ATMIYA UNIVERSITY

"EVALUATION OF ANTI-DIABETIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF OROXYLUM INDICUM PLANT"

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ABSTRACT

Background

It is calculated that 180 million people throughout the world have diabetes mellitus. The ethnobotanical information reports approximately 1000 plants that are likely to possess antidiabetic activity. Roots, leaves, seeds, fruits, and stems of *Oroxylum indicum* have been utilized as a monodrug or a component of certain compound drug preparations in the Indian Ayurvedic medicine system. To confirm the reproducibility quality of plant material, proper regulation of starting material is important. Thus, there has been an increase in the standardization of selected herbal plants of potential medicinal value. Identification of herbal drugs by pharmacognostic studies is more reliable. *O. indicum* is a very potent medicinal plant in Ayurveda. It stands out as one of the most multipurpose plants, having an inclusive variety of therapeutic responses.

Rationale

The current learning designed an ***in vitro and in vivo*** *study to assess the antidiabetic activity* of stem extracts of *Oroxylum indicum* and evaluate the pharmacognostic and phytochemical screening of leaf and stem parts of the *Oroxylum indicum* plant.

Materials & Methods

Several quality control parameters, like morphological study, transverse section, powder microscopic evaluation, leaf constant parameters such as stomatal index, physicochemical evaluations (moisture content, ash values, extractive values), and preliminary phytochemical screening, were undertaken. A specified dose of acarbose, aqueous, and methanolic stem extracts of *O. indicum* was used for the practical. The absorbance values were taken in a spectrophotometer at 540 nm and 546 nm for α-

amylase and α-glucosidase enzymes, respectively. Male Wistar rats, weighing 180-220 g, were used to screen antidiabetic activity on streptozocin-induced diabetic rats. The animals were acclimatized for two weeks before the initiation of the study. Diabetes was induced in overnight-fasted rats via a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 55 mg/kg body weight, prepared in 0.1 M sodium citrate buffer (pH 4.5). Rats exhibiting fasting blood glucose levels exceeding 250 mg/dL were considered diabetic and subsequently included in the experimental protocol. The parameters such as BW (body weight), Vu (urine volume), BGL (blood glucose level), and Ins (serum insulin) were estimated to study the anti-diabetic potential of the extract. During the experiment, all parameters were determined on days 0, 7, 14, 21, and 28. To also investigate alkaline phosphatase (ALP), alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) for antidiabetic activity. Phytochemical analysis revealed the presence of flavonoids and phenolic compounds in the extract of methanol. Flavonoid content was quantified using colorimetry, while chromatographic techniques, including HPTLC fingerprinting and HPLC profiling, were employed for further characterization of flavonoids in the extract.

Results

Dose-dependent % inhibition of α-amylase and α-glucosidase enzymes is observed with both extracts. Though equated with water extract, methanolic extract shows a higher % inhibition. Following the induction of diabetes, diabetic animals exhibited a significant increase in food and water intake, urinary output, and blood glucose levels compared to normal controls. Conversely, a marked reduction in body weight and circulating insulin levels was observed in the diabetic group, indicating metabolic disturbances associated with diabetes. A 500mg dose of methanolic extract shows a decline in blood glucose level,

an increase in body weight, little effect in restoring insulin sensitivity, managing blood glucose side by side, and helps in maintaining Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP). Analysis of total phenolic content by spectroscopic method is 240.34mg gallic acid/g of dried extract. The flavonoid content estimated by the colorimetric method was found to be 1.62% w/w. Chromatographic fingerprinting using HPTLC revealed distinct spots corresponding to flavonoid compounds, while HPLC profiling confirmed the flavonoid concentration as 1.618% w/w.

Conclusion

The current findings contribute valuable information toward the standardization and identification of *Oroxylum indicum*, supporting its potential use in traditional medicine. In vitro studies demonstrated antidiabetic activity of both aqueous and methanolic stem extracts. In vivo results indicated that the methanolic extract exhibited significant antidiabetic effects. Phytochemical screening confirmed the presence of flavonoids and phenolic compounds, warranting further investigation for detailed characterization and potential therapeutic application.

KEYWORDS: *Oroxylum indicum*, Antidiabetic activity, α-glucosidase, α-amylase, Acarbose, Streptozotocin (STZ), phytochemical screening

INTRODUCTION

Diabetes mellitus, commonly referred to as diabetes, encompasses a group of metabolic disorders characterized by chronic hyperglycemia, an abnormal elevation in blood sugar levels that typically results from defects in insulin secretion, insulin action, or a combination of both. [1] It is a multifactorial and progressive condition that poses significant global health, economic, and societal burdens.[2] Although a definitive cure remains elusive, diabetes can be effectively managed through pharmacological interventions, lifestyle modifications, and ongoing medical supervision, thereby minimizing complications and enhancing patient quality of life.[3, 4]

Advancements in biomedical research, therapeutic innovations, and improved understanding of the disease's molecular and physiological basis have contributed to greater effective diabetes management strategies.[5] The integration of public health initiatives, increased awareness, preventive screening, and expansion of healthcare infrastructure are vital components in addressing the rising global prevalence of this condition. [6, 7]

Diabetes affects individuals across all age groups, with most forms being chronic and necessitating long-term management.[8] In 2022, over half of the entities diagnosed with diabetes were not receiving pharmacological treatment, highlighting a significant gap in disease management. Notably, the prevalence of diabetes is rising more rapidly in low- and middle-income countries (LMICs) compared to high-income nations, attributed to urbanization, sedentary lifestyles, and dietary transitions.[9]

A considerable proportion of diabetic individuals, particularly in LMICs, remain undiagnosed, thereby increasing the risk of complications. In 2021, diabetes directly accounted for approximately 1.6 million deaths worldwide, with nearly 47% of these deaths occurring before the age of 70.[10]

Additionally, an estimated 5,30,000 deaths due to kidney disease were attributable to diabetes, and hyperglycemia was implicated in roughly 11% of cardiovascular-related fatalities. India, in particular, bears a substantial share of the global diabetes burden, with approximately 77 million adults currently diagnosed with the disease and an additional 25 million classified as prediabetic. The prevalence of diabetes tends to increase with advancing age, and while it affects both sexes, epidemiological data suggest a marginally higher incidence among females in certain regions. [11]

The World Health Organization (WHO) estimates that 80% of the world's population relies on herbal medicine.[12] The use of plants, their parts, and isolated phytochemicals for treating and managing various diseases has been in practice since ancient times. Alternative systems of medicine, based on plant extracts, have thrived throughout the ages and are still practiced by a large population to manage diabetes. The World Health Organization Expert Committee on Diabetes also suggested that medicinal herbs be further investigated, as they are repeatedly considered to be less toxic and have fewer side effects.[13]

Characterization of microscopy of similar-looking herbs of different species of the same genus can be differentiated by studying the details of cell structure and their general arrangement, stone cells, types of trichomes, and cell components such as crystals, grains, etc.[14]

Oroxylum indicum (Bignoniaceae), also known as 'Sonapatha', has been an important herb in Ayurvedic medicine and the indigenous medical system for thousands of years. Roots, leaves, and stems of *Oroxylum indicum* find application in the Indian Ayurvedic system of medicine, utilized as a standalone therapeutic agent or as an ingredient in specific compound drug preparations for the management of diverse disorders, in addition to their role as a tonic and Rasayana drug. Leaves are used externally to delicately an enlarged spleen, alleviate headaches and ulcers, and are stated to have analgesic and antimicrobial activity. The leaves are reported

to contain flavones and their glycosides, in cluding baicalein and scutellarein. Leaves also contain anthraquinone and aloe-emodin.[15]

Oroxylum indicum exhibits a diverse range of pharmacological activities validated by both *in vitro* and *in vivo* research.[16] The world is increasingly moving towards plant products that are supported by ethnotraditional therapeutic use, as they may be measured as quite fewer side effects compared to many currently available synthetic medicines." [17]

Researchers evaluated the antidiabetic properties of *Oroxylum indicum* in experimental animal models.[18] They specifically studied the hypoglycemic activity of root, leaf, and stem bark extracts in Wistar albino rats with alloxan-induced diabetes.[19]

REVIEW OF LITERATURE

Diabetes Mellitus: An Overview

Diabetes mellitus is a chronic and progressive metabolic disorder characterized by sustained hyperglycemia, or elevated blood glucose levels, due to either insufficient insulin production, impaired insulin action, or a mixture of both.[20] This condition carries a heightened risk of early death and is linked to multiple health complications, notably affecting the cardiovascular system, neuropathy, nephropathy, and retinopathy.[21] Recent studies have also established a link between diabetes and certain types of cancer. Proactive management and prevention strategies are critical to reducing the risk of complications and improving quality of life.[22]

Insulin: The Central Hormone in Glucose Regulation

Insulin is a peptide hormone synthesized and secreted by the beta cells located in the islets of Langerhans in the pancreas.[23] It plays a central role in regulating blood glucose levels by promoting the uptake of glucose into adipose tissue, muscle, and liver cells for energy metabolism.[24] In the liver, insulin stimulates glycogenesis (the formation of glycogen) and inhibits gluconeogenesis (the production of glucose from non-carbohydrate sources). Additionally, it modulates lipid and protein metabolism, making it essential for maintaining metabolic homeostasis.[25]

Types of Diabetes

Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes is an autoimmune disorder in which the immune system mistakenly targets and destroys the insulin-producing beta cells in the pancreas. This leads to a complete lack of insulin in the body. While it commonly appears in childhood or teenage years, it can arise at any stage of life. Managing this condition necessitates continuous insulin therapy to regulate

blood sugar levels effectively and prevent life-threatening complications such as diabetic ketoacidosis.

Type 2 Diabetes Mellitus (T2DM)

Type 2 diabetes is the most prevalent form of diabetes, accounting for nearly 90% of all cases. It is primarily characterized by insulin resistance—where the body's cells do not respond effectively to insulin—and/or a relative insulin deficiency. The pancreas may still produce insulin, but not in adequate amounts to meet metabolic demands. Risk factors include obesity, sedentary lifestyle, aging, genetic predisposition, and family history. Although it typically occurs in adults, an increasing number of children and adolescents are being diagnosed due to rising obesity rates.

Gestational Diabetes Mellitus (GDM)

Gestational diabetes is a transient form of diabetes that develops during pregnancy, typically in the second or third trimester. It results from hormonal changes that cause insulin resistance. Although glucose levels often return to normal postpartum, women with a history of GDM are at increased risk of developing type 2 diabetes later in life. In some instances, hyperglycemia detected during pregnancy may be undiagnosed type 2 diabetes.[26]

Prediabetes

Prediabetes is a metabolic state characterized by blood glucose concentrations that exceed the established norm yet remain below the diagnostic threshold for diabetes mellitus. This intermediate hyperglycemic condition reflects underlying insulin resistance and/or impaired pancreatic beta-cell function. Individuals diagnosed with prediabetes exhibit a significantly increased likelihood of developing type 2 diabetes mellitus (T2DM), particularly in the presence of additional risk factors such as obesity, sedentary lifestyle, and genetic predisposition. Early identification and intervention through lifestyle modification or

pharmacologic therapy are critical to delaying or preventing the progression to overt diabetes and its associated complications.[27]

Other Forms of Diabetes

Other, fewer communal forms of diabetes include:

- **Monogenic diabetes**: Resulting from a mutation in a single gene, such as maturity-onset diabetes of the young (MODY).
- **Secondary diabetes**: Arising from medical conditions (e.g., pancreatitis, cystic fibrosis) or as a consequence of medications (e.g., glucocorticoids, antipsychotics).
- **Post-pancreatectomy diabetes**: Occurs following surgical removal of the pancreas, leading to insulin deficiency.[28]

Pathophysiology of Hyperglycemia

Regardless of the type, uncontrolled diabetes results in elevated blood glucose levels—**hyperglycemia**—which exerts toxic effects on multiple organ systems. Prolonged hyperglycemia leads to **oxidative stress**, **chronic inflammation**, and **endothelial dysfunction**, contributing to microvascular (small vessel) and macrovascular (large vessel) complications. The organs most susceptible include the kidneys (nephropathy), eyes (retinopathy), nerves (neuropathy), and the cardiovascular system.[29]

Table 1: Summary Classification of Diabetes Types

Type	**Etiology**	**Usual Onset**	**Management Strategy**
Type 1 Diabetes	Autoimmune destruction of beta cells	Childhood or adolescence	Insulin therapy, lifestyle, and education

Type 2 Diabetes	Insulin resistance ± beta cell dysfunction	Adulthood (increasing in youth)	Lifestyle, oral hypoglycemics, insulin
Gestational Diabetes	Hormonal changes causing insulin resistance	Pregnancy	Diet, exercise, insulin if necessary
Monogenic/Secondary	Genetic mutation or secondary causes	Variable	Depends on the underlying cause

[30]

Signs and Symptoms of Diabetes[31]

The clinical manifestations of diabetes can vary but commonly include:

- Polydipsia (excessive thirst)
- Polyuria (frequent urination)
- Fatigue and generalized weakness
- Polyphagia (increased hunger)
- Blurred vision
- Slow wound healing and frequent skin infections
- Unintended weight changes
- Mood disturbances
- Numbness or tingling in extremities (peripheral neuropathy)
- Dizziness and headaches
- Muscle cramps
- Increased incidence of infections (e.g., urinary tract, oral candida)[32]

Diagnostic Criteria for Diabetes

Diagnosis of diabetes is based on measuring plasma glucose concentrations using one or more of the following validated tests:

1. Glycated Hemoglobin (A1C) Test

- **Measures**: Average blood glucose over the past 2–3 months.
- **Fasting**: Not required.
- **Interpretation**:
    - Normal: <5.7%
    - Prediabetes: 5.7–6.4%
    - Diabetes: ≥6.5% (confirmed with a second test)[33]

2. Fasting Plasma Glucose (FPG) Test

- **Measures**: Glucose levels after an overnight fast (minimum 8 hours).
- **Interpretation**:
    - Normal: <100 mg/dL
    - Prediabetes: 100–125 mg/dL
    - Diabetes: ≥126 mg/dL on two separate occasions[34]

3. Oral Glucose Tolerance Test (OGTT)

- **Procedure**: Blood glucose is tested before and two hours after ingesting a glucose-rich beverage.
- **Interpretation**:
    - Normal: <140 mg/dL after 2 hours
    - Prediabetes: 140–199 mg/dL
    - Diabetes: ≥200 mg/dL after 2 hours[35]

4. Random Plasma Glucose Test

- **Indication**: Used when symptoms are present.
- **Interpretation**:

- Diabetes: ≥200 mg/dL with classic hyperglycemic symptoms[36]

Complications of Diabetes

Diabetes can lead to a wide range of complications that can be life-threatening or significantly impair quality of life. These are generally categorized into **microvascular** and **macrovascular** complications.[37]

1. Microvascular Complications

These arise due to damage to small blood vessels and include:

- **Diabetic Retinopathy**: Damage to the retina leading to vision impairment and blindness.
- **Diabetic Nephropathy**: Kidney damage that may progress to chronic kidney disease or end-stage renal failure.
- **Diabetic Neuropathy**: Nerve damage resulting in pain, numbness, or weakness, particularly in the extremities.
- **Diabetic Foot**: Ulcers and infections due to neuropathy and poor circulation, potentially leading to amputations.[38]

2. Macrovascular Complications

These involve large blood vessels and significantly increase cardiovascular risk:

- **Cardiovascular Disease (CVD)**: Includes coronary artery disease, myocardial infarction, and heart failure.
- **Stroke**: Ischemic or hemorrhagic events caused by cerebrovascular compromise.
- **Peripheral Artery Disease (PAD)**: Reduced blood flow to limbs, often resulting in pain and mobility issues.[39]

3. Additional Complications

Other diabetes-related health issues include:

- **Oral health problems**: Gum disease, dental infections.

- **Skin conditions**: Bacterial or fungal infections.
- **Sexual dysfunction**: Erectile dysfunction, reduced libido.
- **Bladder dysfunction**: Urinary retention or incontinence.
- **Bone and joint disorders**: Osteoporosis, limited joint mobility.
- **Mental health disorders**: Depression, anxiety, diabetes-related distress.
- **Impaired immunity**: Increased susceptibility to infections.[40]

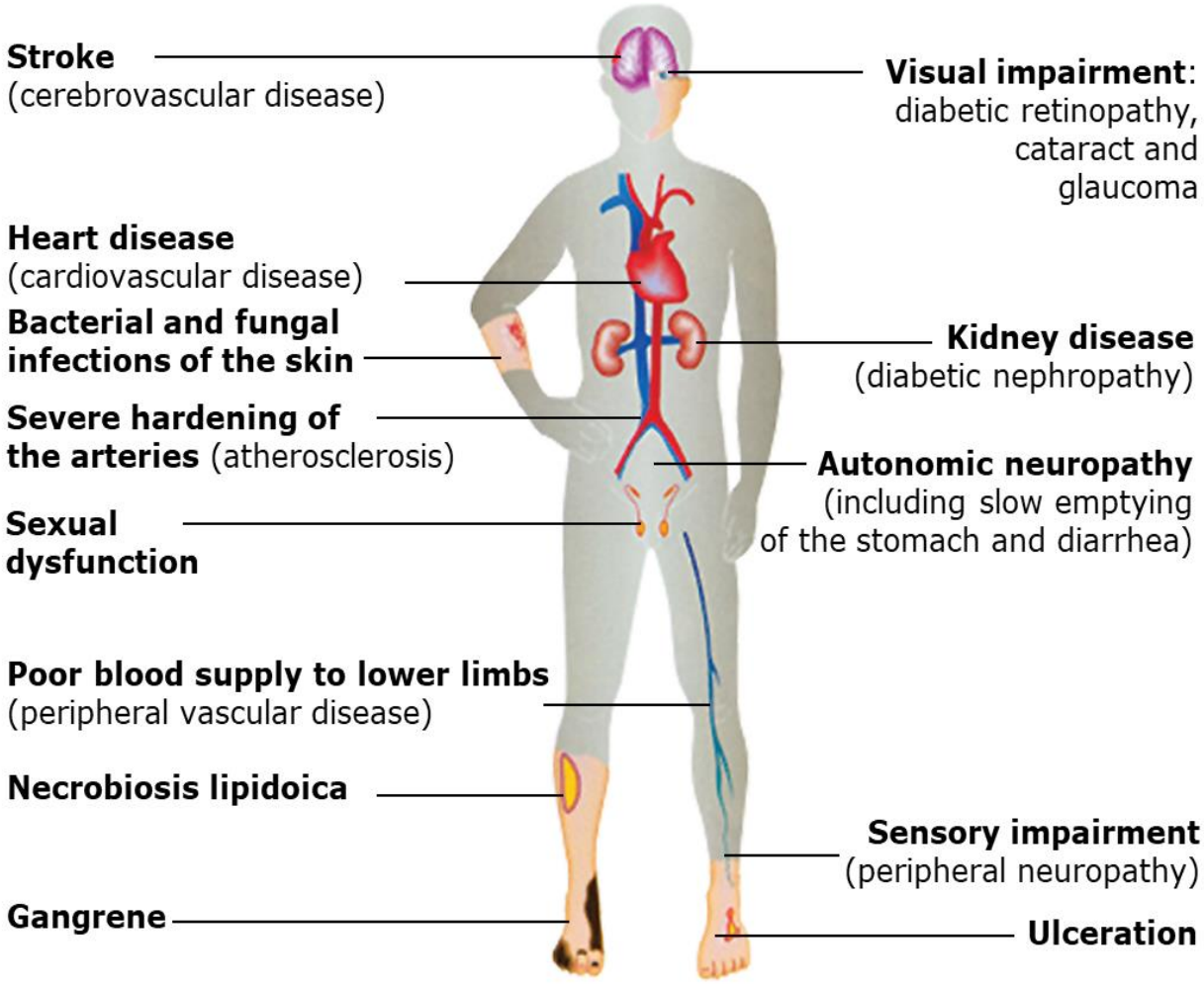


Image 1: Diabetes complications[41]

Diabetes and Medicinal Plants

The WHO has enumerated 21,000 plants, of which medicinal applications are present worldwide. As an alternative to synthetic agents, numerous plants with hypoglycemic activity have been recognized worldwide.[42]

Due to their demonstrated effectiveness, favourable safety profiles observed in clinical settings, and relative affordability, medicinal plants have long been integrated into various traditional medicine systems worldwide for managing conditions such as diabetes. Numerous plant species contain bioactive compounds that have shown potential in modulating blood glucose levels and alleviating diabetes-related complications. As such, medicinal plants present a promising adjunctive strategy in diabetes management. Incorporating these natural therapeutic agents—alongside standard medical treatments and healthy lifestyle interventions—may support a more comprehensive and holistic approach to diabetes care.[43]

Ethnobotanical research has identified nearly 1,000 plant species with reported antidiabetic properties. Multiple medicinal plants have exhibited antidiabetic efficacy through diverse mechanisms, including regulation of glycemia, enhancement of insulin secretion, and mitigation of oxidative stress. Both in vitro and in vivo studies are actively investigating plant-derived compounds for their ability to lower blood glucose, stimulate insulin release, improve peripheral glucose utilization, and preserve pancreatic β-cell function.[44]

These plants contain terpenoids, coumarins, flavonoids, phenolic compounds, and other compounds that have antihyperglycemic, antioxidant, and insulin-sensitizing properties.[45]

The following table shows the well-supported antidiabetic activity of plants.

Table 2: Medicinal plants used in diabetes

No.	Vernacular name	Part used	Active constituent
1	Neem (*Azadirachta indica*; Meliaceae)	Leaves, flowers & seeds	Azadirachtin and nimbin
2	Bitter gourd or Bitter melon (*Momordica charantia*; Cucurbitaceae)	Fruit pulp, seed, leaves, and all parts	Momordicin 1&2, cucurbitacin B
3	Garlic (*Allium sativum*; Alliaceae)	Bulbs	Allicin,allin
4	Onion (*Allium cepa*; Alliaceae)	bulb	Sulfenic acid, S-methyl cysteine sulphoxide
5	Babhul (*Acacia arabica*; leguminosae)	Leaves, seeds, bark, gum	Arabic acid
6	Holy basil (*Ocimum sanctum*; labiatae)	Whole plant	Eugenol, carvecrol
7	Methi/Fenugreek (*Trigonella foenum graecum*; Fabaceae)	Leaves, seeds	Lecithin, Vitexin, trigonilline,
8	Mammejo (*Enicostemma axillare*; Gentianaceae)	Whole plant	Swertiamarin
9	Gurmar or madhunasini (*Gymnema Sylvestre*, Apocynaceae)	Leaves	Gymnemic acid
10	Indian Blackberry or Jamun (*Syzygium cumini*, Myrtaceae)	Fruits, Seeds	Polyphenolic & flavonoids

11	Indian Kino Tree or biyo (Pterocarpus marsupium)	Wood	Catechin
12	Amla – (*Emblica Officinalis*, Phyllanthaceae)	Fruits	Tannins & Vitamin C
13	Bael (*Aegle marmelos*, Rutaceae)	Fruits	Dietary fibres & anti-oxidants
14	Tinospora, Galo, Guduchi (*Tinospora cordifolia*, Menispermaceae)	Stem	Terpenoids,
15	Guar gum (*Cyamopsis tetragonoloba*, Leguminosae)	Stem	Gums & derived products
16	Curry tree (*Murraya koenigii,* Rutaceae)	Leaf	Inorganic elements
17	Cinnamon (*Cinnamomum zeylenica*, Lauraceae)	Bark	Volatile oil
18	Aloe (*Aloe vera*, Liliaceae)	Leaf	Anthraquinones

[46]

Recent systematic reviews and meta-analyses indicate that most herbal formulations are generally well-tolerated and may function as beneficial adjuncts to standard diabetes management. However, further high-quality, long-term clinical trials are necessary to more conclusively determine their efficacy and safety profiles in human populations.[47]

Oroxylum indicum Plant

Oroxylum indicum, belonging to the family **Bignoniaceae**, is known by various botanical synonyms, including *Oroxylum flavum* Rehder, *Arthrophyllum reticulatum* Blume ex Miq., *Bignonia lugubris* Salisb., *Spathodea indica* (L). Pers., *Bignonia indica* L., *Bignonia pentandra* Lour., *Bignonia quadripinnata* Blanco, *Bignonia tuberculata* Roxb. Ex DC., *Calosanthes indica* (L.) Blume, *Hippoxylon indica* (L.) Raf., and *Bignonia tripinnata* Noronha.

Frequently denoted as the "Tree of Damocles" in English and "Sonapatha" or "Shyonak" in Hindi, *Oroxylum indicum* is a semi-deciduous tree categorized by sparse branching and can grow up to 18–20 meters in height.[48] The trunk measures approximately 40 cm in diameter. Its stem bark is pale brown to greyish-brown, soft, and spongy, highlighting abundant corky lenticels. The inner bark is particularly golden-yellow, which is the beginning of its vernacular name "Sonapatha" (sona meaning gold).[49]

Table 3: Synonyms of *Oroxylum indicum*

Sr. No.	Language	Common Name
1	English	Midnight horror, Indian trumpet flower, broken bones plant, Tree of Damocles, Indian calosanthes, Indian caper, Indian trumpet
2	Hindi	Shyonak, Sonapatha, Patrorna, Shallaka, Putivriksha, Shuran, Sauna, Son, Aralu, Vatuk, Urru, Bhut-Vriksha, Dirghavrinta, Kutannat
3	Sanskrit	Shyonaka, Nat, Katvanga, Sukanasa, Prithu Simba, Tuntuka, Dirghavrinta, Mandukparana, Kutannata, Patroma, Katammar, Bhalluka, Mayurjangha
4	Gujarati	Tentu
5	Kannad	Ane-mungu Patagani, Bunepaale, Sonepatta, Tigadu, Alangi, and Tattuna

6	Telugu	Dundilamu, Manduka-paramu, Chettu, Nemali, Pampena, Pampini
7	Bengali	Krong-Sa-Bang (Marma Tribe), Naori Chilana (Chakma Tribe), Kaak-Rakung (Halam Tribe), Tou-Kharung Tripura Tribe), Kanai Dingi (Garo Tribe)
8	Tamil	Achi, Arandei, Cori-Konnai, Venga maram, Palai-yutaicci, Pana, Puta-Puspam (the flower), Pei-maram, Peiarlanke, Palagaipayani, Peruvaagai
9	Marathi	Tetu, Tayitu
10	Assamese	Bhatghila, Dingdinga, or Toguna

[15, 50]

Taxonomical Classification

Kingdom : Plantae

Division : Angiosperms

Class : Eudicots

Sub-class : Asterids

Order : Lamiales

Family : Bignoniaceae

Genus : Oroxylum

Species : *O. indicum*

Terrestrial Spreading

Bignonia indica (Sonapatha) is widely distributed across the Indian subcontinent, excluding arid regions. It predominantly occurs in the foothills of the Himalayas at elevations ranging from 1000 to 1200 meters, in areas of Bhutan and Nepal. In India, it is commonly found in the Western and Eastern Ghats as well as the Northeastern states. Beyond the Indian subcontinent,

it is grown in various provinces of China, including Guangxi, Yunnan, Sichuan, Fujian, Guangdong, and Guizhou. Its distribution also extends to several Southeast and East Asian countries such as Taiwan, the Philippines, Vietnam, Cambodia, Indonesia (Java and Sumatra), Laos, Thailand, Myanmar, Malaysia, and Sri Lanka.[51]

Botanical Description

Sonapatha is a small, deciduous tree typically reaching heights between 8 and 15 meters, characterized by branching near the upper canopy. The bark is light brown and soft, exuding a green sap and often marked by numerous corky lenticels. Leaves are arranged oppositely and measure 3–7 cm in length, exhibiting 2–3 pinnate divisions; the pinnae are robustly attached, with a cylindrical rachis. Each leaf comprises 2–4 pairs of leaflets, which are 6–12 cm long and 4–10 cm wide, ovate to elliptic, acuminate, and glabrous. The leaflet base can be rounded, sometimes cordate with a wedge-shaped (cuneate) indentation, and scattered glands are frequently observed on the underside; lateral petioles measure 6–15 mm. [50] The inflorescence is a terminal, erect raceme, ranging from 25 up to 150 cm in length, with a branched peduncular rachis. Flowers are bisexual, numerous, and malodorous, arranged in sizable, erect clusters, with pedicels varying from 6 to 30 mm in length. The calyx is leathery, oblong-campanulate, glabrous, and measures approximately 4 cm. The corolla, fleshy and up to 10 cm in length, has lobes about 4 cm long, with crisped and subequal margins, displaying a reddish hue externally and a yellow to pink tone internally.

There are five stamens, slightly protruding beyond the corolla tube; one is distinctly shorter. The filaments are cottony at their base, and all stamens are inserted at the throat of the flower. The ovary is superior, bicellular, and contains numerous ovules. Fruit is a capsule, straight, measuring 0.3–0.6 meters long and 5–9 cm wide, tapering at both ends, flat, about 8 mm thick, and semi-woody in texture. Seeds are numerous, winged around the entire margin except at the

base, and typically measure about 6 cm. Floral buds are 2–4 cm in length, 1.5–2 cm in diameter, brown to violet in color, water-containing in early stages, and becoming woody upon fruit maturation. [52, 53]

Image 2: *Oroxylum indicum* plant

Image 3: Leaf part of *Oroxylum indicum*

Image 4: Flower part of *Oroxylum indicum*

Ethnomedicinal uses

The curative uses of different parts of *Oroxylum indicum* (Sonapatha) are integrated into both classical literature and current research.

Root bark

The root bark of *Oroxylum indicum* is recognized for its acrid, bitter, and astringent qualities, and traditionally classified as cooling, aphrodisiac, and tonic. [54] It is used in Ayurveda to stimulate appetite and address gastrointestinal disturbances, including conditions associated with "vata" and biliousness. Clinical uses encompass fevers, bronchitis, intestinal parasites, dysentery, vomiting, leucoderma, asthma, inflammatory disorders, as well as proctological issues. Root bark-based formulations are notably prescribed for diarrhoea and rheumatism. The combination of powdered root bark and sesame oil is administered as a digestive tonic. Pharmacological studies further validate its carminative, stomachic, tonic, diaphoretic,

and astringent actions, supporting its utility in digestive complaints, cough, diarrhoea, and as part of nootropic (cognitive enhancing) preparations[55]

Root

Oroxylum indicum roots are characterized by sweet, bitter, and astringent properties, alongside cooling and anti-inflammatory effects. Beyond functioning as an astringent and refrigerant, the root exhibits expectorant, appetizing, digestive, anthelmintic, and diuretic actions. Traditional indications include management of dropsy, cough, neuralgia, asthma, bronchitis, anorexia, various digestive complaints (such as dyspepsia, flatulence, colic, diarrhea, dysentery), gout, vomiting, leukoderma, wounds, rheumatoid arthritis, and fever. The tonic derived from the root is employed for its antiarthritic, antidiabetic, and febrifuge effects. In both traditional and modern contexts, root decoctions are routinely utilized for diarrheal disorders and as adjuvants in the treatment of rheumatologic and bronchopulmonary diseases.[54]

Bark

The bark is esteemed for its diuretic and stomachic properties. It is mainly used to address diarrhoea and dysentery and, when applied as a paste, is traditionally used for wound healing, non-healing ulcers, and certain skin conditions such as scabies and oral lesions. Reports also indicate its antiseptic and astringent benefits, with additional applications in gynecological and respiratory disorders

Leaves

Oroxylum indicum leaves are prescribed specifically for envenomation (notably snakebite) in traditional Indian medicine. Externally, leaves are applied to treat an enlarged spleen, ulcers, headaches, and as analgesics and antimicrobials. Internal administration includes their use as stomachic and carminative agents for flatulence. Decoctions of the leaves are utilized in the treatment of rheumatic pain, enlarged spleen, ulcers, cough, and bronchitis. Phytochemical

evaluations highlight their content of anthraquinones and emollient agents, supporting these traditional uses. [56]

Seeds

Seeds exert purgative effects and are taken orally for throat infections and hypertension. A paste derived from seeds is topically used for boils and wounds; the seed powder is sometimes used by women to induce conception. Seeds yield a non-drying oil utilized in the perfume industry. Additionally, a mixture of ground seeds with fire soot is applied locally for prompt relief of tonsillar pain. Some accounts also report antimicrobial, laxative, and conception-promoting properties.[57]

Fruits

Fruits are described as acrid, sweet, stomachic, and anthelmintic; traditionally, they are administered for diseases of the throat and heart, piles, bronchitis, cough, jaundice, smallpox, and gastrointestinal complaints including dyspepsia and diarrhea. Both immature and mature fruits are used, with claims for improvement of appetite and as expectorants and agents useful in leukoderma.[58]

Ayurvedic and Classical Formulations:

Oroxylum indicum, under the name Bignonia indica or Shyonaka, is a vital constituent of numerous Ayurvedic formulations, including Dasamula, Amritarista, Dantyadyarista, Narayana Taila, Dhanvantara Ghrita, Brahma Rasayana, and Chyavanaprasha Awaleha. Its inclusion in both classical preparations and modern proprietary nootropic medicines such as Mentat underlines its enduring role in herbal therapeutics. [59]

Summary of Core Activities

- **Root bark and root:** Digestive, carminative, tonic, anti-inflammatory, antimicrobial, antiarthritic, and nootropic uses.
- **Bark:** Diuretic, stomachic, antiseptic, and topical for wounds/ulcers.
- **Leaves:** External applications for spleen, ulcers, headache; internal for digestive and rheumatic ailments.
- **Seeds:** Purgative, antimicrobial, and conception aid; topical applications for skin and throat.
- **Fruits:** Anthelmintic, expectorant, stomachic, used in cardiovascular, dermatologic, and gastrointestinal disorders.

These multifaceted traditional applications are largely corroborated by recent pharmacological studies, though further clinical investigation is required to define standardized dosages and safety profiles across human populations

Chemical constituents

Roots

The roots and root bark of *Oroxylum indicum* are characterized by a notable concentration of flavones, particularly **oroxylin A** (5,7-dihydroxy-6-methoxyflavone), **baicalein** (5,6,7-trihydroxyflavone), and **chrysin** (5,7-dihydroxyflavone). Additional constituents identified in several analyses include **pterocarpan**, **rhodioside**, p-hydroxyphenylethanols, and various cyclohexanols, while the root bark, especially is reported to contain dihydrobaicalein and to yield minor amounts of alkaloids, tannic acid, sitosterol, and galactose.[60]

Root bark

Bark of the root is reported to contain chrysin, baicalein hhand oroxylin-A. Bark also gave dihydrobaicalein. Heartwood yielded beta-sitosterol and an isoflavone, prunetin. The bark also contains traces of an alkaloid, tannic acid, sitosterol and galactose.[56]

Stem

Oroxylin A, chrysin, scutellarin, baicalein, baicalein-6-*O*-glucuronide, dihydrobaicalein, ellagic acid, dihydrooroxylin-A, methyl-3,4,5-trihydroxy-6-(5-hydroxy-6-methoxy-4-oxo- 2-phenyl-chroman-7-yloxy)-tetrahydro-2H-pyran-2-carboxylate, 5-hydroxyl-7-methoxy-2- (2-methoxy-6-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yoloxy)phenyl)- 4H-chromen-4-one, or-oxyloside, hispidulin, apigenin, ficusal, balanophonin, 2-(1-hydroxymethylethyl)-4H, 9H-naphtho [2,3-β]furan-4,9-dione, salicylic acid, ρ - hydroxybenzoic acid, protocatechuic acid, isovanillin, β-hydroxypropiovanillon, oroxylin, pinostrobin, stigmast-7-en-3-ol, and 3,4′,5,7 tetra-hydroxy flavonol (kaempferol), dehydro-iso-α-lapachone, and lapachol were isolated from the stem of Sonapatha. Additionally, 5,6,7-trimethoxy flavone-8-*O*-β-D-glucopyranoside, oroxylin A-7-*O*-β-D-glucuronide butylester, 6-methoxy-baicalein, oroxylin-A-7-*O*-glucoside, 5,7-dihydroxy–flavone, baicalein, and 6-methoxy-7-glucuronide have also been separated from the stem of Sonapatha.[61]

Stem Bark

Stem bark contains flavones oroxylin A (5,7-dihydroxy-6-methoxy flavone), chrysin, baicalein, and its 6 and 7-glucuronide, scutellarin-7-rutinoside, traces of alkaloid, tannic acid, sitosterol, and galactose, baicalein, biochanin-A, ellagic acid. Ethyl acetate extract of the root of *O. indicum* is reported to contain two flavonoids: i) 2,5-dihydroxy-6,7-dimethoxy flavone and ii) 3,7,3',5' tetramethoxy-2-hydroxy flavone.[62, 63]

Leaves

Leaves are known to contain flavones and their glycosides, baicalein (5,6,7-trihydroxy flavone) and its 6 and 7-glucuronides, chrysin (5,7-dihydroxy flavone), scutellarein and its 7-glucuronides, anthraquinone and aloe-emodin, chrysin-7-O-glucuronide, chrysin-diglucoside, and irridoids. From the methanol extract of the leaves of *O. indicum*, chrysin-7-O-glucuronide, chrysin diglucoside, and baicalein were separated.[16, 64]

Flowers and Fruits

Phytochemical studies confirm the presence of **baicalein** and **chrysin** in the flowers. Fruits are reported to contain **oroxylin A**, **orlumin A**, **chrysin**, and **ursolic acid**, marking a distinct profile compared to vegetative tissues.[65]

Seeds

The seeds of *Oroxylum indicum* demonstrate a particularly diverse flavonoid profile, including **chrysin**, **baicalein**, **baicalein-7-O-glucoside**, **baicalein-7-O-diglucoside** (oroxylin B), with some studies also observing an unknown flavonoid. Beyond flavonoids, the seeds yield a non-drying oil (~20% yield) and various other compounds: **oroxylin A**, **scutellarin**, **baicalein-6-glucoside** (tetuin), **oroxin A and B**, **quercetin**, **apigenin**, **kaempferol**, quercetin-3-O-arabinoside, **lupeol**, **β-sitosterol**, **daucosterol**, **pinocembrin**, **adenosine**, galactose, and several triterpenoids. Cardiac glycosides, steroidal saponins, and anthraquinone derivatives have also been isolated from the seeds in some analyses.[66]

Table 4: Summary of Major Phytoconstituents by Plant Parts

Plant Part	Major Phytoconstituents
Roots and Root Bark	Oroxylin A, baicalein, chrysin, pterocarpan, rhodioside, dihydrobaicalein, alkaloids, tannic acid, sitosterol, galactose
Heartwood	Beta-sitosterol, prunetin, alkaloids

Stem/Stem Bark	Oroxylin A, chrysin, baicalein, scutellarin, baicalein-glucuronides, ellagic acid, biochanin-A, isovanillin, tannic acid
Leaves	Baicalein, chrysin, scutellarein, anthraquinones, aloe-emodin, glycosides, irridoids
Flowers	Baicalein, chrysin
Fruits	Oroxylin A, orlumin A, chrysin, ursolic acid
Seeds	Chrysin, baicalein, oroxin A/B, baicalein-glucosides, quercetin, apigenin, kaempferol, lupeol, pinocembrin, sitosterol, cardiac glycosides, oils

Pharmacological Activities

Sonapatha has shown various pharmacological activities in different study systems in vitro and in vivo, as listed below.

1. **Free Radical Scavenging and Antioxidant Activities**

The antioxidant likely of *Bignonia indica* extracts has been extensively evaluated using multiple in vitro assays that measure free radical scavenging and reducing capabilities. Various solvent extracts of the stem bark demonstrate dose-dependent enhancement of antioxidant activity, with the chloroform extract exhibiting the highest capacity, then after alcohol extracts, however petroleum ether extracts show the lowest activity.

Methanolic extracts from different plant parts also effectively scavenge key free radicals such as hydroxyl, nitric oxide, superoxide anion, and DPPH radicals in vitro. Specifically, leaf extracts obtained through different solvents inhibit free radical formation in a concentration-dependent manner; among these, the ethanol extract consistently shows superior radical scavenging and reducing power.

Similarly, leaves extracted with ethyl acetate, methanol, and water display concentration-dependent DPPH radical scavenging, with ethyl acetate extracts being most potent, followed sequentially by methanol and aqueous extracts. Stem bark extracts obtained via hexane, chloroform, ethyl acetate, and water exhibit analogous concentration-dependent DPPH inhibition, where the ethyl acetate fraction demonstrates superior efficacy over the others.

Furthermore, Pet. ether, dichloromethane, and methanol extracts of *B. indica* significantly scavenge DPPH and superoxide radicals, with the methanol extract revealing the highest potency. Ethanol, chloroform, and aqueous stem bark extracts inhibit multiple free radical species—including DPPH, superoxide anion, hydroxyl, and nitric oxide radicals—in a concentration-dependent fashion. Notably, maximal DPPH radical scavenging occurs at 200 µg/mL for both chloroform and ethanol extracts, hydroxyl radical inhibition peaks at 6000 and 8000 µg/mL respectively, while superoxide radical scavenging reaches its maximum at 2500 µg/mL for these extracts. Nitric oxide radical scavenging requires substantially higher concentrations, up to 20,000 µg/mL, to achieve maximal inhibition.

The ferric reducing antioxidant power (FRAP) of stem bark extracts also increases in a dose-dependent manner with extract concentration, with 2500 µg/mL yielding maximal ferric ion reduction across chloroform, ethanol, and aqueous fractions. Regarding seeds and fruits, ethanol and aqueous seed extracts exhibit inhibitory activity against DPPH radicals, although fruit extracts are comparatively less effective in such antioxidant assays.

Collectively, these findings substantiate that Oroxylum indicum harbors a rich pool of bioactive phytochemicals such as flavonoids (e.g., baicalein, chrysin, oroxylin A), phenolics, and other secondary metabolites that contribute to its robust antioxidant capacity. The variability in antioxidant potency across solvents correlates with differential solubility and extraction efficiency of these compounds. These results provide a scientific basis supporting the

traditional use of *O. indicum* in conditions associated with oxidative stress, and highlight the potential for developing standardized antioxidant formulations from specific plant extracts.[67]

2. Anti-inflammatory and analgesic activity

Oroxylum indicum exhibits significant anti-inflammatory properties, demonstrated through multiple experimental models. In biochemical assays, various extracts of the plant inhibited key inflammatory enzymes cyclooxygenase (COX) and lipoxygenase (LOX). Notably, the dichloromethane extract of the root at 50 µg/mL achieved complete (100%) inhibition of leukocyte LOX activity, reflecting strong potential to modulate inflammation-mediated pathways involved in leukotriene synthesis.

Cell culture studies using macrophage lines reported that *O. indicum* extracts suppress pro-inflammatory cytokine production induced by lipopolysaccharide (LPS). Specifically, extracts inhibited the secretion of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), indicating their capacity to attenuate inflammatory signaling cascades triggered by endotoxins.

In vivo, anti-inflammatory efficacy was validated using established rodent models of inflammation. The aqueous leaf extract demonstrated dose-dependent reduction of carrageenan-induced rat paw edema, with a 300 mg/kg oral dose being more effective than 150 mg/kg. Similarly, decoctions from root and stem bark reduced paw edema in rats, corroborating traditional uses in inflammatory conditions.

Further, oral administration of aqueous root extract in doses of 100, 200, and 400 mg/kg over four to seven days significantly attenuated colitis induced by dinitrobenzene sulfonic acid, illustrating broader anti-inflammatory effects in gastrointestinal inflammation models.

Analgesic activity parallel to anti-inflammatory effects was observed: 500 mg/kg of aqueous extract diminished acetic acid-induced writhing in mice comparably to 50 mg/kg aminopyrine, a standard analgesic.

Ethanol extracts of *O. indicum* also reduced both mouse ear and paw edema at doses of 250 and 300 mg/kg, with the higher dose yielding maximum anti-inflammatory and analgesic effects.

Hydroalcoholic and methanolic stem bark extracts inhibited carrageenan-induced paw edema in a dose-dependent fashion (100–400 mg/kg), with histological examinations confirming reduced cellular infiltration and attenuated inflammation in treated rats.

These findings converge to demonstrate that *Oroxylum indicum* possesses potent anti-inflammatory and analgesic activities mediated through inhibition of pro-inflammatory enzymes (COX, LOX), suppression of cytokines in immune cells, and attenuation of edema and inflammatory cell infiltration in vivo. The dose-dependent efficacy and convergence across biochemical, cellular, and animal models validate its traditional therapeutic applications and encourage further development toward standardized anti-inflammatory agents.[68, 69]

3. Anti-Allergic and Antiasthmatic Effect

Allergic rhinitis and asthma, closely linked respiratory disorders, affect approximately 40% of the global population, adversely impacting quality of life and work performance, with prevalence rising worldwide. Research on the antiallergic and antiasthmatic effects of *Oroxylum indicum* is relatively limited but promising.

Oroxylin A, a flavone constituent isolated from *Oroxylum indicum* (Sonapatha), has been investigated for its antiallergic and antiasthmatic efficacy in vitro and in vivo. In vitro studies using rat RBL-2H3 mast cells sensitized with monoclonal anti-dinitrophenyl (DNP)-specific mouse IgE demonstrated that oroxylin A inhibited antigen-induced degranulation in a dose-dependent manner, as evidenced by a reduction in the release of β-hexosaminidase. The maximal inhibitory effect was observed at 30 μM concentration.

In vivo, female Balb/c mice treated with intraperitoneal injections of oroxylin A (1 and 5 mg/kg body weight) prior to ovalbumin (OVA) challenge exhibited significant attenuation of allergic asthma symptoms. Specifically, oroxylin A administration reduced eosinophil accumulation in bronchoalveolar lavage fluid by 51% and 84% at these doses, respectively. Histopathological examination of lung tissue revealed diminished peribronchiolar eosinophilic infiltration correlating with oroxylin A dose. Furthermore, it alleviated OVA-induced mucin overproduction in the lungs, a hallmark of airway hypersecretion in asthma.

At the molecular level, oroxylin A suppressed the expression of key Th2 cytokines—interleukin (IL)-4, IL-5, and IL-13—as well as interferon-gamma (IFN-γ) and IL-2 in lung tissue, suggesting modulation of the immune response critical to allergic inflammation. These combined effects underpin oroxylin A's potential as a therapeutic agent for allergic asthma and related hypersensitivity conditions

4. Antimicrobial Activity

The antimicrobial properties of *Oroxylum indicum* have been documented in various solvent extracts targeting a wide spectrum of microbial pathogens.

The dichloromethane extract of Sonapatha exhibits broad-spectrum antibacterial activity, inhibiting both Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative species including *Escherichia coli* and *Pseudomonas aeruginosa*. It also demonstrates antifungal activity against *Candida albicans*, dermatophytes, and wood root fungi.Alcoholic extracts of the root and stem suppress the growth of bacterial pathogens like *Klebsiella*, *Escherichia coli*, *Proteus*, *Pseudomonas*, and *Staphylococcus aureus*, with the stem extract showing superior efficacy over root extract.[70]

Methanol extracts of the stem bark are active against a wide range of Gram-positive bacteria (*Bacillus cereus*, *Bacillus megaterium*, *Sarcina lutea*) and Gram-negative bacteria (*Salmonella*

paratyphi, *Salmonella typhi*, *Shigella boydii*, *Vibrio mimicus*). Additionally, antifungal activities have been reported against *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus niger*.

These findings illustrate the broad antimicrobial spectrum of *Oroxylum indicum* and support its traditional use in managing infections and related ailments. [57].

5. Anthelmintic Effect

The methanolic extract of *Oroxylum indicum* stem bark has been extensively evaluated for its anthelmintic properties against the cestode *Hymenolepis diminuta* through both in vitro and in vivo models. Juvenile and adult *H. diminuta* worms were exposed to 10, 20, and 30 mg/mL concentrations of the stem bark extract. Notably, exposure to 30 mg/mL resulted in mortality of juvenile worms as rapidly as 0.25 ± 0.00 hours post-treatment, demonstrating a potent dose-dependent anthelmintic effect. Scanning electron microscopy (SEM) of the treated worms revealed significant morphological alterations, including wrinkling of the scolex, distortion of the integument, and erosion of the microtriches, indicating structural damage to the parasite's tegument affecting its viability.

In vivo experimentation using *H. diminuta*-infected Wistar rats showed that oral administration of 1000 mg/kg body weight of the *O. indicum* stem bark extract achieved a 79.3% reduction in eggs per gram (EPG) of feces and a 70.8% decrease in adult worm counts. These findings suggest enhanced efficacy of the extract against juvenile parasite stages relative to adult worms, aligning with the rapid in vitro mortality observations.

Parallel investigations have assessed the anthelmintic activities of methanolic stem bark extract and aqueous fruit extract of *Oroxylum indicum* against the earthworm *Pheretima posthuma*, a commonly used model organism for such studies. Concentrations ranging from 10 to 100

mg/mL induced paralysis and mortality of earthworms in a clear concentration-dependent manner, further supporting the extract's broad-spectrum anthelmintic potential.

Moreover, the anthelmintic efficacy of *Oroxylum indicum* has been compared favorably against ivermectin—a benchmark deworming drug—specifically targeting equine strongyle eggs in vitro. Results indicate that *O. indicum* extracts exhibit comparable inhibition, highlighting their promise as a botanical alternative or adjunct in managing helminth infections in veterinary contexts.

Collectively, these data underscore *Oroxylum indicum* stem bark and fruit extracts as potent anthelmintic agents acting through morphological disruption and motility impairment of parasitic helminths. The dose-dependent efficacy, rapid onset of mortality in juvenile stages, and in vivo fecal egg count reductions consolidate its traditional use and suggest scope for developing standardized phytotherapeutics for human and animal helminthiasis. [71]

6. Hepatoprotective Effect

The hepatoprotective potential of *Oroxylum indicum* extracts has been demonstrated in multiple models of liver toxicity. Petroleum ether, chloroform, ethanol, and aqueous extracts of the leaves have shown significant protection against carbon tetrachloride (CCl_4)-induced hepatic damage. This was evidenced by marked reductions in serum biomarkers such as aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin, with the ethanol extract exhibiting superior efficacy compared to other solvents. Similarly, methanolic stem bark extract administered at 500 mg/kg body weight effectively mitigated CCl_4-induced hepatic injury, reducing serum enzyme levels and histopathological damage, including necrosis.

Aqueous root bark extracts at doses of 100, 200, and 400 mg/kg also attenuated paracetamol-induced hepatotoxicity in Wistar rats in a dose-dependent manner, reflected biochemically by

decreased AST, ALT, and ALP levels and corroborated by improved liver histology. Among stem bark extracts, the ethyl acetate fraction was most potent in protecting against CCl_4-induced liver damage, surpassing petroleum ether, chloroform, ethanol, and aqueous extracts in reducing serum liver enzymes and pathological alterations.

Whole plant methanol-dichloromethane extracts demonstrated hepatoprotection both in vitro in rat liver explants and in vivo in chronic liver injury models by normalizing biochemical parameters and alleviating hepatic swelling and injury. In acetaminophen-induced hepatotoxicity, aqueous-methanol stem bark extracts conferred dose-dependent protection by lowering serum AST, ALT, and lipid peroxidation (LOO) levels.

Additionally, aqueous and ethanol extracts of Sonapatha mitigated hepatotoxic effects induced by the antitubercular drug AKT-4 in Wistar rats, normalizing liver enzymes (ALT, AST, LDH, total bilirubin) and elevating endogenous antioxidants such as catalase, superoxide dismutase (SOD), and glutathione (GSH). Histopathology confirmed prevention of drug-induced hepatocyte degeneration, fatty changes, and hemorrhage. The 70% ethanol stem bark extract reduced serum ALT, ALP, γ-glutamyltransferase (GGT), and bilirubin levels post CCl_4 exposure, affirming its hepatoprotective role.

Notably, seed extracts inhibited progression from nonalcoholic fatty liver disease to steatohepatitis in vitro and in vivo by modulating NF-κB signaling, decreasing its transcriptional activity and phosphorylation while increasing levels of its inhibitor IκB, thereby attenuating the inflammatory cascade implicated in hepatic injury.

Collectively, these findings validate the traditional use of *Oroxylum indicum* in liver disorders and its incorporation in diverse Ayurvedic formulations for hepatoprotection.

7. Gastroprotective Effect

Hydroalcoholic, petroleum ether, chloroform, ethyl acetate, and n-butanol extracts of the root bark at 300 mg/kg significantly reduced ethanol-induced gastric ulceration in rats, with petroleum ether, n-butanol, and chloroform fractions showing superior efficacy compared to hydroalcoholic and ethyl acetate extracts. These extracts also decreased lipid peroxidation and enhanced endogenous antioxidants (SOD, catalase, GSH). Hexane and acetone stem bark extracts exhibited mild to moderate protective effects in various gastric ulcer models, with isolated flavonoids such as chrysin displaying notable antiulcer activity. Butanol, petroleum ether, and ethanol stem bark extracts inhibited ethanol-induced gastric ulcers with inhibition indices above 86%, underscoring their gastroprotective potential. [72, 73]

8. Cardioprotective Effect

Sonapatha root bark methanol extract administered orally at 200 and 400 mg/kg protected Sprague Dawley rats from doxorubicin-induced cardiotoxicity. This was demonstrated by normalization of electrocardiogram parameters (ST-segment, QRS complex) and significant reduction of cardiac injury markers, including creatinine phosphokinase (CPK), AST, and lactate dehydrogenase (LDH). Antioxidant enzyme levels (GSH, glutathione peroxidase, SOD) increased while lipid peroxidation decreased, with histopathological analysis confirming attenuation of myocardial degeneration and necrosis.

9. Antidiabetic Effect

Oroxylum indicum extracts exhibit significant antidiabetic potential across diverse experimental models involving chemically induced diabetes in rodents and in vitro enzymatic and cellular assays. Oral administration of aqueous and ethanol extracts at doses of 300 and 500 mg/kg body weight for 21 days significantly lowered serum glucose levels in alloxan- and dexamethasone-induced diabetic Wistar rats, demonstrating robust glycemic control.

Methanolic extracts derived from the heartwood notably inhibited α-glucosidase (GAA) activity, an enzyme pivotal in the digestion of carbohydrates and glycoprotein biosynthesis that is typically upregulated in diabetic states. Correspondingly, 50% hydroalcoholic extracts of O. indicum demonstrated comparable α-glucosidase inhibition in vitro, positioning these extracts as promising modulators of postprandial hyperglycemia.

Further mechanistic insights are provided by studies on cultured 3T3-L1 mature adipocytes, where O. indicum extracts improved insulin sensitivity, suggesting enhancement of peripheral glucose uptake and utilization. In streptozotocin-induced diabetic rats, oral administration of 250 mg/kg 50% ethanol extract for 28 days significantly reduced fasting blood glucose, low-density lipoprotein (LDL), and glycosylated hemoglobin (HbA1c) levels, while elevating high-density lipoprotein (HDL). Insulin resistance indices—homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI)—were significantly improved, reflecting restoration of insulin responsiveness.

The seeds of O. indicum, extracted with 90% ethanol, inhibited rat intestinal α-glucosidase activity but showed only non-significant reductions in fasting glucose when administered alone (50 and 250 mg/kg) in alloxan-induced diabetic mice. However, co-administration with the α-glucosidase inhibitor acarbose markedly potentiated glucose-lowering effects. This synergistic phenomenon was replicated in streptozotocin-induced diabetic murine models with combined seed extract and acarbose administration.

In prediabetic mice, seed extract treatment enhanced antioxidative defenses by elevating glutathione (GSH), superoxide dismutase (SOD), and HDL levels, alongside reductions in lipid peroxidation (LOO) and LDL, indicating mitigation of oxidative stress associated with diabetes progression.

Supplementing these findings, administration of 300 mg/kg aqueous and methanol leaf extracts reduced key metabolic parameters—including glucose, total cholesterol, triglycerides, protein, urea, and creatinine—in alloxan-induced diabetic rats. Additionally, 50% ethanol seed extract at doses of 200 and 400 mg/kg for 21 days attenuated serum glucose, triglyceride, and cholesterol levels in glibenclamide-induced diabetic models.

Collectively, these studies substantiate the antidiabetic efficacy of Oroxylum indicum through multiple pathways: enzymatic inhibition of carbohydrate digestion, enhancement of insulin sensitivity, reduction of hyperglycemia and dyslipidemia, and antioxidative action mitigating oxidative stress. These pharmacodynamic attributes validate the plant's traditional use and support its potential development as an adjunct or alternative therapeutic option in diabetes management.[74]

10. Anti-obesity Effect

Sonapatha bark extracts in hexanes, dichloromethane, ethyl acetate, and methanol inhibited lipid accumulation and lipase activity dose-dependently in 3T3-L1 adipocytes, with ethyl acetate extract being most effective. Similarly, 95% ethanol fruit extracts suppressed lipid and carbohydrate accumulation and downregulated key adipogenic genes including fatty acid synthase (FAS), sterol regulatory element-binding protein 1c (SREBP-1c), peroxisome proliferator-activated receptor γ2 (PPARγ2), glucose transporter 4 (GLUT4), and leptin, indicating its antiobesity potential.

11. Anticancer Effect

Aqueous and methanol extracts of stem bark induced apoptosis in multiple human cancer cell lines (breast MDA-MB-435S, hepatic Hep3B, prostate PC-3). Petroleum ether extract showed greater cytotoxicity (IC_{50} ~112 µg/mL) in HeLa cells with apoptosis markers, including membrane blebbing and DNA fragmentation. Stem bark petroleum and chloroform extracts

demonstrated dose-dependent cytotoxicity in MDA-MB-231 and MCF-7 cells, with apoptosis confirmed by DNA fragmentation assays. Methanol leaf extracts selectively induced apoptosis in HeLa cells without affecting normal cells, causing cell cycle arrest at G1/S and increased p53 expression.

Ethanol extracts from wild and in vitro cultured twigs also exhibited dose-dependent toxicity in cervical and hepatic carcinoma cells. Root bark extracts showed lethality in brine shrimp assays and cytotoxicity in MCF-7 cells. Ethanol extracts of leaves, bark, pods, and seeds displayed concentration-dependent cytotoxicity, with seeds being most potent in clonogenic assays. These extracts inhibited migration and induced apoptosis via caspase activation and gene expression modulation (MMP-9, ICAM-1, Rac1 suppression; RhoA elevation).

Mechanistically, methanol leaf extract downregulated HPV18 oncoproteins E6/E7, activated caspase-8/3, and increased pro-apoptotic gene expression (p53, pRb, Fas/FasL) in cervical cancer cells, alongside modulating cytokines (increased IL-12, reduced IL-6), supporting potent anticancer activity.[75-78]

12. Wound Healing Effect

The Methanol root bark extract enhanced partial burn wound healing in mice, increasing wound contraction, collagen synthesis, and accelerating re-epithelialization. Ethanol stem bark extracts dose-dependently promoted full-thickness excision wound healing, improving collagen and DNA synthesis while reducing lipid peroxidation. Topical application downregulated pro-inflammatory markers NF-κB and COX-2, facilitating tissue repair.

13. Neuroprotective and Nootropic Activity

In vivo studies demonstrated that Oroxylum indicum extracts improved spatial memory in scopolamine-induced memory impairment models, enhancing brain acetylcholine levels and antioxidant enzyme activities, supporting its cognitive and neuroprotective potential.

14. Effect against COVID-19 Infection

Bignonia indica active constituents show promising antiviral potential against SARS-CoV-2. Molecular docking and surface plasmon resonance experiments revealed that oroxylin A binds the human ACE2 receptor, potentially blocking viral entry. Baicalein inhibited SARS-CoV-2 replication in vitro with IC_{50} ~10 μM, reduced viral-induced lung injury in hACE2 transgenic mice, and protected cultured Vero cells. Additional constituents, including baicalein-7-O-diglucoside, chrysin-7-O-glucuronide, oroxindin, and scutellarein, demonstrated binding to the viral main protease (Mpro), suggesting their ability to inhibit viral replication and restrain infection progression.

Table 5: Summary of pharmacological activities of the plant

Sr. No.	**Pharmacological Activity**	**Plant Part/ Extract Studied**	**Key Findings / Notes**
1	Anti-inflammatory	Leaves (aqueous), Stem bark, root	Significant activity in animal models; reduces paw edema.
2	Analgesic (pain relief)	Ethanol extract (bark/leaves)	Effective in pain attenuation in the hot plate, tail immersion tests.
3	Antioxidant	Fruits, Stem bark, Leaves, Root	Various extracts (ethanol, water, methanol) show antioxidant effects.
4	Antimicrobial	Root bark, Stem bark, Leaf	Shown activity against a range of microbes in vitro.
5	Anticancer/ Antiproliferative	Stem bark, roots, isolated flavonoids	Inhibits cancer cell growth; contains compounds like baicalein, oroxylin A, chrysin.

6	Anti-diabetic	Stem bark	Modulates glucose and insulin resistance in diabetic models.
7	Hepatoprotective	Roots, Stem bark, Leaves	Protects against liver toxins and damage in animal studies.
8	Wound healing	Stem bark, Leaf	Promotes faster healing in animal models.
9	Anti-ulcer	Root, Stem bark	Reduces gastric ulceration in animal models.
10	Immunostimulant	Root, Stem bark	Enhances immune response in various test models.
11	Anti-arthritic	Bark, leaves	Used traditionally and tested for inflammation/joint disorders.
12	Antimutagenic/ Photocytotoxic	Extracts of varied parts	Shown in lab studies; potential for cancer prevention.
13	Antidiarrheal/ Antidysenteric	Root bark, stem, seeds	Treats diarrhea & dysentery, traditional and experimental evidence.

Inference

Bignonia indica is a very potent medicinal plant in Ayurveda. It stands out as one of the most versatile plants, exhibiting a wide range of therapeutic properties. This Ayurvedic plant is the inimitable basis of many kinds of complexes having varied biological compounds. Relatively little systematic research has focused on the probable therapeutic claims of these compounds, and a later wider search is required to determine their medicinal usefulness. Antioxidant activity assays were performed on all anatomical parts of the plant, whereas germicide activity evaluations were specifically conducted on the stem bark and root bark. Anthelmintic,

antiulcer, immunomodulatory, and gastroprotective studies have been done in the root bark. Anti-inflammatory activity was evaluated on the leaves and stem bark, while antihepatotoxic and antimutagenic studies were conducted on the leaves and fruits, respectively. Though plant extracts from several parts of *Bignonia indica* have been allotted several therapeutic uses from time ancient, the possibility of renovating these biological activities into current drugs may be discovered more individually after extensive research of proper standardization and a thorough understanding of its bioactive constituents, mechanism of action, and toxicity, this line would be consistent with prevailing comprehensive scientific movements. While current data are promising, further studies, especially human clinical trials and molecular mechanism investigations, are essential to translate this potential into validated pharmaceutical applications. Standardization of extracts, bioavailability studies, and formulation development are areas of future interest. [79]

RATIONALE

To date, there is no research on the anti-diabetic activity of the *Oroxylum indicum* plant stem, nor is there any effort on the pharmacognostic assessment and phytochemical investigation of the stem and leaf.

Therefore, the extant study purposes to evaluate the *in vitro* and *in vivo* anti-diabetic activity of *Oroxylum indicum* stem extract using a streptozotocin (STZ)- induced model in diabetic rats and to evaluate the pharmacognostic parameters and phytochemical study of the stem and the leaf of the *Oroxylum indicum* plant.

OBJECTIVES

The present-day study was supported with the following objectives.

1. Authentication of the *Oroxylum indicum* plant
2. Pharmacognostic study of stem & leaf of *Oroxylum indicum* plant.
3. Evaluation of physico-chemical properties of stem & leaf of *Oroxylum indicum* plant.
4. Preparation of crude extracts of stem & leaf of *Oroxylum indicum* by the successive solvent extraction method.
5. Investigation of phytochemical screening of the stem & leaf of the *Oroxylum indicum* plant.
6. Estimation of total phenol in stem.
7. Investigation of total flavonoids in the stem extract of methanol.
8. Investigation of *in Vitro* antidiabetic activity of stem extracts by α-Amylase Inhibition Activity and α-glucosidase Inhibitory Activity.
9. Investigation of *in vivo* antidiabetic activity of active stem extract by streptozotocin-induced diabetic rat models.
10. Determination of the type of phytoconstituents present in the active extract using chromatographic techniques of HPTLC & HPLC methods.

MATERIAL & METHOD

4.1 Procurement of plant material

The fresh leaves and stems of *Oroxylum indicum* were collected in December from Rajkot, Gujarat, India. Authentication of the plant is done in the Botany Department, Shri M & N Virani Science College, Rajkot, Gujarat, India. A voucher specimen number AIP/20/02 was submitted to the herbarium department.

4.2 Chemicals and Solvents Requirement

4.2.1 Solvents- Acetic anhydride, Acetone, Benzene, Butanol, Chloroform, Ethanol, Ethyl acetate, Glacial acetic acid, Methanol, Petroleum ether (60-80°C), Toluene (Analytical Rasayan, S.D. Fine Chem. Limited)

4.2.2 Chemicals- Sodium hydroxide, Chloral hydrate, concentrated HCl, Ferric chloride, Sulphuric acid, Iodine, Potassium iodides, Picric acid, Nitric acid, α- naphthol, Vanillin, gelatin, sodium chloride (Loba Chemie)

4.3 Preparation of leaf & stem extracts

Leaves and stems of *Oroxylum indicum* were dried in the shade and pulverized. 100 g of pulverized leaves were used for successive Soxhlet extraction by solvents in cumulative order of polarity, viz., petroleum ether, toluene, chloroform, and methanol.

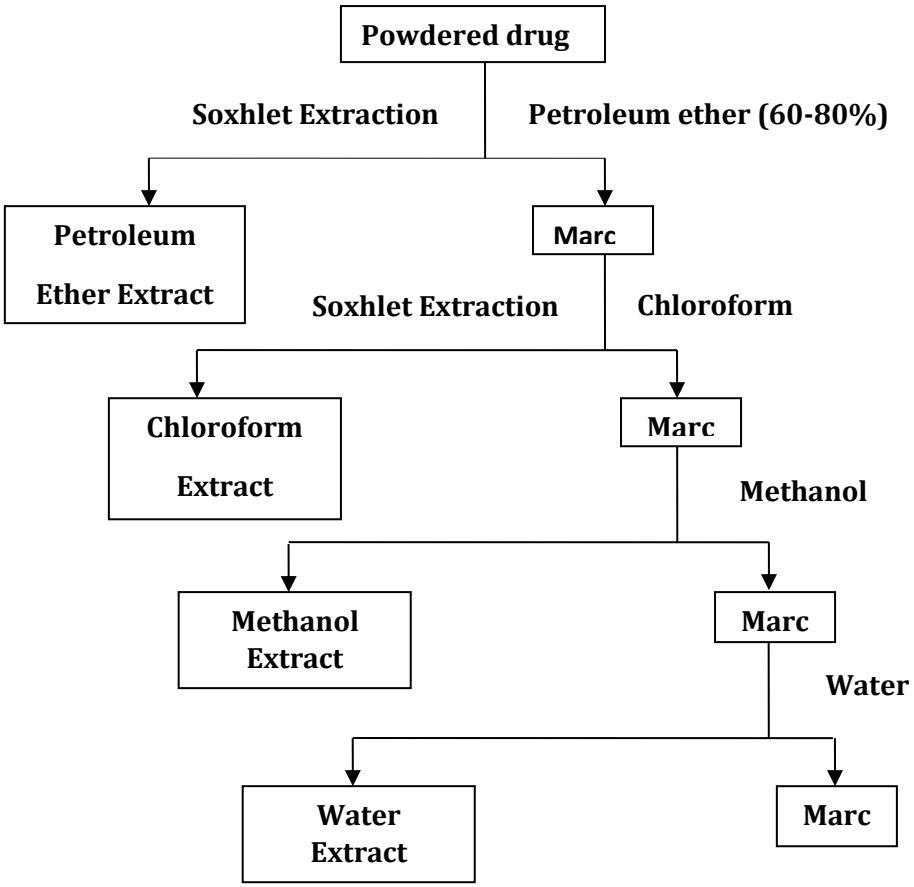


Figure 1: Successive solvent extraction of the leaf & stem

Each time previously, the ground plant mass was desiccated using a hot air oven at a temperature maintained below 50 °C to prevent degradation of thermolabile constituents. The dried marc was then subjected to maceration with liquid for 1 day to acquire the extract of water. Each extract was subsequently concentrated by evaporating the solvent through distillation, followed by drying to completeness in a water bath. Extracts were assessed, and the ratio was calculated as mentioned: air-dried mass of the drug powder material. Several extracts of *Oroxylum indicum* were evaluated for phytochemical screening.

4.4 Microscopic and Histological Techniques

4.4.1 Study of Transverse Sections

The leaves and stems of *Bignonia indica* were heated with liquid until soft. Freehand slices of the leaves were cut, transferred to slides, cleaned by boiling with chloral hydrate, and mounted

in glycerin. The tissues and different types of cells were distinguished using distinction staining techniques [13].

4.4.2 Photomicrography

Photographs at various magnifications were captured using an Olympus microscope projection system equipped with an image card. To examine crystals and lignified cells, divergent light was utilized.[14]

4.4.3 Surface preparation of the leaf

Place a few small pieces of the leaf in a solution of chloral hydrate in the test tube. Boil in a water bath until sufficiently transparent. Mount in a glycerin solution and examine microscopically.

4.4.4 Determination of leaf constants

Quantitative analysis of leaf morphology was employed to investigate ultrastructural characteristics that are not readily discernible through conventional light microscopy techniques. These included-

Stomatal Number- This refers to the mean count of stomata present per square millimeter on the leaf's epidermal surface.

Stomatal Index- It denotes the ratio, expressed as a percentage, of epidermal cells that have differentiated into stomata to the total number of epidermal cells, including the stomata themselves.

$$I = \frac{S}{E + S} \times 100$$

S = number of stomata per unit area and

E = number of ordinary epidermal cells in the same unit area

4.4.5 Powder microscopy

Leaves and stems of *Oroxylum indicum* were subjected to oven drying at 60°C for a duration of 4 to 6 hours to ensure complete removal of moisture content. Subsequently, the dried plant material was finely ground using an electric grinder to obtain a uniform powder, which was sieved to pass through a 60-mesh screen, yielding the 60# powder suitable for microscopic evaluation.

For microscopic examination, a small quantity of the powdered sample was placed on a clean glass slide, and a few drops of chloral hydrate solution were added. The preparation was gently covered with a cover slip and subjected to mild heating over a micro-Bunsen burner. Care was taken to avoid vigorous boiling to prevent damage or distortion of cellular structures. The clearing process facilitated by chloral hydrate rendered the sample transparent, allowing enhanced visualization of anatomical features under the microscope.

Upon completion of clearing, a drop of glycerol was applied to the slide. The glycerol served to inhibit crystallization and preserve tissue morphology during cooling, thereby maintaining specimen clarity.

Further histochemical staining was conducted to identify specific constituents within the powdered material. Treatment with phloroglucinol followed by the addition of concentrated hydrochloric acid selectively stained lignified cell wall components, highlighting sclerenchyma and other lignified tissues. Iodine solution was applied separately to detect the presence of starch grains within the sample, producing a characteristic coloration confirming starch localization.

To assess the occurrence of calcium oxalate crystals, a portion of the powdered sample was mounted with glycerin alone, without additional reagents. The crystalline structures were then observed microscopically based on their distinct birefringence and morphology.[80]

4.5 Physicochemical analysis

Physicochemical parameters of the air-dried powdered drug were evaluated, including moisture content, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, alcohol-soluble extractive, and petroleum ether-soluble extractive values.[81]

4.5.1 Petroleum ether soluble extractive

A precisely weighed quantity of powdered drug (5 g) was subjected to maceration with 100 mL of petroleum ether in a sealed conical flask. The mixture was frequently agitated during the initial 6 hours of maceration and subsequently allowed to remain undisturbed for an additional 18 hours at room temperature. Following extraction, the solution was filtered, and an aliquot of 25 mL of the filtrate was transferred to a shallow evaporating dish. The solvent was evaporated to dryness under controlled conditions. The resulting residue was further dried in an oven maintained at 105°C until constant weight was achieved. The mass of the dried residue was recorded, and the percentage of petroleum ether-soluble extractive relative to the original air-dried drug weight was calculated.

4.5.2 Determination of Chloroform-Soluble Extractive

Similarly, 5 g of powdered drug was macerated with 100 mL of chloroform in a closed flask under identical conditions of agitation (first 6 hours) and standing period (18 hours). Post maceration, filtration was performed, and 25 mL of the filtrate was evaporated to dryness in a shallow dish. The dry residue was oven-dried at 105°C to constant weight, and its mass was used to calculate the percentage of chloroform-soluble extractive of the initial air-dried powdered drug.

4.5.3 Determination of Methanol-Soluble Extractive

An equivalent procedure was employed using methanol as the extraction solvent. Five grams of powdered drug were macerated with 100 mL of methanol in a closed container, agitated frequently during the first 6 hours, and left undisturbed for 18 hours. The resulting extract was filtered, and 25 mL aliquots were evaporated to dryness. The residue was dried at 105°C to constant mass, enabling calculation of the methanol-soluble extractive percentage.

4.5.4 Determination of Water-Soluble Extractive

For aqueous extractive determination, 5 g of powdered drug was combined with 50 mL of water heated to 80°C in a Stoppered Erlenmeyer flask. The mixture was thoroughly shaken and then allowed to stand undisturbed at room temperature for 24 hours. Post maceration, the extract was filtered through Whatman filter paper, and 25 mL of the filtrate was evaporated to dryness in a shallow dish. The residue was oven-dried at 105°C until constant weight was obtained. The percentage of water-soluble extractives was then calculated relative to the original air-dried drug sample.

4.5.5 Determination of Total Ash Value

Approximately 2 to 3 grams of air-dried powdered drug were accurately weighed into a previously ignited and tarred Gooch crucible. The sample was incinerated gradually in a muffle furnace at a temperature not exceeding 450°C until complete combustion was achieved and the ash appeared free of carbon (white or nearly white). The crucible was cooled in a desiccator and weighed. In cases where carbon-free ash could not be attained upon initial incineration, the residue was treated with hot water to remove soluble salts, filtered through ashless filter paper, and the combined residue and filter paper were re-incinerated under the same conditions. The total ash content was expressed as a percentage of the weight of the air-dried drug.

4.5.6 Determination of water-soluble ash

To determine the water-soluble ash content of a sample, the process begins by calculating its total ash. The resulting ash is then boiled with distilled water, allowing any water-soluble minerals and salts to dissolve. After boiling, the mixture is filtered to separate the insoluble residue. This residue is subsequently dried, ignited, and weighed to obtain the mass of water-insoluble ash. The water-soluble ash is found by subtracting the weight of the insoluble ash from the total ash. This value is then expressed as a percentage of the original sample's weight, providing insight into the amount of inorganic material that is soluble in water.

4.5.6 Determination of Acid-Insoluble Ash

The total ash obtained as described above was boiled with 25 mL of 2M hydrochloric acid for 5 minutes. The insoluble portion was collected on an ignited Gooch crucible or ashless filter paper, washed with hot water until neutral, then incinerated at 450°C, cooled in a desiccator, and weighed. The acid-insoluble ash was calculated as a percentage of the original air-dried drug weight, representing the siliceous matter present.

4.5.7 Determination of Loss on Drying (Moisture Content)

A clean, dry, and glass-stoppered weighing bottle was initially weighed (W1). Approximately 1-2 g of the powdered drug sample was added, and the combined weight was recorded (W2). The stopper was removed, and the sample was dried in an oven at a prescribed temperature until a constant weight was attained. After cooling in a desiccator to ambient temperature, the bottle with the dried sample was weighed again (W3). The loss on drying was determined as the difference between the weights before and after drying $[(W2 - W3) \times 100]/(W2 - W1)$, expressed as a percentage of the initial moisture content.

4.6 Phytochemical Screening

The extracts of *Bignonia indica* were evaluated by qualitative chemical examination.[82, 83]

4.6.1 Alkaloids

Aliquots of the acidic filtrates were treated carefully with specific alkaloid reagents and observed for characteristic precipitates, indicating the presence of alkaloids:

- *Mayer's Test:* Addition of Mayer's reagent (potassium mercuric iodide) to the filtrate resulted in the formation of a yellowish cream precipitate, confirming alkaloids.
- *Wagner's Test:* Treatment with Wagner's reagent (iodine in potassium iodide) yielded a brown to reddish-brown precipitate, indicative of alkaloids.
- *Dragendorff's Test:* Dragendorff's reagent (potassium bismuth iodide solution) produced a red precipitate upon interaction with alkaloids.
- *Hager's Test:* Addition of Hager's reagent (saturated picric acid solution) led to yellow-colored precipitate formation, affirming the presence of alkaloids.[84]

4.6.2 Carbohydrates

Carbohydrate detection involved aqueous dissolution and filtration of extracts, followed by classical colorimetric tests:

- *Benedict's Test:* Filtrates treated with Benedict's reagent and heated in a water bath produced an orange-red precipitate, indicating reducing sugars.
- *Molisch's Test:* Treatment with alcoholic α-naphthol solution, followed by careful addition of concentrated sulfuric acid along the test tube walls, generated a violet ring at the interface, signifying carbohydrates.
- *Fehling's Test:* Hydrolyzed and neutralized filtrates heated with equal volumes of Fehling's A and B solutions formed a red precipitate, confirming carbohydrates.

- *Barfoed's Test:* Heating filtrates with Barfoed's reagent in a water bath led to orange-red precipitate formation indicative of reducing sugars.

4.6.3 Flavonoids

Detection of flavonoids was performed using:

- *Magnesium-Hydrochloric Acid Test:* Ethanolic extract (2–3 mL) was treated with small magnesium ribbon pieces and 1 mL concentrated hydrochloric acid. Appearance of pink, red, or reddish coloration indicated flavonoids.
- *Alkaline Reagent Test:* Extracts treated with a few drops of sodium hydroxide produced an intense yellow color, which turned colorless upon dilute acid addition, confirming flavonoid presence.
- *Shinoda Test:* Extracts treated with fragments of magnesium metal and dropwise addition of concentrated hydrochloric acid developed a magenta coloration, characteristic of flavonoids.[85]

4.6.4 Phenols

A chemical test for phenol detects the presence of the phenolic –OH group through characteristic reactions that yield distinct color changes or precipitates. The most common laboratory tests for phenol are as follows:

1. Ferric Chloride Test: Dissolve a small amount of the sample in water or ethanol. Add a few drops of neutral ferric chloride solution. The appearance of violet, blue, green, or red color indicates the presence of phenol.
2. Bromine Water Test: Add bromine water dropwise to an aqueous or acetic acid solution of the suspected phenol. Disappearance of the brown color of bromine and formation of a white precipitate (2,4,6-tribromophenol) confirms phenol.

3. Phosphomolybdic acid reagent was employed for phenol detection. A drop of ethanolic extract stained on filter paper was exposed to ammonia vapor; the emergence of blue coloration confirmed phenolic compounds.[86]

4.6.5 Saponins

Assessment of saponins was performed by:

- *Froth Test:* Methanol and aqueous extracts were diluted with distilled water (20 mL) and shaken vigorously for 15 minutes. The formation of persistent foam approximately 1 cm in height indicated saponins.
- *Haemolytic Test:* A mixture of 0.2 mL extract and 0.2 mL of 10% v/v blood in normal saline was centrifuged. A red supernatant, compared to a pale-yellow supernatant in the control (blood plus saline), indicated haemolysis caused by saponins.[87]

4.6.6 Tannins (Phenolic compounds)

- Nsbd *Ferric Chloride Test:* Extracts treated with 5% neutral ferric chloride solution produced a bluish-black coloration, confirming phenolic content.
- *Lead Acetate Test:* Addition of 10% lead acetate solution resulted in yellow precipitate formation; indicative of flavonoids commonly associated with tannins.
- *Vanillin-Hydrochloric Acid Test:* Reaction with vanillin hydrochloride reagent generated a pinkish-red color, confirming tannins.

4.6.7 Steroids and Triterpenoids

- *Libermann-Burchard Test:* One milliliter ethanolic extract was mixed with 1 mL chloroform and 2–3 mL acetic anhydride, followed by addition of 1–2 drops

concentrated sulfuric acid. Development of dark green coloration signified steroids, while dark pink to red coloration indicated triterpenoids.

- *Salkowski Test:* Treatment of chloroform extract with concentrated sulfuric acid, followed by standing, produced a red lower layer (steroids) or yellow coloration in the lower layer (triterpenoids). [88]

4.7 Thin Layer Chromatography Analysis

Thin layer chromatography was performed on glass plates measuring 5 × 15 cm with a uniform thickness of 0.25 mm, coated with silica gel G as the stationary phase. Prior to sample application, the TLC plates were activated by heating at 110°C for 30 minutes to remove moisture and ensure optimal adsorption characteristics.

Extracts and their respective bioactive fractions were applied as small spots onto the activated plates. Development of the chromatograms was carried out in a saturated TLC chamber using appropriate mobile phase solvent systems tailored to separate the phytoconstituents within the samples effectively.[89]

Upon completion of chromatographic development, the plates were air-dried and visualized under ultraviolet (UV) light at wavelengths of 254 nm and 366 nm to detect UV-active compounds exhibiting fluorescence quenching or emission. Additionally, the chromatograms were subjected to derivatization by spraying with suitable chemical reagents specific for different classes of phytochemicals, enhancing visualization and aiding in tentative compound identification.[90]

The retention factor (Rf) values of resolved spots were calculated, providing qualitative insight into the composition and profiling of the plant extracts and fractions under investigation.[91]

4.8 Evaluation of *in vitro* anti-diabetic activity of various extracts of the stem

4.8.1 Inhibition of α-amylase Enzyme

α-amylase (0.5 mg/ml) was mixed with the sample at various concentrations (100-500 µg/ml) to which 1% of starch solution and 100 µl of 0.2 mM of phosphate buffer (pH 6.9) were added. The response was allowed to be carried out at 37°C for five minutes and completed by the addition of 2 ml of 3,5-dinitrosalicylic acid reagent. The reactant solution was heated for 15 minutes at 100°C and diluted with 10 mL of distilled water in an ice bath. α-amylase activity was checked by measuring color intensity at 540 nm in a spectrophotometer.[92]

4.8.2 Inhibition of α-glucosidases Enzyme

The inhibitory activity was checked by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M Tris buffer (pH 8) and various concentrations of the sample (100-500 mg/ml). The reactant solution was incubated at 37°C for 10 min. The reaction was begun by adding 1 ml of α-glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. Then the reaction was concluded by the addition of 2 mL of 6 N HCl. The intensity of the color was measured at 540 nm in a spectrophotometer.[93, 94]

The results were expressed as % inhibition using the formula:

% Inhibitory activity = (Ac-As)/Ac ×100

Where Ac is the absorbance of the control, and As is the absorbance of the sample.

The inhibitory concentration (IC_{50}) is defined as the concentration of an inhibitor required to reduce the biological activity by 50% under the given assay conditions. IC_{50} values were calculated by plotting the percentage of inhibition against the logarithm of inhibitor concentration and calculating the values through logarithmic regression analysis derived from mean inhibitory responses. [74, 95, 96]

Statistical Analysis

All analyses were performed in quintuplicate (n=5), and the results are presented as mean ± standard error of the mean (SEM). The IC_{50} values were derived from dose-response curves plotted as log inhibitor concentration versus percentage inhibition.[97, 98]

4.9 *In vivo* anti-diabetic activity (Induction of Diabetes in experimental animals)

Male Wistar rats weighing between 180 and 220 grams were used to assess the antidiabetic potential of the test compounds. The animals were housed under standardized laboratory conditions maintained at a temperature of 22 ± 2°C and relative humidity of 55%, with a controlled 12-hour light/dark photoperiod.[99] Throughout the experimental duration, the rats had unrestricted access to standard laboratory rodent chow and water ad libitum. Prior to commencement of the study, all animals underwent an acclimatization period of two weeks to minimize environmental stress and physiological variability.[100]

All experimental protocols involving animals were performed in strict accordance with institutional ethical standards and received approval from the Institutional Animal Ethical Committee, ensuring compliance with established guidelines for the care and use of laboratory animals.

Following overnight fasting, diabetes mellitus was experimentally induced by a single intraperitoneal administration of freshly prepared Streptozotocin (STZ) at a dose of 55 mg/kg body weight. The STZ was dissolved in 0.1 M sodium citrate buffer (pH 4.5) immediately before injection to preserve its stability and efficacy.

Post-STZ injection, the animals were provided free access to standard feed and water. To mitigate the risk of hypoglycemic episodes during the early post-induction phase, a 5% glucose solution was supplied for 18 hours. Subsequently, a 10% glucose solution replaced the drinking water, which was made available in bottles attached to the cage grills for a further 24-hour period.[101]

On the fifth day following STZ administration, fasting blood glucose levels were measured via tail vein sampling to confirm diabetes induction. Rats demonstrating fasting plasma glucose concentrations exceeding 250 mg/dL were deemed diabetic and selected for subsequent experimental procedures.[102]

The confirmed diabetic animals were randomly allocated into five groups, each consisting of six rats. Designated treatments were administered daily over a 28-day period, during which various pharmacological parameters related to glycemic control and metabolic status were monitored. [96]

Table 6: Grouping of rats

Group	**I**	**II**	**III**	**IV**	**V**
Treatment	**Control**	**Disease control**	**Standard**	**OI 250**	**OI 500**
Dose	**Vehicle**	**10% Glucose**	**10% Glucose + Glibenclaimide 5mg/kg**	**10% Glucose + Methanolic Extract of oroxylum indicum 300 mg/kg**	**10% Glucose + methanolic Extract of oroxylum indicum 500 mg/kg**
No. of animals	**6**	**6**	**6**	**6**	**6**
Duration of Treatment	**90**	**90**	**90**	**90**	**90**
Route of Administration	**p.o**	**p.o**	**p.o**	**p.o**	**p.o**
				Total number	**30**

After induction and establishment of the diabetic condition, rats were grouped based on the treatment regime, including normal rats, as follows:

1. NC (Normal control, vehicle) group in which rats maintained a systematic diet and intake water ad libitum; this group consists of non-diabetic rats, they remain untreated throughout the study, and allow for the assessment of normal physiological parameters in healthy rats.
2. The DC (Diabetic control) grouping, in which rats maintained a systematic diet and intake water ad libitum, serves as a positive control to evaluate the progression of diabetes without treatment.
3. PC (positive control) group, animals were medicated with the standard drug Glimenclaimide (5 mg/kg b.w.), this group serves as a standard group to compare the antidiabetic activity of the plant extract
4. The plant extract group, to given rats with methanolic extract (300 mg/kg b.w.), is the experimental group, which will be used to assess the antidiabetic activity of the plant according to dose.
5. The plant extract group, to given rats with methanolic extract (500 mg/kg b.w.), is the experimental group, which will be used to assess the antidiabetic activity of plants according to dose.

The parameters such as BW (body weight), Vu (urine volume), BGL (blood glucose level), and Ins (serum insulin) were estimated to study the anti-diabetic potential of the extract. During the experiment, all parameters were determined on days 0, 7, 14, 21, and 28. The BWs were assessed on a digital balance in all the groups. Vu was determined by collecting the urine at 5-hour intervals.[103] Blood glucose levels (BGL) were measured using the tail-flick method with the aid of a glucometer. Under light ether anesthesia, blood samples were obtained from the retro-orbital sinus of the animals using standard procedures. Plasma insulin concentrations were measured utilizing a commercial insulin radioimmunoassay kit based on the enzyme-linked immunosorbent assay (ELISA) method. For serum collection, supplementary blood

samples were drawn into plain, clot-activator tubes and allowed to clot at room temperature for 30 minutes.[104] The clotted samples were then centrifuged at 2000 rpm for 10 minutes, and the pure supernatant (serum) was wisely collected for further biochemical estimation of liver enzymes—alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)—per the manufacturer's protocols (Sigma-Aldrich, Merck KGaA, Life Science, India).[105]

Table 7: Parameters to be measured

Parameters	1. Blood glucose level (BGL) 2. Body weight (b.w.) 3. Oral glucose tolerance level (OGTT) 4. Insulin 5. Alkaline phosphatase (ALP) 6. Alanine aminotransferase (ALT) 7. Aspartate aminotransferase (AST), all were determined

[106]

Statistical analysis

The final results were expressed as mean ± standard error of the mean (SEM) or standard deviation (SD). Statistical analysis of the data is accomplished with GraphPad Prism version 8.02. One-way analysis of variance (ANOVA) followed by Student's t-test was employed to assess differences among groups. Dunnett's multiple comparison test was used to determine statistical significance between the drug-treated groups and the negative control group, with $p < 0.05$ considered statistically significant.[107]

Blood Glucose Estimation

All the animals were delivered orally in a 0.5%, w/v NaCMC aqueous Suspension form through oral gavage. A small aliquot of the test sample was collected from the tail vein at 2 hours post-administration. Immediately following collection, the blood sample was analyzed to measure

glucose concentrations using a glucometer (Accu-Chek, Roche Diagnostics India Pvt. Ltd., Mumbai). Blood glucose levels were stated as mg/dl.[108]

Body weight

The animals with a weight range between 180-220 g were identified for the study and were acclimatized for the required period and maintained with a normal diet and water. The weights of the rats are presented in the Table.[109]

Measurement of insulin level

Blood samples were collected from the retro-orbital venous plexus of the experimental rats on days 0, 7, 14, 21, and 28 post-treatments. Samples were immediately centrifuged at 4000 rpm for 10 minutes at 4°C to separate serum. The insulin concentration in serum was quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits specific for rat insulin, strictly adhering to the manufacturer's instructions for reagent preparation, incubation times, and detection. Optical density readings obtained from microplate readers were used to calculate insulin levels based on standard curves generated with known concentrations.[110]

Measurement of Blood Glucose Levels in Oral Glucose Tolerance Test (OGTT)

Before OGTT, animals were subjected to a 12-hour fasting period with free access to water. Subsequently, a glucose solution was administered orally at a dose of 2 g/kg body weight. Blood glucose levels were monitored at baseline (pre-glucose administration) and at specified time points post-glucose load—commonly at 30, 60, and 120 minutes. Glucose quantification was performed using a validated glucometer (Accu-Chek, India), with blood samples obtained from tail vein puncture. Peak glucose levels were typically observed at approximately 30 minutes following glucose challenge in both normal and diabetic cohorts. Treatment with standard antidiabetic agents and plant extract formulations elicited a statistically significant ($p < 0.05$) reduction in hyperglycemia at 30- and 60-minute intervals compared to diabetic control.

Concurrently, blood samples were collected for serum separation and subsequent determination of circulating glucose via glucometer and insulin concentrations using rat-specific ELISA kits (Invitrogen, Cat. No. ERINS; Thermo Fisher Scientific, India).[111]

Measurement of AST, ALP & ALT

Blood samples collected via retro-orbital plexus puncture on days 0, 7, 14, 21, and 28 were centrifuged at 4000 rpm for 10 minutes to yield serum for biochemical assays. Activities of hepatic enzymes—namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)—were quantified using commercially available ELISA kits calibrated for rat serum. Assay procedures were conducted following the protocols prescribed by the manufacturers, including sample and reagent volumes, incubation periods, and detection methods. Enzyme activity values were calculated based on optical density readings derived from standard calibration curves.[112]

Investigation of the LD50 value

In an acute toxicity study, administration of a single oral dose of 2000 mg/kg of extract to rats did not cause any signs of toxicity or mortality within a 2-week observation. Hence, the extract's LD50 was estimated to be greater than 2000 mg/kg.

The safety performance of the *Oroxylum indicum* plant was evaluated using standard toxicological tests recommended by the OECD test guidelines. The test results indicated the absence of toxicity in plant extracts at various doses. [113]

Estimation of Total Phenolics

The concentration of phenolics in plant extracts was determined using the spectrophotometric method.[114] Methanol solutions of the extracts in a concentration of 1 mg/ml were used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of a methanol solution of extract, 2.5 ml of 10% Folin-Ciocalteu’s reagent dissolved in water, and 2.5 ml of 7.5%

$NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using a spectrophotometer at λmax=765 nm. The samples were prepared in triplicate for each analysis, and the mean value of absorbance was obtained (results of absorbance for methanol are tabulated in Table). The same procedure was repeated for the standard solutions of gallic acid, and the calibration line was construed (results of absorbance of different concentrations of gallic acid for the calibration curve are tabulated in Table 4.10 and represented as Fig. 4.14). Total phenolic content of extracts was expressed as mg/gm gallic acid equivalent. The experiment was conducted in triplicate, and the results were expressed as mean ± SD values (100).

Determination of flavonoids

Flavonoid compounds were calculated by the $AlCl_3$ colourimetric method, with rutin as the standard reference compound. One gram of air-dried pulverized plant material was extracted with twenty-five mL of methanol for 24 hours, then filtered. The volume of the filtrate was adjusted to 25 mL with methanol, and it was used for analysis.

For the assay, two mL of the sample solution was mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of purified H_2O. The reaction mixture was incubated at room temperature for 30 minutes. Absorbance was then measured at 415 nm using a colorimeter. The flavonoid compounds were measured and expressed as grams per 100 grams of dry matter, using rutin as the standard.[115]

HPLC Analysis of Total Flavonoids

- **Chemicals:** Methanol (HPLC grade), Quercetin (standard), Phosphoric Acid, Water (HPLC grade), and Acetonitrile.

- **Instruments:** HPLC system equipped with UV detector, analytical balance, sonicator, vacuum filtration unit, and micropipettes.
- **Glassware:** Volumetric flasks, filter paper (0.45 µm), and measuring cylinders.

Table 8: Reagents used in HPLC

Manufacturer	Grade	Reagent
Merck	HPLC	Methanol
Sigma-Aldrich	HPLC	Acetonitrile
Himedia	AR	Phosphoric Acid
Sigma-Aldrich	Standard	Quercetin

Standard Solution Preparation

A stock solution of quercetin (100 µg/mL) was prepared by dissolving 10 mg of quercetin in 100 mL of methanol. Different concentrations of standard solutions of 10, 20, 40, 60, 80, and 100 µg/mL were prepared from the stock solution to generate a calibration curve. Filter all solutions through a 0.45 µm membrane filter and sonicate for five minutes before use.

Sample Solution Preparation

Precisely 100 mg of the methanolic extract of Oroxylum indicum stem was weighed and soluble in 100 mL of methanol. The resulting solution was filtered through a 0.45 µm membrane filter and used for HPLC analysis.

HPTLC analysis of methanolic extract

Instrument: CAMAG Linomat 5

Stationary Phase: Plate size (X x Y) 10.0 x 10.0 cm, Material HPTLC plates silica gel 60 F 254, Manufacturer E. MERCK KGaA. 171118

Mobile Phase: solvent Methanol

Illumination instrument: CAMAG Visualizer (254 nm remission, 366 nm remission, white light)

Post-Chromatographic Derivatization: 10% alcoholic 0.1 mL potassium acetate

RESULTS

Pharmacognostic Study of Leaf

5.1 Organoleptic features of the leaves of *Oroxylum indicum* were observed.

Type - Compound leaf (2-4 leaflets)

Colour - Upper surface: dark green and lower surface: light green

Odour - Characteristic

Taste - Characteristic

Size - 10-15cm long and 3-4cm wide.

Shape - ovate or elliptic

Margin - entire

Surface - flat, glabrous

Venation - pinnate

Apex - Acuminate

Base - Immature leaflets have a symmetrical base, and mature leaflets are

Symmetrical base

5.2 Histology study of the leaf

The transverse section of a leaf is dorsiventral, which includes the following characteristics. The outer surface consists of a single sheet of rectangular-celled upper epidermis with a covered thick cuticle, which is devoid of stomata (Figure 1). The lower surface of the leaf

consists of epidermal cells of rectangular shape enclosed with a thick cuticle, bearing unicellular covering trichomes and anomocytic stomata.

In the lamina region, a single layer of palisade cells is located just beneath the upper epidermis, consisting of compact and elongated cells (Figure 2). The spongy parenchyma consists of 5 to 7 layers of roughly organized cells and intercellular spaces, which house rosettes of calcium oxalate crystals.

Midrib part consists of collenchyma tissue is observed overhead the lower epidermis and beneath the upper epidermis. A large collateral vascular bundle is centrally located within the midrib, comprising xylem and phloem, surrounded by discontinuous patches of pericyclic fibres (Figure 3).

Powder microscopy of the leaf reveals polygonal to rectangular epidermal cells, anomocytic stomata (Figure 4), unicellular trichomes (Figure 5), and reticulate xylem vessels (Figure 6).

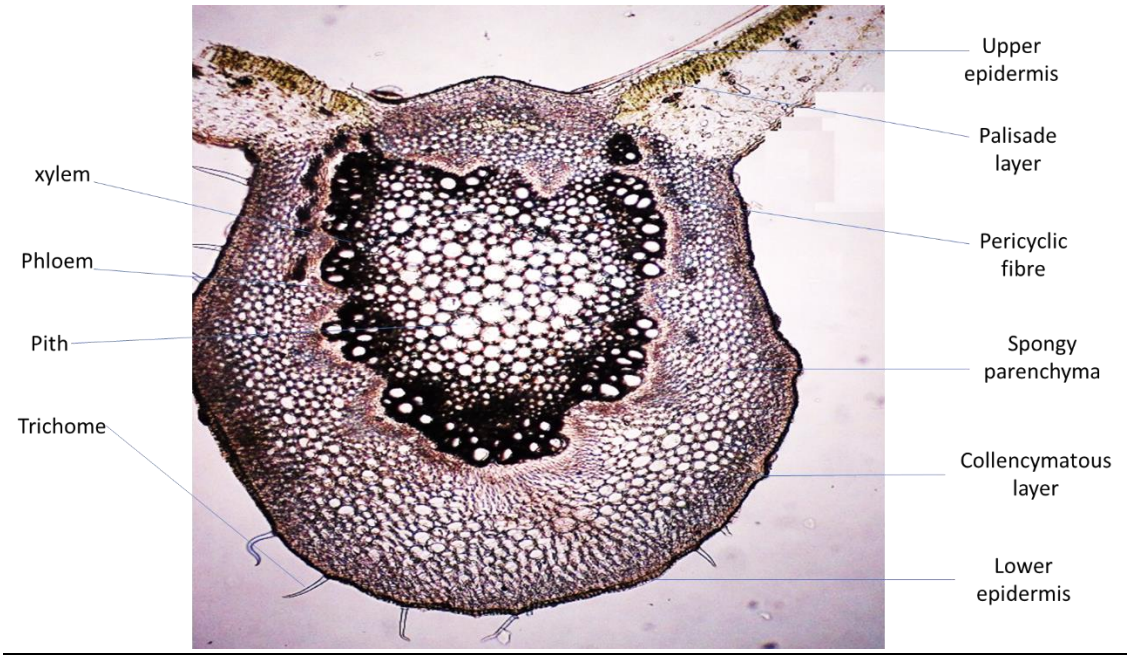


Figure 2: Transverse section of leaf

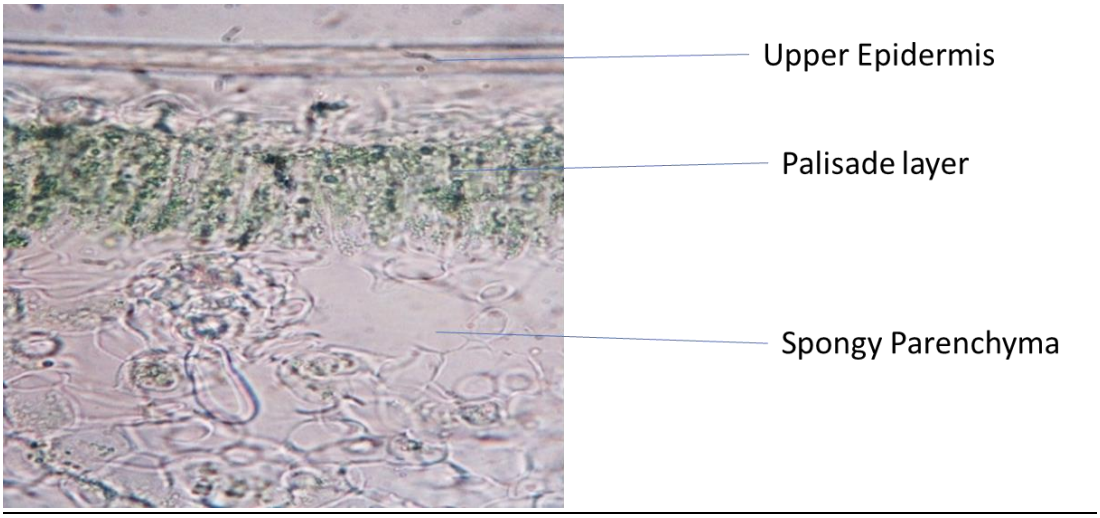


Figure 3: Lamina region of leaf

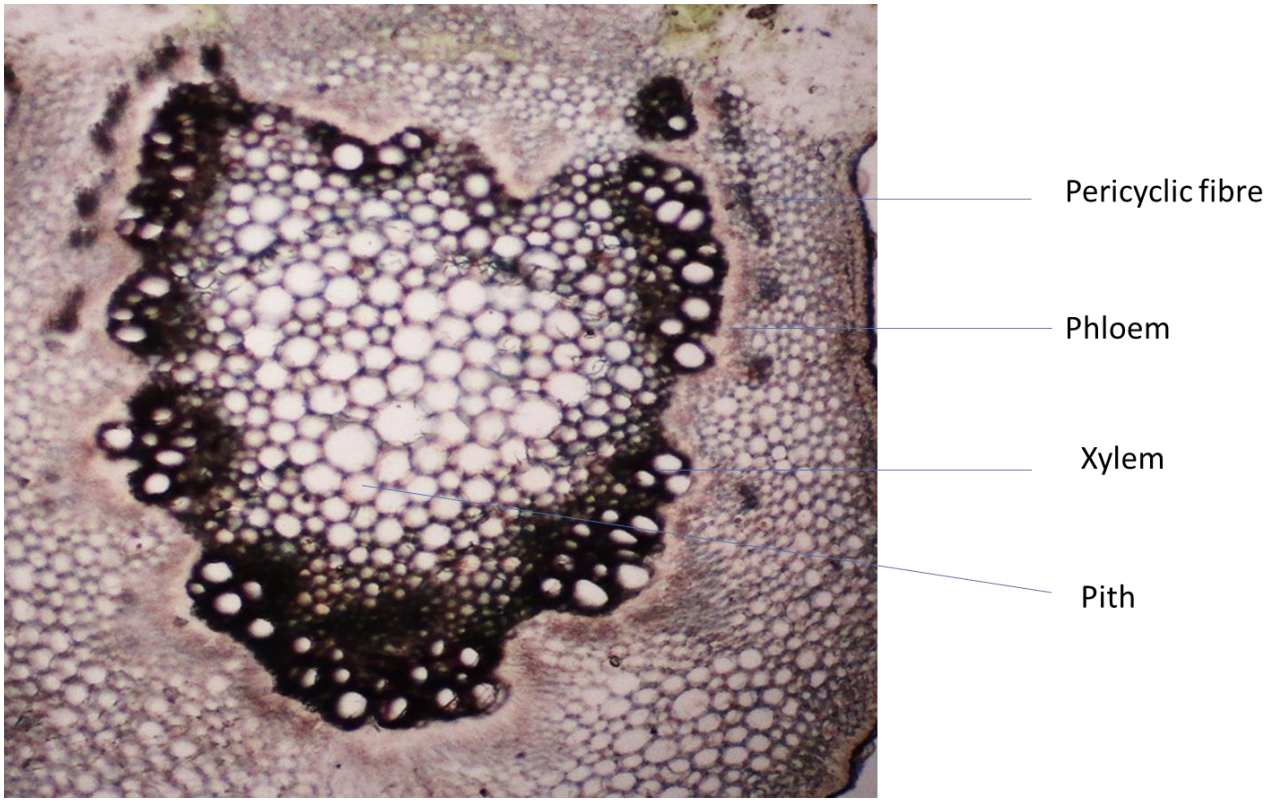


Figure 4: Vascular bundle of leaf

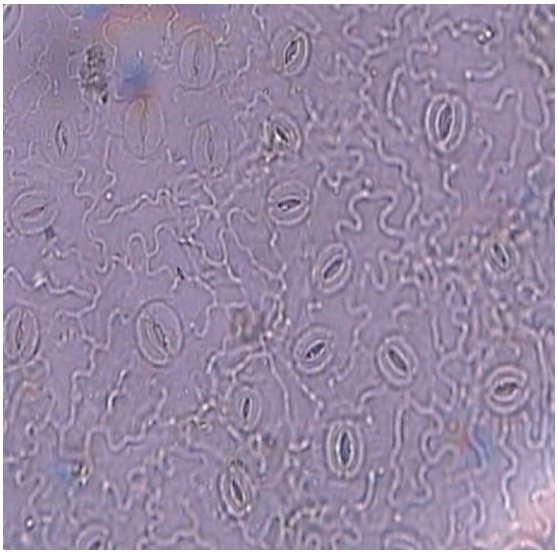


Figure 5: Anomocytic stomata of a leaf

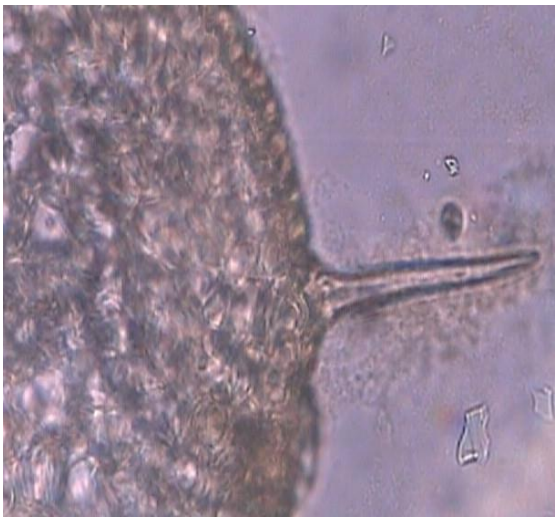
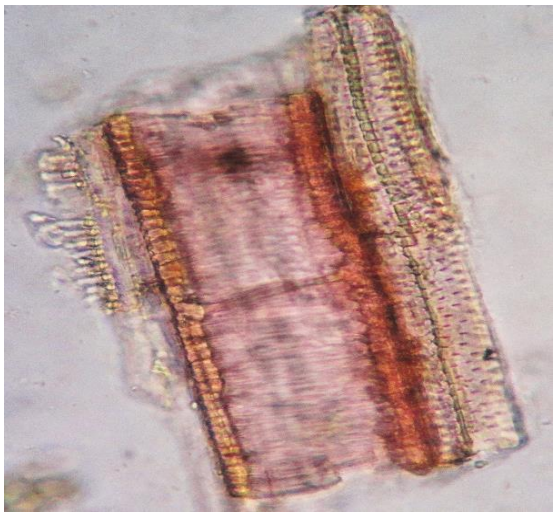


Figure 6: Trichome of a leaf



. Figure 7: Reticulate xylem vessel

Determination of Leaf Constants

The surface parameters of leaves of *Oroxylum indicum* were measured (Table 1).

Table 9: Surface data of leaf

Leaf constant	**Value**
Stomatal No	30/mm^2
Stomatal Index	24.19

Vein-islet No.	16.45/mm^2
Vein-termination No.	22.70/mm^2
Palisade Ratio	6.25

5.3 Physicochemical analysis of the leaf

The physical parameters of powdered leaves of *Oroxylum indicum* were evaluated. It includes moisture content, ash values, and extractives values (Table 2).

Table 10: Physical parameters of leaves of *Oroxylum indicum*.

Physical Parameter	**%w/w (Air dried drug)**
moisture content	9.8
Total Ash	9
Water Soluble Ash	4.7
Acid Insoluble Ash	0.7
Ethanol Soluble Extractives	11.5
Water Soluble Extractives	22.6
Petroleum Ether Soluble Extractives	5.4

Table 11: Different leaf extracts with their yield

Sr. No.	**Extract**	**Consistency**	**Colour**	**Yield (%w/w)**
1	Petroleum ether	Non-sticky	Green	6.7% w/w
2	Chloroform	Non-sticky	light yellow	1.4% w/w

3	Methanol	Slightly sticky	Brown	6.1% w/w
4	Water	Sticky	Brownish black	10.4% w/w

5.4 Phytochemical screening of leaf

The phytochemical screening of the leaves of *Oroxylum indicum* was investigated by performing chemical tests of various extracts. Outcomes are specified in Table 3.

Table 12: Phytochemical screening of leaf extracts –

Plant constituent	**EXTRACTS**				
Test/Reagent Used	**Extract of petroleum ether**	**Extract of toluene**	**Extract of Chloroform**	**Extract of methanol**	**Extract of water**
1) Alkaloids					
Dragendroff's reagent	–	–	–	–	+
Hager's reagent	–	–	–	–	+
Wagner's reagent	–	–	–	–	+
Mayer's solution	–	–	–	–	+
2) Steroids					
Liebermann-Burchard's test	–	–	–	–	–
3) Saponins					
Foam test	–	–	–	–	–
4) Flavonoids					
Shinoda test	–	–	–	+	–
Sodium hydroxide test	–	–	–	+	–
5) Phenolic contents and tannins					

$FeCl_3$ solution	–	–	–	+	+
Lead acetate test	–	–	–	+	+
6) Carbohydrate					
Molisch's reagent	–	–	–	–	+
Fehling solution	–	–	–	–	+
Benedict's solutions	–	–	–	–	+

5.5 Organoleptic study of the stem

Organoleptic structures of the stem of *Oroxylum indicum* were observed.

Morphology

Size: 3-20 mm diameter, 10-20 inches in length

Shape: Cylindrical

Color: light brown & wood is brownish

Odor: indistinct

Fracture: short

Extra feature: twisted & gradually tapering.

The external surface is white.

Surface: longitudinally

5.6. Histology study of the stem

The **transverse section** of the stem is circular in outline.

Epiblema is a single outermost layer made of parenchymatous cells.

Cortex: Cortex is next to epiblema & consists of parenchymatous cells are sufficient intracellular space. It contains starch grains, lignified cells. Cortex is followed by discontinuous patches of lignified pericyclic fibres.

Vascular bundle: Phloem consists of phloem parenchyma & phloem fibres are present. Phloem fibres are available as thick-walled. Xylem consists of tracheids, xylem fibres, and intercellular space.

Pith: Pith occupies ½ half of the stem. The pith is made up of large parenchymatous cells and intercellular space.

The histology of the powder of *Oroxylum indicum* stem reveals distinct cellular structures and inclusions critical for its identification. Key features include:

- **Sclereids**: Lignified sclereids, thick-walled.
- **Fibers**: Groups of elongated, lignified fibers.
- **Xylem Vessel**: Pitted vessels
- **Parenchyma**: Parenchymatous cells containing
- **acicular calcium oxalate crystals** (needle-shaped) and occasional starch grains.

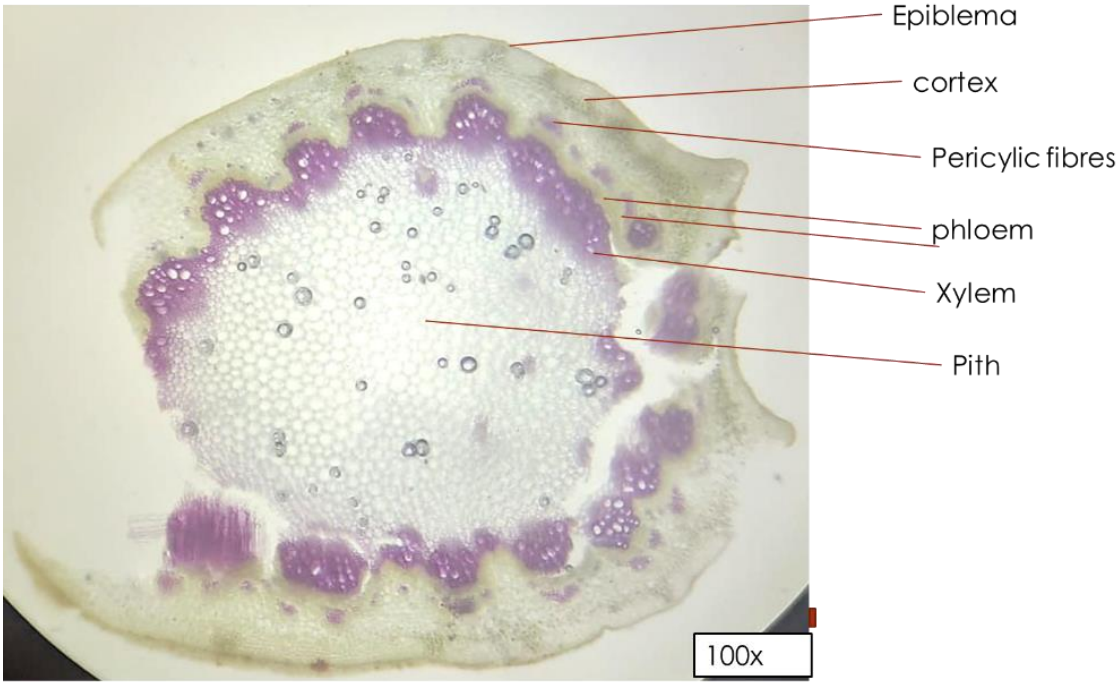


Figure 8: transverse section of stem

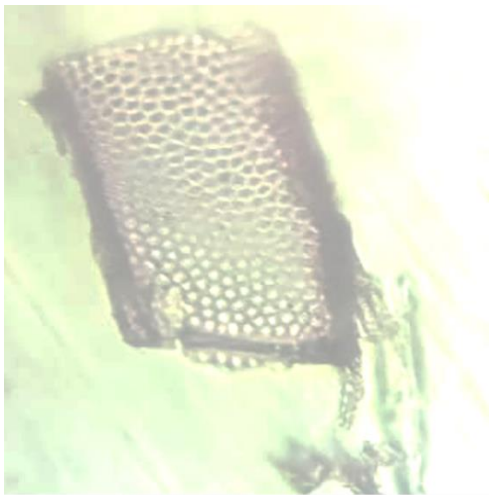


Figure 9: Pitted xylem vessels

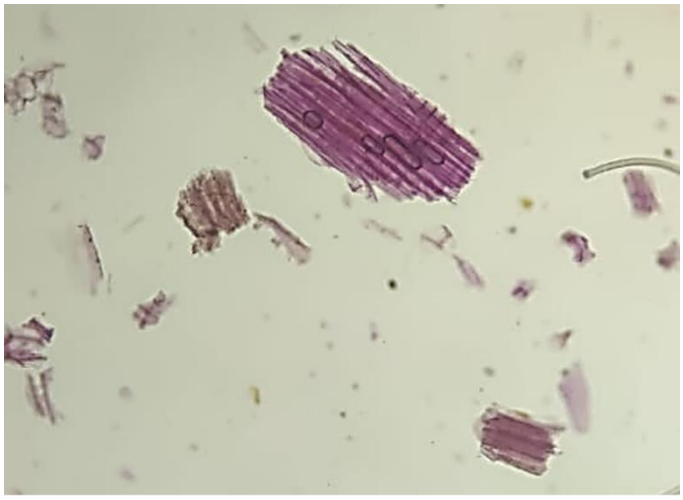


Figure 10: fibre cells

5.7 Physicochemical analysis of the stem

Physico-chemical parameters of the pulverized stem of *Oroxylum indicum* were evaluated. It includes LOD, ash values, and extractives values.

Table 14: Physical variable of the stem part

Physical Parameter	%w/w (Air dried drug)
Moisture content	4.8
Total Ash	10
Water Soluble Ash	5.2
Acid Insoluble Ash	2.7
Ethanol Soluble Extractives	11.5
Water Soluble Extractives	24.6
Petroleum Ether Soluble Extractives	6.4

Table 10: Various stem extracts with their yield

Sr. No.	Extract	Consistency	Colour	Yield (%w/w)
1	Petroleum ether	Non-sticky	Yellow	0.5% w/w
2	Chloroform	Non-sticky	Dark yellow	0.6% w/w
3	Methanol	Slightly sticky	Brown	5.3% w/w
4	Water	Sticky	Brownish black	9.1% w/w

5.8 Phytochemical screening of the stem

Table 15: Phytochemical screening of stem extracts

Plant constituent	EXTRACTS				
Test/Reagent Used	Extract of petroleum ether	Extract of toluene	Extract of Chloroform	Extract of methanol	Extract of water

1) Alkaloids					
Dragendroff's reagent	–	–	–	–	+
Hager's reagent	–	–	–	–	+
Wagner's reagent	–	–	–	–	+
Mayer's reagent	–	–	–	–	+
2) Steroids					
Liebermann-Burchard's test	–	–	–	–	–
3) Saponins					
Foam test	–	–	–	–	–
4) Flavonoids					
Shinoda test	–	–	–	+	–
Sodium hydroxide test	–	–	–	+	–
5) Phenolic contents and tannins					
Ferric Chloride solution	–	–	–	+	+
Lead acetate test	–	–	–	+	+
6) Carbohydrate					
Molisch's reagent	–	–	–	–	+
Fehling solution	–	–	–	–	+
Benedict's reagent	–	–	–	–	+

5.9 *In vitro* anti-diabetic activity of stem extracts

5.9.1 α-amylase Inhibitory Activity

Dose-dependent % inhibition of α-amylase enzyme is observed with both extracts. The IC_{50} values of aqueous extract, methanolic extract & acarbose were 530.12±15.34, 266.71±4.56, and 85.02±1.12. Nevertheless, related to water extract, methanolic extract shows a higher % inhibition of the α-amylase enzyme. (Figure 12)

5.9.2 α-glucosidase Inhibitory Activity

Dose-dependent % inhibition of the α-glucosidase enzyme is observed with both extracts. The IC_{50} values of aqueous extract, methanolic extract & acarbose were 545.77±77, 246.34±34, and 89.75±1.07. Nevertheless, related to water extract, methanolic extract shows a higher % inhibition of the α-glucosidase enzyme. (Figure 13)

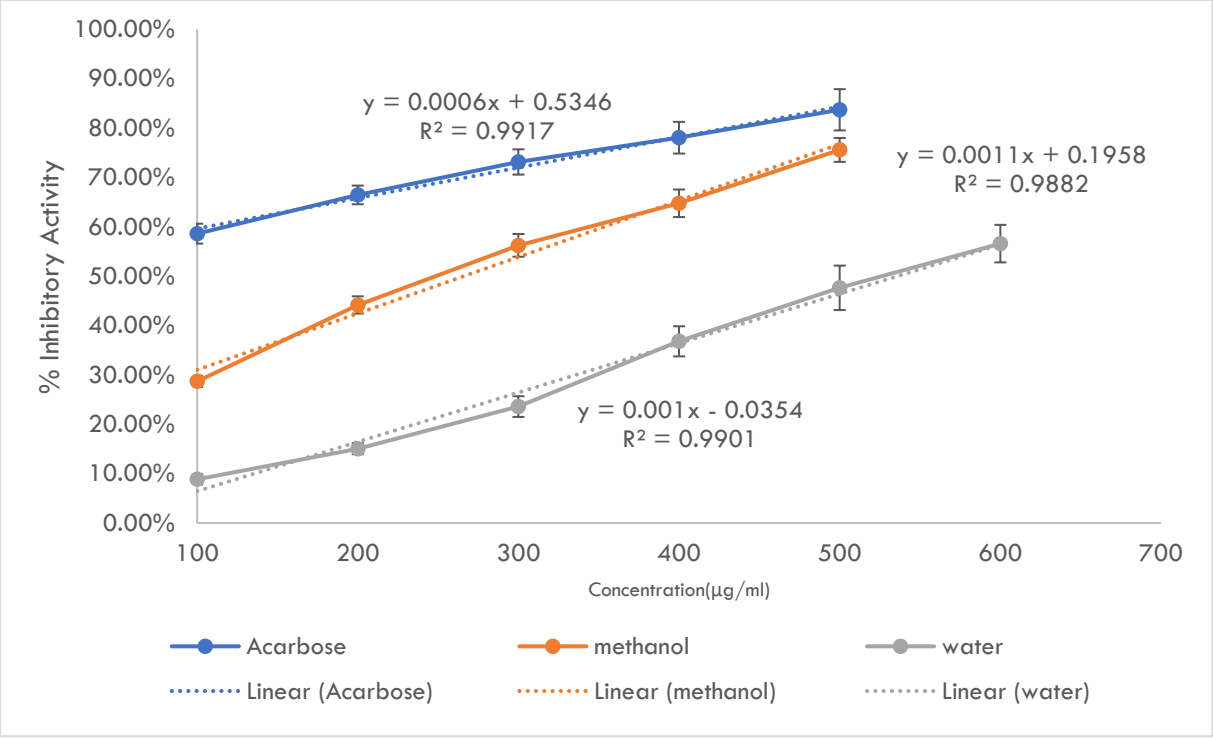


Figure 11: In vitro α-amylase inhibitory activity of the stem extract of *O. indicum*

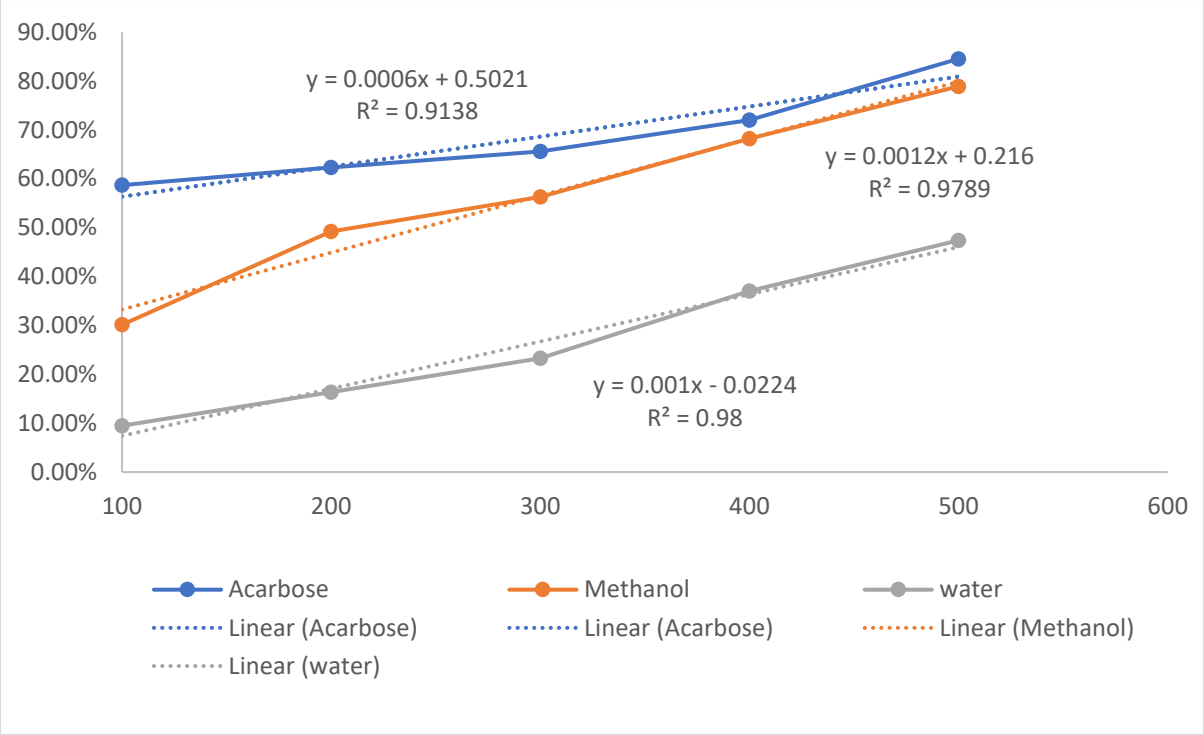


Figure 12: *In vitro* α-glucosidase inhibitory activity of the stem extract of *O. indicum*

5.10 *In vivo* anti-diabetic activity in streptozotocin-induced diabetic rats

5.11 Parameters to be measured

5.11.1 Blood Glucose Level measurement (BGL)

Table 16: Blood Glucose Level measurement (BGL)

Blood Glucose level	0 hr	72 hr	7 days	14 days	21 days	28 days
Group-I (Normal Control mg/dl)	87.33 ± 2.58	86.16 ± 3.65	86.83 ± 2.48	87.16 ± 1.47	86.66 ± 1.63	88 ± 1.41
Group-II (Positive Control mg/dl)	87.5 ± 2.42	351.6 ± 12.8	476.8 ± 13.1	518.8 ± 14.9	544 ± 15.0	553.6 ± 12.5
Group -III (Standard)	86.83 ± 1.47	365.3 ± 24.3	276 ± 15.5	212.5 ± 14.2	168.5 ± 12.2	136.6 ± 8.93
Group -IV (300mg)	86.66 ± 3.01	393.1 ± 20.0	325.3 ± 17.2	276.6 ± 18.9	230.8 ± 12.4	177.1 ± 8.72
Group - V (500mg)	87.08 ± 2.42	299.0 ± 20.1	291.2 ± 13.6	273.7 ± 10.2	229.8 ± 57.9	150.3 ± 6.74

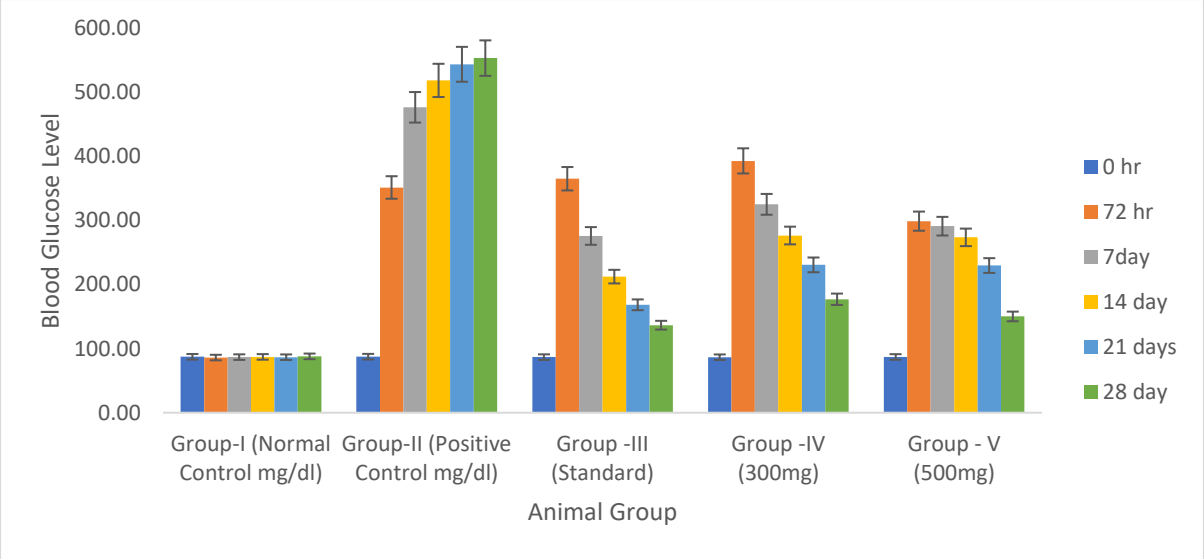


Figure 13: Blood Glucose Level Estimation

Normal Control - This group showed no significant changes in glucose levels, indicating that the rats maintained metabolic stability under normal physiological conditions.

Positive Control - This suggests that the positive control (likely untreated or treated with a hyperglycemic agent) induced a severe hyperglycemic state.

Standard - This indicates that the standard treatment effectively reduced hyperglycemia over time.

Dose (300mg) - This group exhibited a similar trend to the standard treatment, though the glucose reduction was slightly less pronounced.

Dose (500mg) - This suggests that the 500 mg dose demonstrated greater efficacy in reducing blood glucose levels than the 300 mg dose; however, its hypoglycemic effect was less than that of the standard treatment.

Table 17: Anova calculation of active extract

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	417245.3	4	104311.3	**20.04797**	4.494E-06	**3.006917**
Columns	19946.44	4	4986.611	**0.958395**	0.4567309	**3.006917**
Error	83249.39	16	5203.087			
Total	520441.1	24				

Table 18: t-Test: Two-Sample Assuming Equal Variances

t-Test: Two-Sample Assuming Equal Variances		
	Group - V (500mg)	Group III (Standard)
Mean	221.8947222	207.6388889
Variance	7364.023552	10145.46019
Observations	6	6
Pooled Variance	8754.741868	
Hypothesized Mean Difference	0	
df	10	
t Stat	**0.263895244**	
P(T<=t) one-tail	0.398609575	
t Critical one-tail	**1.812461123**	
P(T<=t) two-tail	0.797219151	
t Critical two-tail	2.228138852	

When linking the standard treatment to the experimental doses, the 500 mg dose was not as much of a real than the standard treatment. The data suggests that increasing the dosage from 300 mg to 500 mg results in a more substantial reduction in blood glucose levels, indicating a dose-dependent effect. t-test is an apply for determining the optimal dosage in future research. This is critical for interpreting the results in a broader pharmacological context. Additionally,

more data points between the 21st and 28th days could provide a clearer understanding of the glucose-lowering kinetics.

Overall, the outcomes accessible in the overhead table suggest that a plant extract dose of 500mg has a comparable hypoglycemic effect to the reference standard in rats. This makes them expectant applicants for further growth in dealings with diabetes. However, it is noteworthy to note that these verdicts are restricted to rat models and may not certainly be explained to humans. Supplementary work is needed to determine the safety, efficacy, and optimal dosing of these conducts in humans. The transformation in the percentage reduction of the blood glucose levels of different groups is significant ($p<0.005$).

5.11.2 Measurement of Body weight (b.w.)

The body weight in this group decreased consistently from 211.33 grams at 0 hours to 162.83 grams by day 28. This marked reduction in body weight indicates that the condition induced in these rats (likely hyperglycemia or another stressor) adversely affected their overall health form and growth. standard treatment mitigated the effects of the induced condition and promoted growth and recovery in the animals. The overall weight gain is slightly less than in the standard treatment group, suggesting a moderate effectiveness of the 300 mg dose. A 500 mg dose was effective in promoting weight gain and recovery and may be slightly more effective than the 300 mg dose. The body weight data recommend that both the standard treatment and the 500 mg dose (Group V) were effective in reversing the weight loss observed in the positive control group. The data indicate that after an initial drop in body weight at 72 hours (possibly due to the onset of treatment or condition), the rats in the treated groups began to recover and gain weight steadily. Overall, the study indicates that the treatments administered (both standard and experimental) effectively mitigate weight loss and promote recovery in rats subjected to a negative health condition, with the 500 mg dose showing considerable promise as an effective therapeutic dose.

Table 19: Measurement of Body Weight

Body Weight	0 hr	72 hr	7day	14 days	21 days	28 days
Group-I (Normal Control mg/dl)	207.5 ± 10.0	211 ± 8.80	215.1 ± 8.75	220.5 ± 10.4	224.8 ± 9.45	228.8 ± 9.62
Group-II (Positive Control mg/dl)	211.3 ± 4.36	197.6 ± 4.50	191.8 ± 6.79	180.8 ± 5.07	171.5 ± 4.84	162.8 ± 6.79
Group -III (Standard)	207 ± 5.40	196 ± 7.32	208.6 ± 6.18	225.1 ± 7.85	238.6 ± 6.65	250.6 ± 6.62
Group -IV (300mg)	206.1 ± 6.11	193.3 ± 6.62	201.6 ± 5.85	210.5 ± 5.82	218.1 ± 5.67	223.6 ± 7.44
Group - V (500mg)	208.6 ± 7.52	195.1 ± 6.52	205.3 ± 7.58	216.8 ± 7.52	224 ± 10.3	236.1 ± 6.43

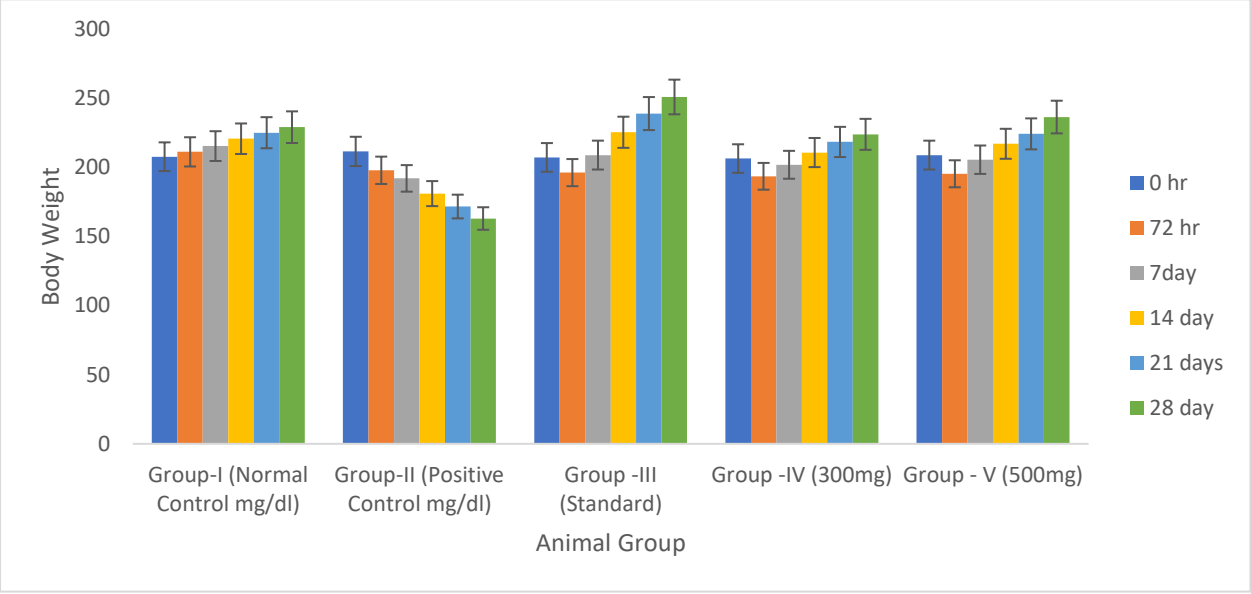


Figure 14: Measurement of Body Weight

5.11.3 Measurement of Insulin

Normal Control - This consistency suggests that the rats in the present group maintained normal glucose metabolism throughout the study, as expected for a control group.

Positive Control - The relatively low insulin levels despite high plasma glucose suggest significant insulin resistance. The body's incapacity to synthesize or utilize sufficient insulin is evident, as blood glucose levels still elevated while insulin levels do not increase appropriately.

Standard - The treatment effectively improved insulin sensitivity and helped maintain glucose homeostasis. The stable insulin levels suggest that the treatment reduced the need for high insulin production, reflecting better glucose utilization.

Dose (300 mg) - may indicate less effectiveness in restoring insulin sensitivity.

Dose (500 mg) - indicating that this higher dose might be more effective than the 300 mg dose in controlling blood glucose, but it still requires a higher level of insulin.

Table 20: Measurement of Insulin level

Insulin	**Group-I (Normal Control mg/dl)**	**Group-II (Positive Control mg/dl)**	**Group -III (Standard)**	**Group -IV (300mg)**	**Group - V (500mg)**
0 day	182.7±4.58	95.6±2.65	99.8±2.48	102.8±2.56	115.7±2.89
3 day	185.6±3.82	90.7±2.58	96.7±1.31	105.7±2.36	112.7±2.74
7 day	187.3±5.61	87.9±1.89	101.8±1.59	109.7±3.18	110.8±3.58
14 day	187.6±4.02	93.7±2.54	97.6±2.59	108.7±3.25	109.7±3.38
21 days	183.7±5.73	89.7±2.41	99.4±3.69	107.6±3.17	111.9±2.75
28 days	184.5±4.83	88.7±1.98	97.5±2.56	108.5±2.98	113.7±3.34

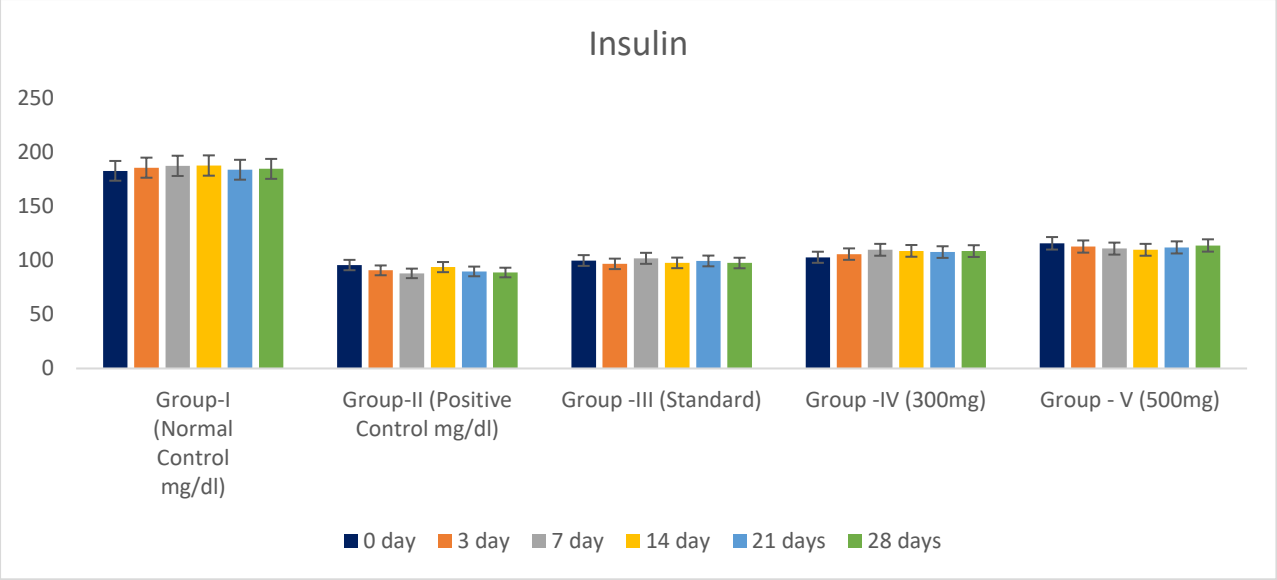


Figure 15: Measurement of Insulin level

5.11.4 Evaluate Oral glucose tolerance level (OGTT)

The Oral Glucose Tolerance Test (OGTT) measures how effectively the body can process glucose over time.

Normal Control - This suggests that the rats in this group maintained normal glucose metabolism.

Positive Control - The consistently high and rising glucose levels indicate severe glucose intolerance and possibly type 2 diabetes-like symptoms, reflecting poor glucose metabolism and insulin resistance.

Standard - This group represents a model of untreated diabetes. The slower rise in glucose levels indicates that the standard treatment improves glucose tolerance, though it may not completely normalize glucose levels.

Dose (300 mg) – Dose provides some glucose tolerance improvement but is not sufficient to prevent a significant increase in glucose levels, indicating suboptimal effectiveness in glucose metabolism management.

Dose (500 mg) - The 500mg Dose is more effective than the 300 mg dose in managing glucose levels, but still not as effective as the standard treatment.

Table 21: Measurement of Oral Glucose Tolerance Test

OGTT	Group I (Normal Control mg/dl)	Group-II (Positive Control mg/dl)	Group -III (Standard)	Group -IV (300mg)	Group - V (500mg)
0 day	100±3.65	199±4.89	135±4.45	180±4.69	165±4.38
3 days	115±3.82	245±6.23	140±6.79	222±7.03	201±6.72
7 days	145±5.3	310±9.8	189±10.36	289±10.6	245±10.29
14 days	179±5.8	375±10.58	219±11.14	345±11.38	289±11.07
21 days	210±5.73	445±14.27	245±14.83	400±15.07	322±14.76
28 days	215±6.23	485±13.65	262±14.21	443±14.45	339±14.14

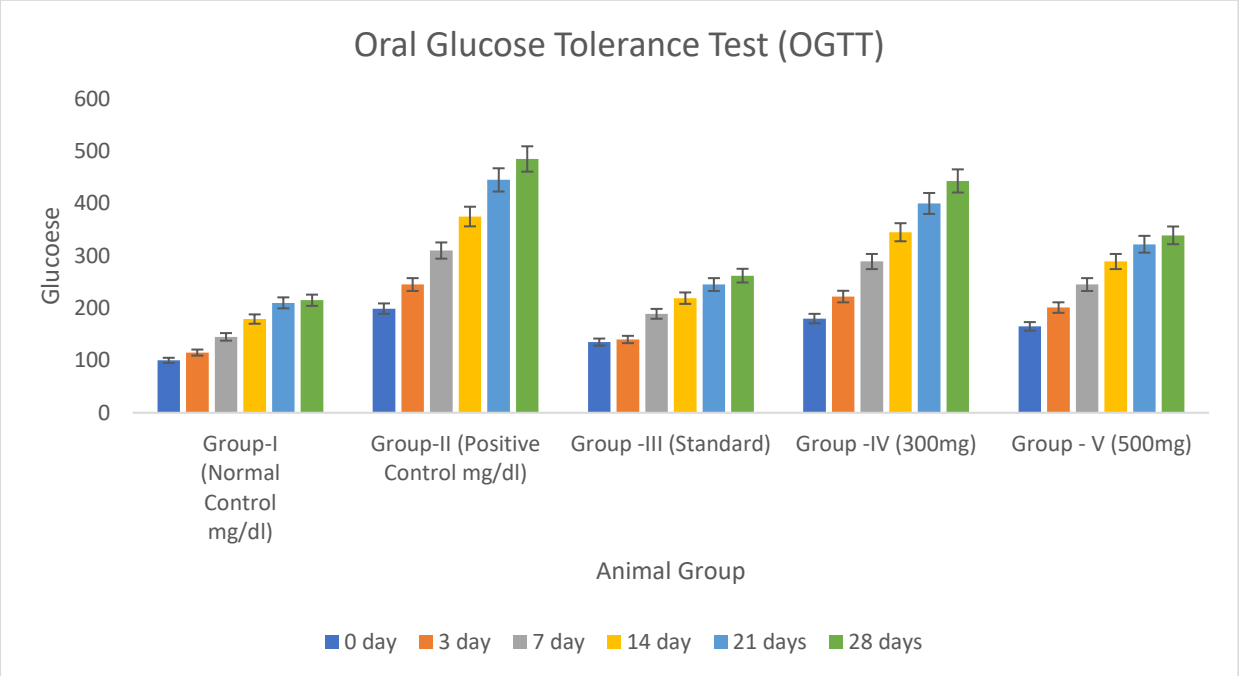


Figure 16: Measurement of Oral Glucose Tolerance Test

5.11.5 Measurement of Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST) is an enzyme in the liver, heart, and muscles. It is commonly used as a biomarker for liver health, with elevated levels indicating liver damage or other tissue damage.

Normal Control - The AST levels remain within the normal range throughout the experiment, indicating no liver or tissue damage. This group represents the healthy control.

Positive Control - The consistently high AST levels indicate liver or tissue damage, reflecting a pathological condition such as untreated diabetes or metabolic stress—this group models liver dysfunction or injury without treatment.

Standard - AST stages continued comparatively steady with slight fluctuations, indicating that the standard treatment helps to maintain liver function and prevent significant damage, resulting in near-normal AST levels.

Dose (300 mg) – It provides partial hepatoprotection but does not completely prevent hepatic stress or injury. It is somewhat effective but not as potent as the standard treatment.

Dose (500 mg) - appears more protective than the lower dose (300 mg), though still not as effective as the standard.

Table 22: Measurement of Aspartate Aminotransferase (AST)

AST	Group-I (Normal Control mg/dl)	Group-II (Positive Control mg/dl)	Group -III (Standard)	Group -IV (300mg)	Group - V (500mg)
0 day	83.24±2.58	129.8±2.89	91.4±2.48	101.8±1.67	95.3±1.63
3 days	84.6±2.42	124.8±2.58	89.7±1.56	102.8±3.65	97.4±2.65
7 days	77.9±1.47	129.5±2.78	96.4±2.56	109.5±2.58	106.7±2.48

14 days	85.9±2.65	127.9±3.42	97.5±3.01	107.8±2.59	99.4±1.47
21 days	81.4±2.42	125.1±3.12	91.7±2.42	108.4±2.43	101.8±3.01
28 days	84.9±2.58	123.7±1.89	86.5±2.36	106.4±3.18	99.8±2.69

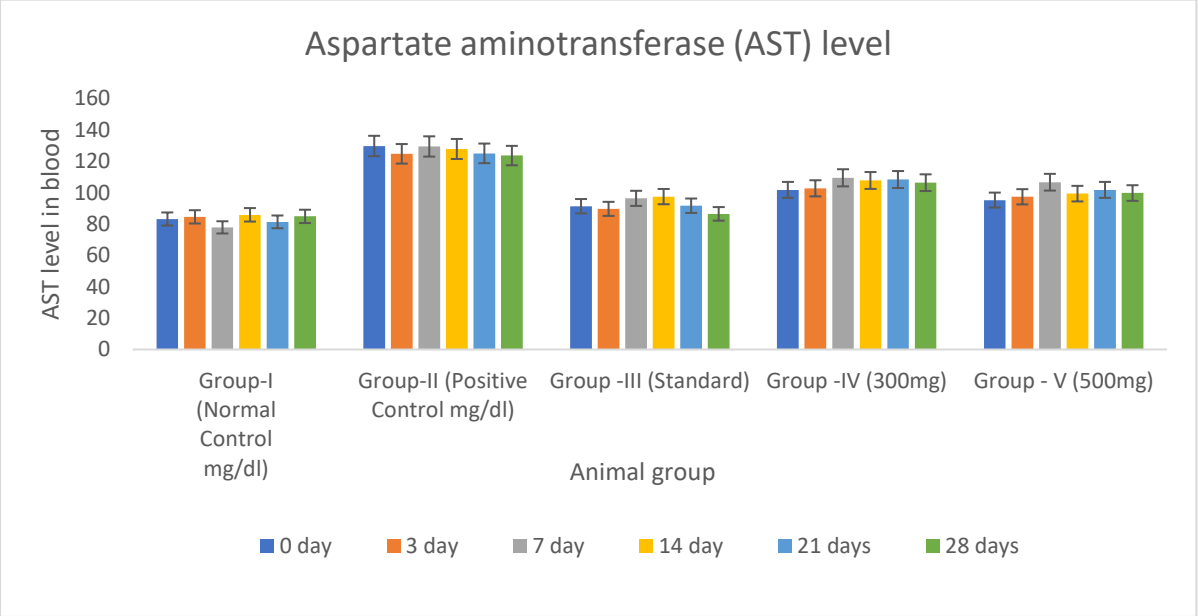


Figure 17: Measurement of Aspartate Aminotransferase (AST)

5.11.6 Measurement of Alanine Aminotransferase (ALT)

Normal Control – This group represents the normal baseline for liver function.

Positive Control —This group has high ALT levels, suggesting liver stress or damage, which could be due to the condition or treatment used in this group.

Standard - they fluctuate within a moderate range, indicating better liver function.

Dose (300 mg) - The levels are higher than the normal controls, suggesting some liver stress.

Dose (500 mg) - some protective or therapeutic effect on liver function, though the ALT continues to exceed that of the normal control group.

Table 23: Measurement of Alanine Aminotransferase (ALT)

ALT	Group-I (Normal Control mg/dl)	Group-II (Positive Control mg/dl)	Group -III (Standard)	Group - IV (300mg)	Group - V (500mg)
0 day	35.7±1.08	74.8±1.39	40.8±0.98	61.7±1.56	51.8±1.05
3 days	38.7±0.92	77.8±1.08	42.8±0.78	62.5±2.15	54.8±1.15
7 days	34.8±0.95	71.6±1.28	45.7±1.06	68.7±1.08	56.7±0.98
14 days	33.7±1.15	79.8±1.92	46.7±1.51	59.7±1.09	50.7±0.94
21 days	31.8±0.92	75.8±1.62	42.8±0.92	62.7±0.93	50.7±1.51
28 days	38.9±1.14	72.6±1.25	41.8±0.86	61.4±1.68	52.7±1.19

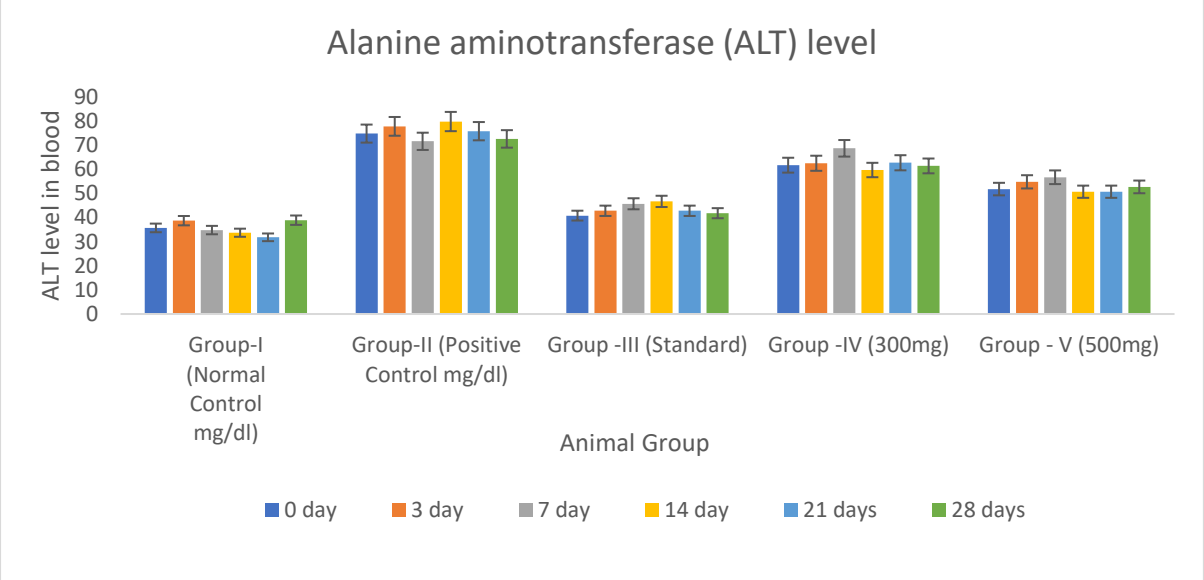


Figure 18: Measurement of Alanine Aminotransferase (ALT)

5.11.7 Measurement of Alkaline Phosphatase (ALP)

Table 24: Measurement of Alkaline phosphatase (ALP)

ALP	Group-I (Normal Control mg/dl)	Group-II (Positive Control mg/dl)	Group -III (Standard)	Group -IV (300mg)	Group - V (500mg)
0 day	180.9±9.02	235.7±8.75	200.7±10.4	205.7±9.45	215.7±9.62
3 days	181.7±4.50	238.7±8.63	202.7±7.56	210.8±9.63	219.7±8.52
7 days	183.7±7.32	234.9±6.18	201.8±7.85	211.8±10.25	219.2±7.96
14 days	186.7±8.97	236.7±8.56	198.4±8.69	207.9±8.65	215.7±7.44
21 days	183.7±7.85	237.8±7.58	199.7±9.56	209.7±10.3	216.7±7.89
28 days	187.5±8.34	239.7±10.25	203.7±10.56	208.4±9.25	217.8±11.18

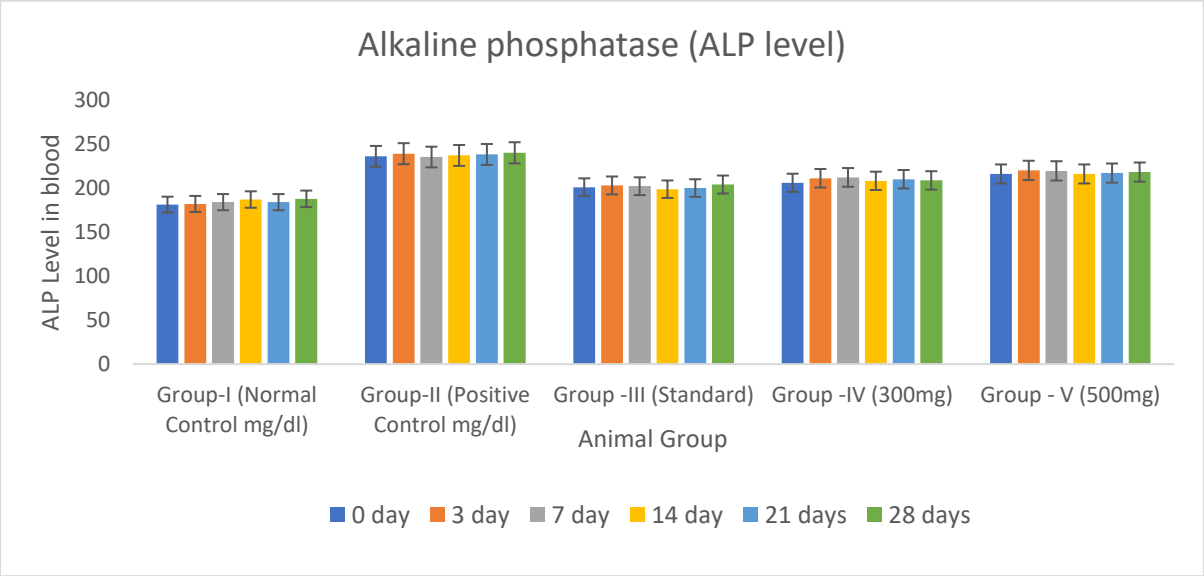


Figure 19: Measurement of Alkaline phosphatase (ALP)

Normal Control - These values reflect normal liver function and bone metabolism.

Positive Control - elevated levels suggest liver or bone damage or stress.

Standard - protective effect on liver and bone function, though not entirely bringing it back to normal.

-Dose (300 mg) - helps reduce some liver or bone stress, but not entirely.

Dose (500 mg) - Remains elevated compared to the normal control group, suggesting that the higher dose of treatment provides a protective effect, maintaining more consistent liver and bone function.

There was no significant variation in ALP levels across all groups. Nevertheless, a significant elevation in ALT levels was observed in diabetic control rats compared to normal controls, as well as in rats treated with the standard and extract. Likewise, AST levels showed a significant increase in diabetic control rats and those receiving the standard and extract treatments compared to the normal control group.

5.12 Measurement of total phenolic content

Table 25: Measurement of total phenolic content

Mean Absorbance ± SD	Concentration of Gallic acid (mcg/ml)
0.051±0.002	**2.5**
0.094±0.003	**5**
0.17±0.002	**10**
0.24±0.002	**20**
0.309±0.003	**25**
0.224±0.003	**Sample**

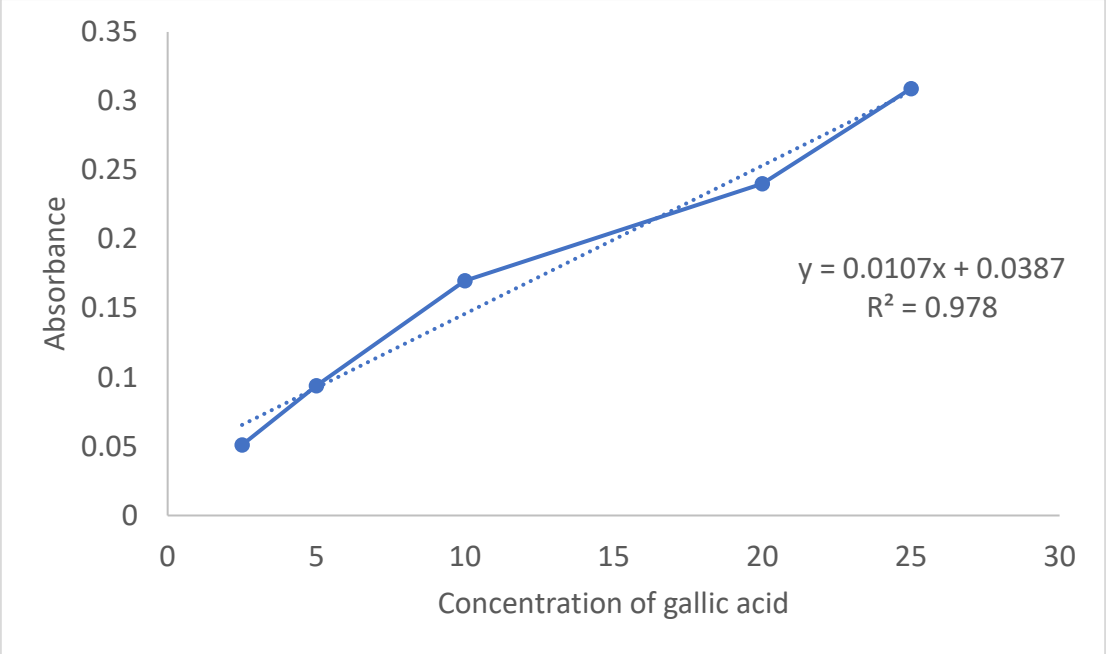


Figure 20: Analysis of total phenolic content of the stem

From the Graph, the Total phenolic content present in the stem part of *Oroxylum indicum* is 240.34mg gallic acid/g.

5.13 Estimation of total flavonoids by the colorimetric method

Table 26: estimation of total flavonoids

Mean Absorbance ± SD	Concentration of Rutin (mcg/ml)
0.167±0.03	0.2
0.307±0.05	0.4
0.46±0.04	0.6
0.623±0.06	0.8
0.78±0.07	1
0.67±0.04	**Test**

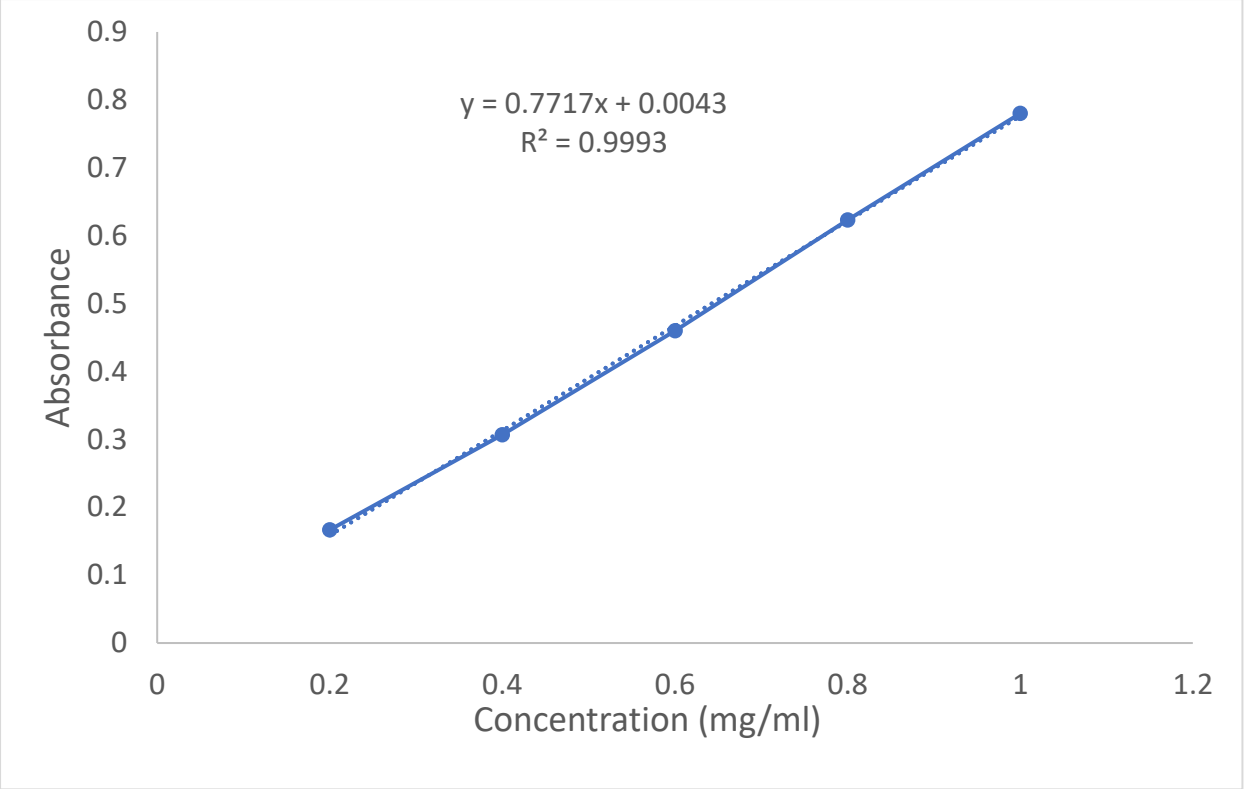
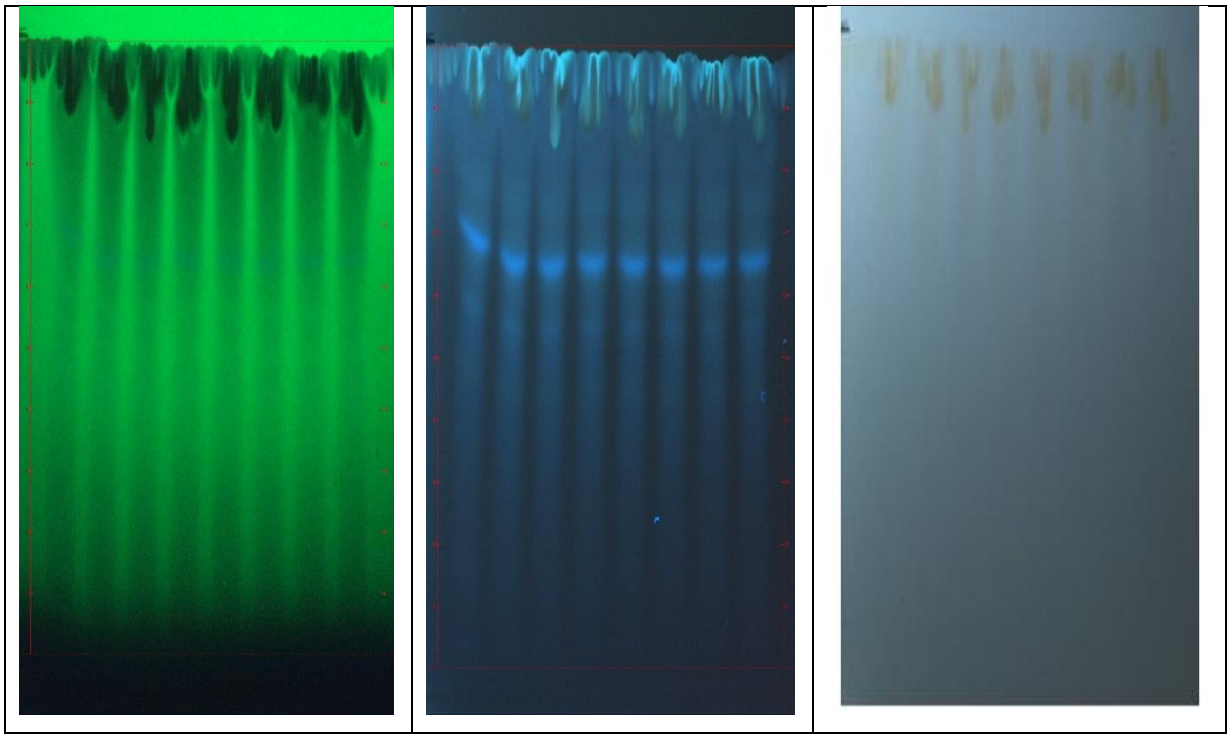


Figure 21: Total flavonoids estimation by the colorimetry method

From the Graph, the Total Flavonoids present in the stem part of *Oroxylum indicum* are **1.62% w/w**.

5.13 HPTLC Fingerprinting of active extract

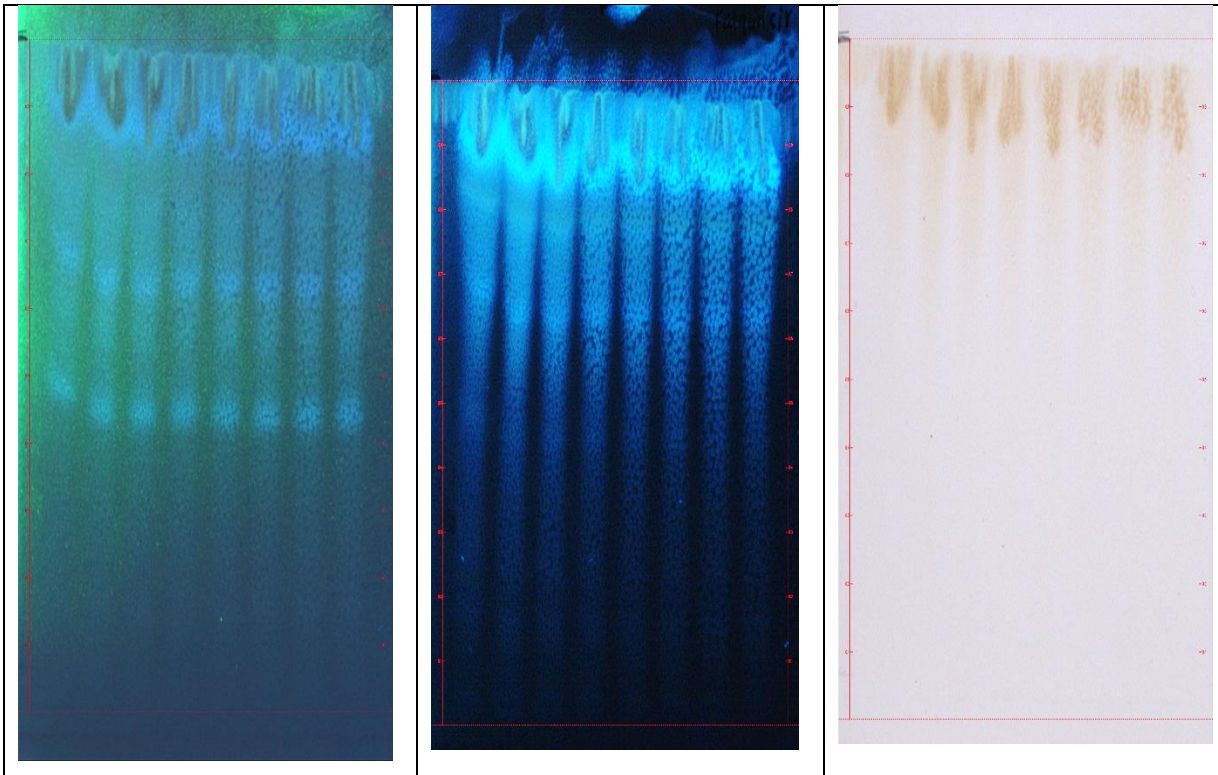


UV254nm	UV366nm	Under white light

Figure 22: HPTLC profile of methanolic extract (before derivatization)

Table 27: HPTLC profile of methanolic extract (before derivatization)

No. of resolved bands	**Before derivatization**		
	UV254nm	UV366nm	Under white light
1	0.18	0.18	0.18
2	0.37	0.4	
3		0.5	
4			



UV254nm	UV366nm	Under white light

Figure 23: HPTLC profile of methanolic extract (after derivatization)

Table 15: HPTLC profile of methanolic extract (after derivatization)

No. of resolved bands	After derivatization		
	UV254nm	UV254nm	Under white light
1	0.18	0.18	0.18
2	0.37	0.37	
3	0.42	0.42	
4	0.53	0.53	

5.14 HPLC Analysis of Total Flavonoids in Methanolic Extract of Oroxylum indicum Stem

- **Stationary Phase:** C18 Column (250 mm × 4.6 mm, 5 µm) (Patel & Patel, 2018)
- **Mobile Phase:** Methanol: Water: Phosphoric Acid (60:38:2, v/v/v)
- **Flow Rate:** 1.0 mL/min
- **Detection Wavelength:** 360 nm (UV-Visible detector)
- **Injection Volume:** 20 µL
- **Run Time:** 15 minutes

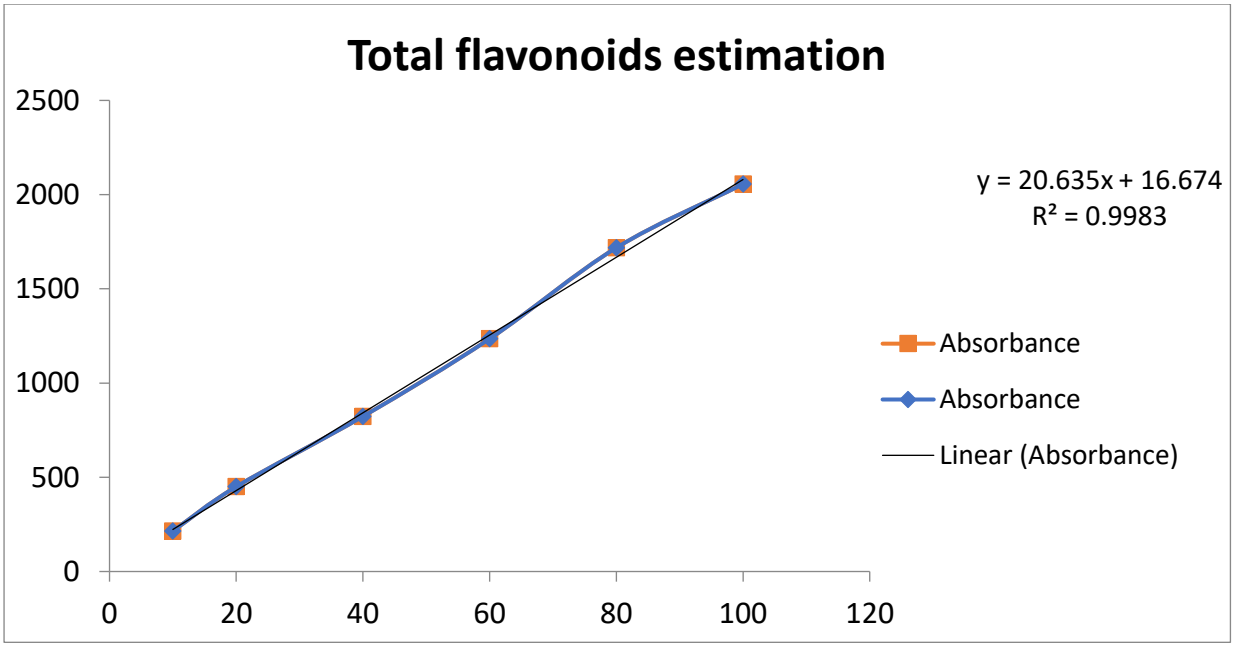


Figure 24: Calibration curve of standard

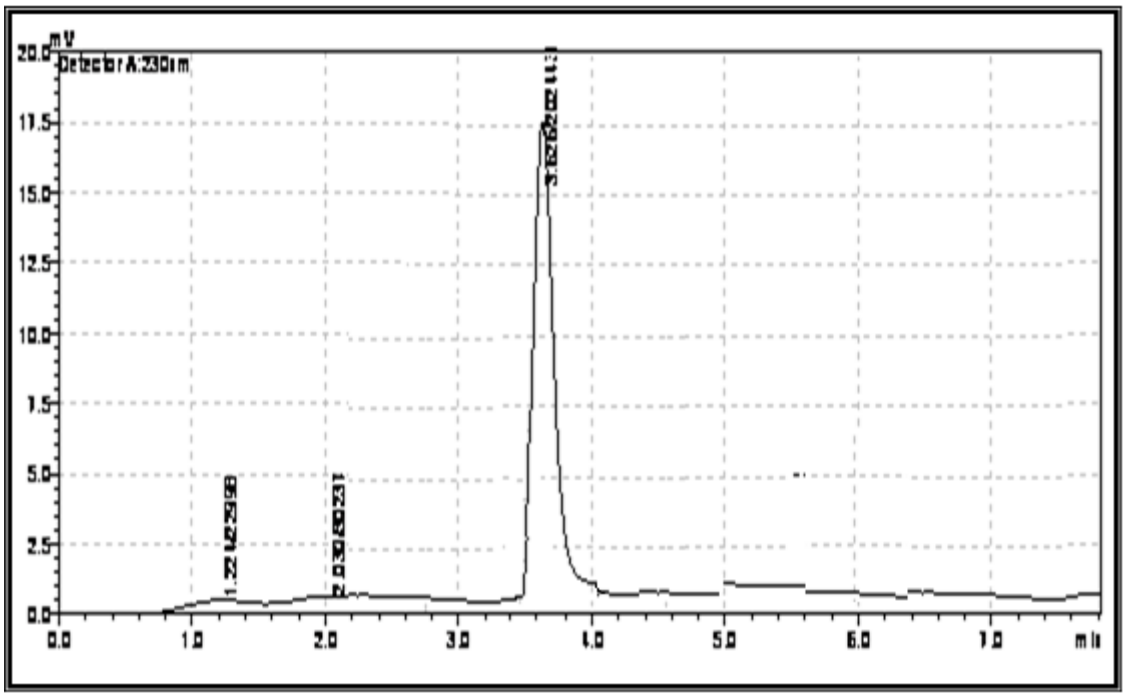


Figure 25: HPLC profile of standard

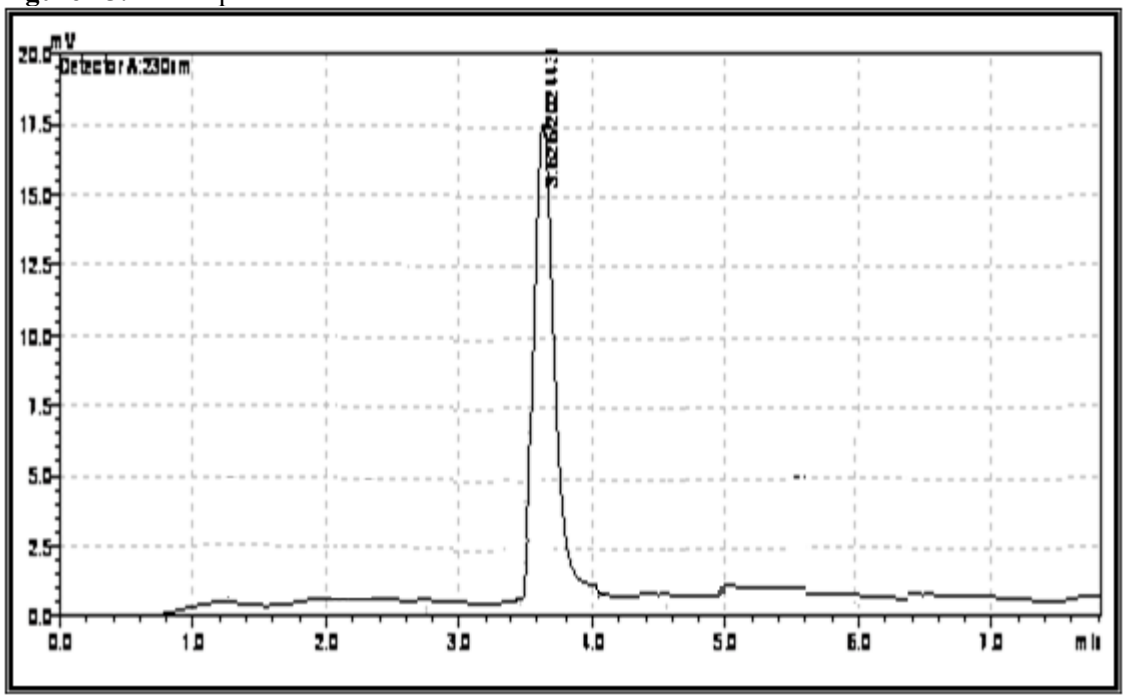


Figure 26: HPLC profile of active extract

The total flavonoid concentration of the extract of methanol was determined spectrophotometrically using a quercetin calibration curve as the standard reference. The

measured flavonoid concentration was **1.618% w/w**, aligning well with values reported in the literature.

DISCUSSION

The present investigation was designed to explore the pharmacognostic characteristics of *Oroxylum indicum*, focusing specifically on its leaves and stems. This comprehensive study aimed to establish a detailed profile that could support the identification, authentication, and standardization of the plant material used in traditional medicine.

Microscopic and Physical Analysis

Microscopic examination of the plant revealed several distinctive anatomical traits. Transverse sections of the leaf displayed structural features that are crucial for botanical identification. Among these were **anomocytic stomata**, which lack subsidiary cells and are surrounded by epidermal cells of similar shape and size. The presence of **unicellular covering trichomes**—hair-like outgrowths on the epidermis—was also noted. These structures play a role in protecting the plant and can serve as diagnostic markers.

Further microscopic observations included **reticulate xylem vessels**, which exhibit a net-like pattern aiding in water conduction, and **rosettes of calcium oxalate crystals**, which are often used as taxonomic indicators. The **stomatal index**, representing the ratio of stomata to epidermal cells, was calculated to provide additional data for species verification.

In addition to anatomical studies, physical parameters were assessed to evaluate the quality and purity of the plant material. These included the determination of **extractive values** in solvents such as water, alcohol, and ether. Extractive values indicate the amount of active constituents soluble in each solvent and are essential for assessing the efficacy of herbal preparations. The study also measured **total ash**, **acid-insoluble ash**, and **water-soluble ash**—parameters that help detect inorganic impurities and ensure the material's authenticity. All calculations were based on **air-dried powdered samples**, which served as the standard reference.

Phytochemical Screening and Solvent Extraction

To isolate bioactive compounds, the leaves underwent **successive exhaustive extraction** using solvents arranged in increasing order of polarity. This method allows for the separation of constituents based on their solubility, thereby providing a clearer understanding of the plant's chemical composition.

The **extractive yields** offered insight into the concentration and nature of phytochemicals present in each solvent. Subsequent **phytochemical screening** revealed that the **methanolic extract** contained **phenolic compounds, tannins, and flavonoids**, all known for their antioxidant and therapeutic properties. Meanwhile, the **aqueous extract** tested positive for **alkaloids, carbohydrates, and phenolic compounds**, suggesting a diverse range of bioactive molecules.

These findings contribute significantly to the **pharmacognostic profile** of *Oroxylum indicum*, enabling its identification even in powdered form. This is particularly useful for distinguishing genuine plant material from adulterants or substitutes in herbal formulations.

Diabetes Mellitus: A Global Health Challenge

Diabetes mellitus is a widespread metabolic disorder characterized by **chronic hyperglycemia**, resulting from insufficient insulin production, insulin resistance, or both. The condition is especially prevalent in **low-income and developing countries**, with **India ranking second globally** in terms of diabetic population.

Several factors contribute to the rising incidence of diabetes, including **poor dietary habits**, **environmental stressors**, **obesity**, and a **sedentary lifestyle**. If not properly managed, diabetes can lead to serious complications such as **diabetic ketoacidosis**, **cardiovascular disease**, **kidney damage (nephropathy)**, **nerve degeneration (neuropathy)**, and **vision impairment (retinopathy)**.

Enzyme Inhibition: A Therapeutic Strategy

One effective strategy for controlling **postprandial hyperglycemia**—the spike in blood sugar following meals—is the inhibition of **carbohydrate-digesting enzymes**, specifically **α-amylase** and **α-glucosidase**. These enzymes are responsible for breaking down complex carbohydrates into simple sugars that are easily absorbed into the bloodstream.

By inhibiting these enzymes, the digestion and absorption of glucose are slowed, resulting in a more gradual increase in blood sugar levels. Pharmaceutical agents such as **acarbose** and **miglitol** are commonly used for this purpose. However, these drugs often cause **gastrointestinal side effects** like bloating, diarrhea, and abdominal discomfort, which can reduce patient compliance. Additionally, they are **contraindicated in individuals with liver or kidney disorders**, limiting their use in vulnerable populations.

***Oroxylum indicum* as a Natural Enzyme Inhibitor**

In this study, both **methanolic and aqueous extracts** of *Oroxylum indicum* stem were evaluated for their ability to inhibit **α-amylase and α-glucosidase**. The results were promising: both extracts showed **significant inhibitory activity**, with the methanolic extract demonstrating **greater potency**.

This enzyme inhibition is attributed to the presence of **polyphenolic compounds**, particularly **flavonoids**, **tannins**, and **phenolic acids**, which are known to interfere with carbohydrate metabolism. These findings suggest that *Oroxylum indicum* could serve as a **plant-based alternative** for managing postprandial blood sugar levels, offering a safer and more tolerable option compared to synthetic drugs.

In vivo antidiabetic activity

The present study evaluated the in vivo antidiabetic potential of the methanolic extract derived from the stem of *Oroxylum indicum* using streptozotocin (STZ)-induced diabetic rats as the experimental model. The findings revealed a significant reduction in fasting blood glucose levels in extract-treated groups compared to diabetic controls, indicating promising hypoglycemic activity.

The observed glucose-lowering effect can be attributed to the rich presence of bioactive compounds in the methanolic extract, particularly **flavonoids**, **tannins**, and **phenolic acids**. These phytoconstituents are known to exert multiple pharmacological actions, including **antioxidant**, **anti-inflammatory**, and **enzyme-inhibitory** effects. In this study, the extract likely contributed to the inhibition of key carbohydrate-digesting enzymes such as **α-amylase** and **α-glucosidase**, thereby slowing the breakdown and absorption of dietary sugars. This mechanism aligns with the therapeutic strategy employed by conventional antidiabetic drugs like acarbose, but with potentially fewer side effects.

Furthermore, the extract-treated groups showed improvements in **body weight**, **lipid profile**, and **general behavior**, suggesting an overall enhancement in metabolic health. These systemic benefits reinforce the therapeutic potential of *Oroxylum indicum* as a multifaceted antidiabetic agent.

Histopathological examination of pancreatic tissue revealed signs of **β-cell preservation or regeneration** in treated animals, which may contribute to improved insulin secretion and glucose regulation. Although the exact molecular pathways remain to be elucidated, the data suggest that the extract may exert both **insulin-sensitizing** and **β-cell protective** effects.

Importantly, no signs of acute toxicity or adverse reactions were observed during the treatment period, even at higher doses. This supports the **safety profile** of the methanolic stem extract and underscores its suitability for further pharmacological development.

Liver Enzyme Analysis and Hepatoprotective Potential

To assess the safety and additional therapeutic benefits of the plant, **liver enzyme levels** were measured in diabetic rats. Elevated levels of **alanine aminotransferase (ALT)** and **aspartate aminotransferase (AST)** were observed in the diabetic control group, indicating **hepatic stress or injury**. These elevations are commonly associated with increased **protein breakdown** and **gluconeogenesis**, which are hallmarks of diabetes.

Interestingly, treatment with ***Bignonia indica*** extracts resulted in only **mild elevations** in ALT and AST, particularly at a dosage of **500 mg/kg**, suggesting **hepatoprotective effects**. No significant changes were noted in **alkaline phosphatase (ALP)** levels, indicating that the liver's excretory function remained stable across treated groups.

Therapeutic Implications and Future Prospects

In summary, the methanolic extract of *Oroxylum indicum* stem exhibits significant antidiabetic activity in vivo, likely mediated through enzyme inhibition, antioxidant defense, and hepatic protection. These findings validate its traditional use in managing diabetes and highlight its potential as a **natural alternative to synthetic antidiabetic drugs**. Future studies should focus on isolating specific active compounds, exploring molecular mechanisms, and conducting clinical trials to confirm efficacy in human subjects.

Phytochemical analysis of the stem extract confirmed the presence of **flavonoids** and other **polyphenolic compounds**, which are likely responsible for both the **antidiabetic** and **hepatoprotective** effects observed. These results underscore the **therapeutic potential** of *Oroxylum indicum* and support its use in the development of **plant-based antidiabetic agents**.

Moreover, the study highlights the importance of **exploring indigenous medicinal plants** as sources of novel treatments. With rising global interest in natural remedies and holistic health,

Oroxylum indicum stands out as a promising candidate for further research and clinical application.

CONCLUSION

Oroxylum indicum is a widely cited drug in Ayurvedic medicine. It's recognized as ace of the utmost multipurpose plants, having a wide range of therapeutic activities.[116] The present study may be useful to supplement the information with regard to its standardization and identification, and in carrying out further research and its use in traditional systems of medicine. Apart from this, these studies have experiential that pharmacognostic and physicochemical parameters are of great value in quality control and formulation development.[117]

Medicinal plants offer a promising natural alternative in the management of diabetes, particularly type 2 diabetes. Their diverse mechanisms of action, safety profile, and accessibility make them valuable either as standalone treatments or complementary therapies.[118]

However, scientific rigor in standardization, clinical validation, and regulatory approval is essential before they can be widely recommended in clinical practice. As the global burden of diabetes rises, integrating traditional plant-based wisdom with modern pharmacology could provide sustainable and effective solutions.[119]

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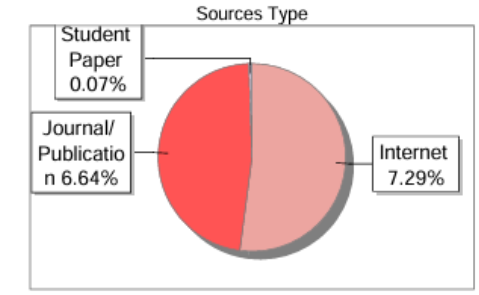

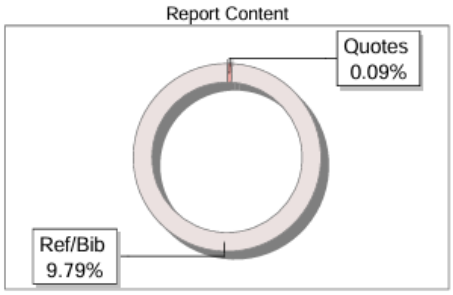

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STUDIES ON PHARMACOGNOSITIC PARAMETERS AND PHYTOCHEMICAL SCREENING OF *OROXYLUM INDICUM* LEAF

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ABSTRACT

Background: To confirm reproducible quality of plant material, proper regulation of starting material is important. Thus, there has been an increase in the standardization of selected herbal plants of potential medicinal values. Identification of herbal drugs by pharmacognostic studies is more reliable. Till date there is no work on pharmacognositic evaluation and phytochemical screening of *Oroxylum indicum* leaf. Therefore, the current study designed to the pharmacognostic parameters of the leaves of *Oroxylum indicum*.

Materials and Methods: Several quality control parameters like morphological study, transverse section, powder microscopic evaluation, leaf constants parameter such as stomatal index, physicochemical evaluations (moisture content, ash values, extractive values), preliminary phytochemical screening were carried out.

Results: Major microscopic characters such as Spongy mesophyll, Upper palisade, stomata and trichomes. Phytochemical screening of several extracts of leaves indicated the presence of phenolic compounds and flavonoids in the methanol extract and in aqueous extract exhibited the presence of alkaloids and phenolic compounds.

Conclusion: The quality control parameters bring referential information for proper identification of the herbal material and will also be useful in preparation of monographs.

Keywords: *Oroxylum indicum*, Pharmacognostic parameters, Preliminary Phytochemical Screening

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In Vitro Evaluation of Antidiabetic Activity of Stem Extracts of *Oroxylum Indicum*

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Abstract

Background: It is estimated 180 million people in the world have diabetes mellitus. The ethnobotanical information reports about 1000 plants that may possess antidiabetic activity. Roots, leaves, seeds, fruits and stems of *Oroxylum indicum* have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine.,

Aim: The current study designed In vitro evaluation of antidiabetic activity of aqueous and methanolic stem extracts of *Oroxylum indicum.*

Materials & Methods: About 100, 200, 300, 400, 500 µg concentrations of acarbose, aqueous, and ethanolic stem extracts of *O. indicum* were used for the study. The absorbance values were taken in spectrophotometer at 540 nm and 546 nm for α-amylase and α-glucosidase enzyme, respectively.

Results: Dose-dependent % inhibition of α-amylase and α-glucosidase enzymes are observed with the both extracts. However, compare with aqueous extract, methanolic extract shows more % inhibition.

Conclusion: In this study, aqueous and methanolic stem extracts of *O. indicum* showed the in vitro antidiabetic activity.

Keywords: *Oroxylum indicum, In Vitro Antidiabetic activity, α-glucosidase, α-amylase, Acarbose*

Introduction

Diabetes is metabolic disorder characterized by hyperglycaemia and the body is unable to maintain adequate insulin secretion. It is estimated 180 million people in the world have DM. That's roughly 6% of the world population. These numbers are estimated to double by 2030.[1] The World Health Organization estimates that 80% of the world's population relies on herbal medicine.[2] The use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice from time immemorial.[3] Alternative systems of medicine based on plant extracts have thrived through the ages and are still practiced by a large population for the management of diabetes. [4] The world health organization expert committee on diabetes also suggested that medicinal herbs be further investigated as they are repetitively considered to be less toxic and side effects. [5] *Oroxylum indicum* (Bignoniaceae) also known as 'Sonapatha' is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years. [6] Roots, leaves and stems of *Oroxylum indicum* have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine for treatment of various disorders as well as used as a tonic and Rasayana drug. [7,8] Leaves are used externally to treat an enlarged spleen and also to alleviate headaches and ulcers and also reported for its analgesic and antimicrobial activity. [9,10] The leaves have been reported containing flavones and their glycosides baicalein and scutellarein. Leaves also contain an anthraquinone, aloe-emodin. [11,12,13]

Certificate

This is to certify that the project proposal No. IAEC/DPS/SU/2315 entitled evaluation of Anti diabetic activity and phytochemical screening of *oroxylum indicum* plant by Mr. Falgun Dhabaliya has been approved/recommended by the IAEC of Department of Pharmaceutical Sciences, in its meeting held on 17th Januarys 2023 and 56 Sprague Dawley male rats have been sanctioned under this.

Authorized by	**Name**	**Signature**	**Date**
Chairman:	Dr. Tirupesh pathani		17/1/23
Member Secretary:	Dr. Riddhi shukla		17/1/23
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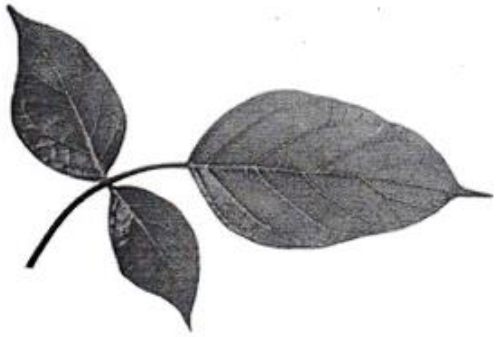
AUTHENTICATION CERTIFICATE

Based upon the organoleptic/macroscopic examination of the fresh sample, it is certified that the specimen given by Mr. Falgun Dhabaliya is identified as below:



Binomial: *Oroxylum indicum.*

Synonym: Indian trumpet tree, Shyonak

Family: Bignoniaceae

Regional name: tentoo

Voucher specimen no.: AU/FOS/LS/09-2022/04

Reference: Flora of Gujarat State

Date: 28/09/2022

Place: Atmiya University, Rajkot.

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