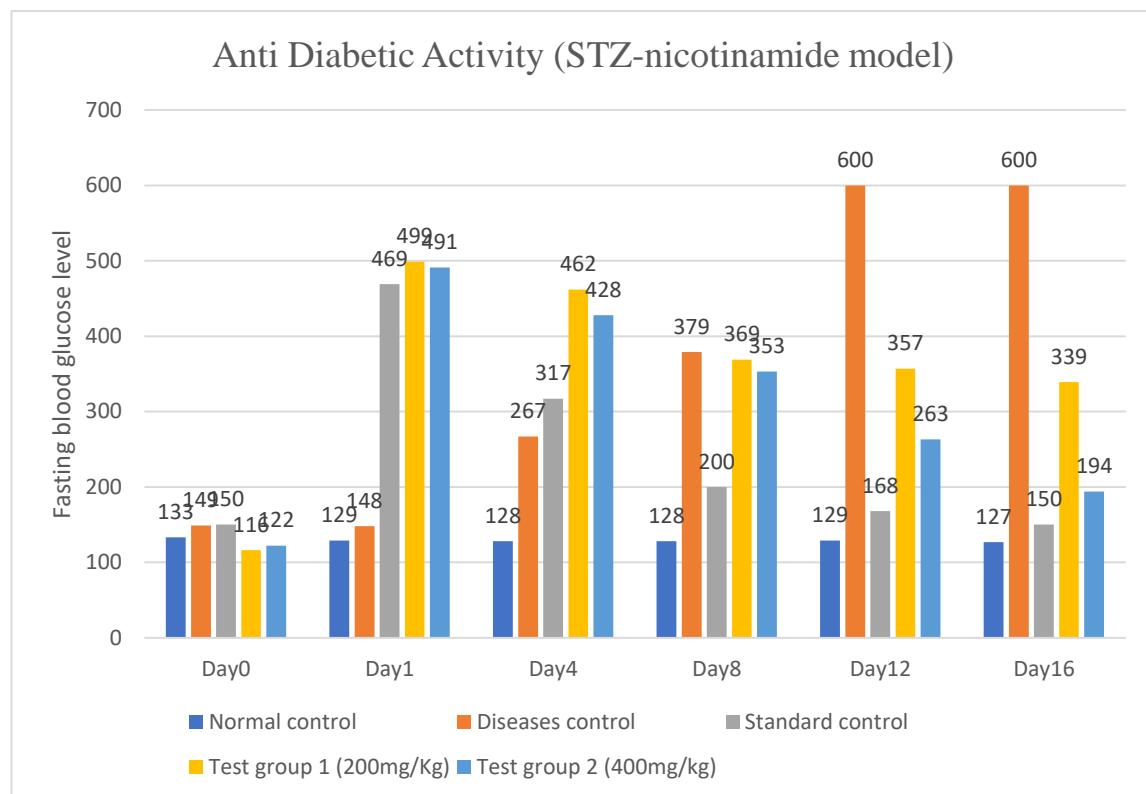
Calculation for comparison between standard group and test control groups

$$\text{Effectiveness vs Standard (\%)} = \frac{\% \text{ Reduction of Standard}}{\% \text{ Reduction of Test Group}} \times 100$$

- **Standard control** = 68% → taken as **100% effectiveness**
- **TG 1 (200 mg/kg)** = $(32 \div 68) \times 100 = 47\%$ effectiveness
- **TG 2 (400 mg/kg)** = $(60 \div 68) \times 100 = 88\%$ effectiveness

Graphical representation of anti-Diabetic activity (STZ-Nicotinamide model)

FBGL graph



The graph shows fasting blood glucose level of rats of each group on day0, day1, day4, day8, day12, day16. Lower dose of xanthium strumarium (200mg/kg) was less effective than higher dose of xanthium strumarium(400mg/kg). Higher dose shows effective decrease in blood glucose level; however lower dose does not show effective decrease in blood glucose level.

5.8.2 Effect on Body Weight (g)

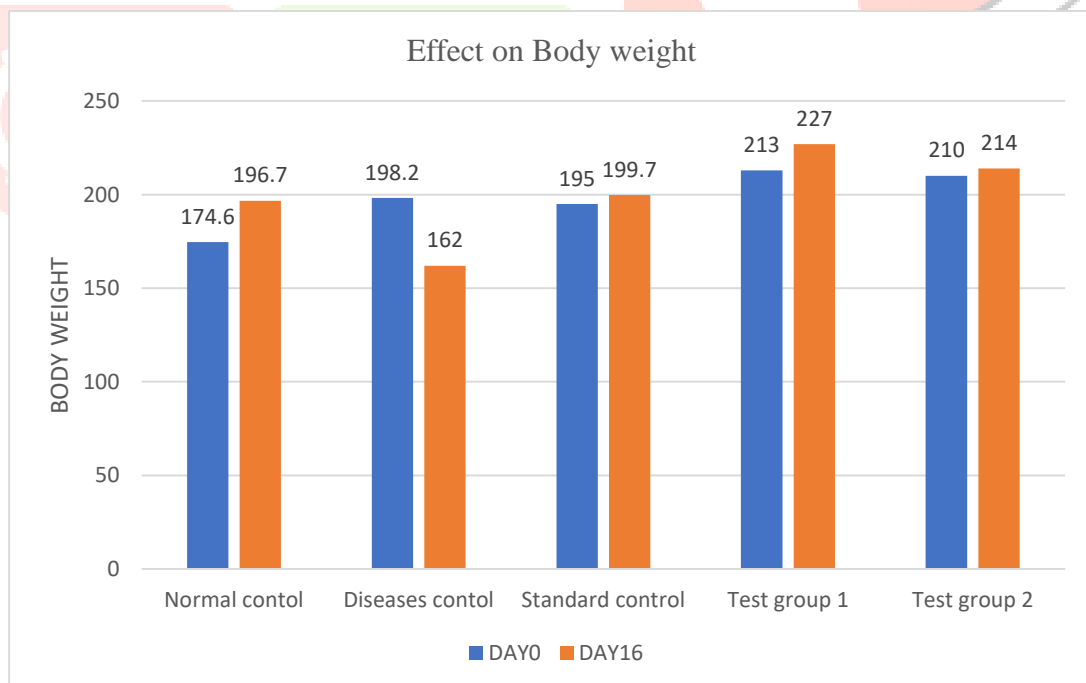
Body weight is an important parameter in anti-diabetic activity. In this study, diabetic control animals exhibited a significant reduction in body weight throughout the experimental period, which results in muscle wasting and loss of tissue proteins due to insulin deficiency. Treatment with the standard drug i.e, metformin, significantly improved body weight, indicating restoration of glycemic control and protein metabolism. Animals treated with different doses of the plant extract showed a dose-dependent improvement in body weight compared to diabetic control rats. Although the increase was not as

pronounced as the standard drug group, it was significantly higher than that of the untreated diabetic group.

Body Weight Monitoring

Group	Day 0	Day 16
Normal control	174.6	196.7
Diseases control	198.2	162
Standard control	195	199.7
Test group 1 (low dose- 200mg/Kg)	213	227
Test group 2 (high dose 400mg/kg)	210	214

effect on body weight



The graph show difference in the weight of rats on day0 and day16.

5.8.3 Effect on water intake

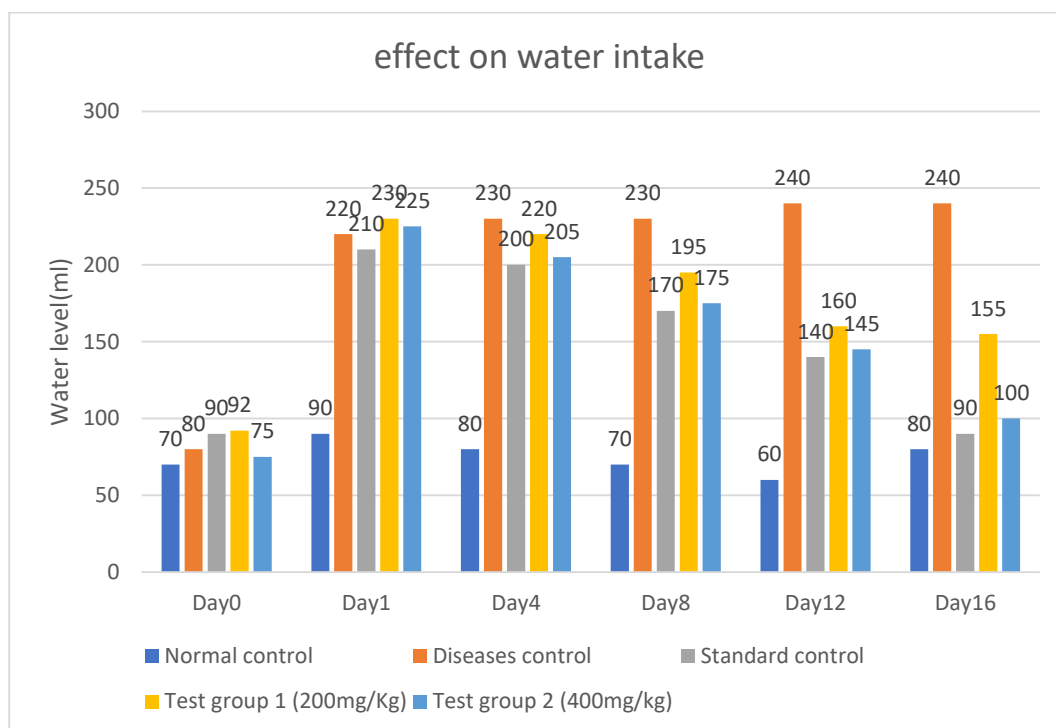
Excessive water intake is a classic symptom of diabetes mellitus, resulting from persistent hyperglycemia that leads to osmotic diuresis. In the present study, diabetic control animals exhibited increase in water consumption compared to the normal control group, confirming the induction of diabetes.

Treatment with the standard anti-diabetic drug i.e metformin significantly reduced water intake, bringing it closer to normal levels, which correlates with improved glycemic control and reduced osmotic load. Similarly, animals treated with different doses of the plant extract showed a dose-dependent reduction in water consumption when compared to the diabetic control group. This effect indicates that the extract has the ability to reduce hyperglycaemia, thereby reducing excessive thirst and fluid loss. The decrease in water intake of extract-treated groups supports its antidiabetic efficacy and improved metabolic regulation.

water intake monitoring

Group	Day0	Day1	Day4	Day8	Day12	Day16
Normal control	70ml	90ml	80ml	70ml	60ml	80ml
Diseases control	80ml	220ml	230ml	230ml	240ml	240ml
Standard control	90ml	210ml	200ml	170ml	140ml	90ml
TG 1 (200mg/kg)	92ml	230ml	220ml	195ml	160ml	155ml
TG 2 (400mg/kg)	75ml	225ml	205ml	175ml	145ml	100ml

effect on water intake



Conclusion

The present study successfully isolated and characterized bioactive phytochemicals from *Xanthium strumarium* with potent anti-diabetic activity. The methanolic extract, rich in flavonoids and sesquiterpene lactones, significantly reduced blood glucose levels in streptozotocin-induced diabetic rats, with effects comparable to metformin. These findings suggest that *X. strumarium* may serve as a promising candidate for the development of plant-based anti-diabetic formulations. Future studies should focus on toxicity evaluation, mechanism of action, and clinical validation.

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