Phytochemical Characterization And In Vitro Evaluation Of Antidiabetic And Anticancer Activities Of Ethanolic Extract Of Terminalia Catappa Red Leaves

Dasrao A. Patil[1*], Dr. Disha Chouhan[2]
[1*] Research Scholar, Institute Of Pharmaceutical Science And Research, Sardar Patel University, Balaghat, Madhya Pradesh, India 481001. Email: dasrao.patil@gmail.com (Corresponding Author)
[2] Professor, Institute Of Pharmaceutical Science And Research, Sardar Patel University, Balaghat, Madhya Pradesh, India 481001.

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Abstract
The present study aimed to investigate the phytochemical profile and evaluate the in vitro antidiabetic and anticancer activities of the ethanolic extract of terminalia catappa red leaves. Preliminary phytochemical screening revealed the presence of key bioactive constituents, including flavonoids, phenolics, tannins, and terpenoids. The antidiabetic potential was assessed through α-amylase and α-glucosidase inhibitory assays, along with a glucose diffusion model. The extract exhibited significant, concentration-dependent inhibition of both enzymes and effectively reduced glucose diffusion, indicating its potential to control postprandial hyperglycemia. The anticancer activity was evaluated against mcf-7 using the mtt assay, which demonstrated a dose-dependent decrease in cell viability with an ic_{50} value of approximately 72.5 µg/ml. Further confirmation of cytotoxicity was obtained through acridine orange/ethidium bromide (ao/eb) staining, which revealed apoptosis induction characterized by nuclear condensation and membrane alterations. Overall, the findings suggest that the ethanolic extract of terminalia catappa red leaves possesses promising antidiabetic and anticancer properties, supporting its potential as a natural therapeutic agent for further pharmacological development.

Keywords: Terminalia Catappa, Red Leaves, Antidiabetic Activity, Anticancer Activity, A-Amylase Inhibition, A-Glucosidase Inhibition, Glucose Diffusion Assay, Mtt Assay, Apoptosis, Ao/Eb Staining, Phytochemicals.
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INTRODUCTION:

Non-communicable diseases such as diabetes mellitus and cancer represent two of the most pressing global health concerns, contributing substantially to morbidity and mortality worldwide. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia arising from defects in insulin secretion, insulin action, or both. Prolonged elevation of blood glucose levels is associated with severe complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy.[1-2] On the other hand, cancer is a multifactorial disease marked by uncontrolled cell division, evasion of programmed cell death, and the ability of abnormal cells to invade surrounding tissues and metastasize to distant organs. Despite advancements in therapeutic strategies, the long-term management of both conditions remains challenging due to drug resistance, adverse effects, and high treatment costs.[3]

In recent years, there has been growing interest in the use of plant-based therapeutics as safer and more cost-effective alternatives to synthetic drugs. Medicinal plants are rich sources of structurally diverse bioactive compounds, including flavonoids, phenolic acids, tannins, alkaloids, and terpenoids, which exhibit a wide spectrum of pharmacological activities. These phytoconstituents are known to possess antioxidant properties that help mitigate oxidative stress, a key factor implicated in the pathogenesis of both diabetes and cancer. Consequently, the exploration of plant extracts for multifunctional therapeutic effects has become an important area of research.[4]

Terminalia catappa, commonly referred to as tropical almond, is a widely distributed medicinal plant belonging to the family Combretaceae. Various parts of this plant have been traditionally utilized in ethnomedicine for the treatment of ailments such as inflammation, liver disorders, skin diseases, and metabolic conditions. The red leaves of *Terminalia catappa*, in particular, are reported to be rich in polyphenolic compounds and other secondary metabolites that contribute to its biological activities. Previous studies have suggested that extracts of this plant exhibit antioxidant, antimicrobial, hepatoprotective, and potential antidiabetic effects.[5]

From a mechanistic perspective, plant-derived compounds can contribute to glycemic control by inhibiting key carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, thereby slowing down the digestion and absorption of glucose. This approach is widely recognized as an effective strategy to manage postprandial hyperglycemia. Similarly, several phytochemicals have demonstrated the ability to exert anticancer effects through mechanisms such as induction of apoptosis, inhibition of cell proliferation, and modulation of signaling pathways involved in tumor progression.[6-8]

Considering these therapeutic potentials, the present study was designed to investigate the phytochemical profile and evaluate the in vitro antidiabetic and anticancer activities of the ethanolic extract of *Terminalia catappa* red leaves. The study aims to provide scientific validation for its traditional use and to explore its potential as a natural source of bioactive compounds for the development of novel therapeutic agents.

MATERIALS AND METHODS:

MATERIALS:

Red leaves of *Terminalia catappa* were collected and authenticated. Ethanol (analytical grade) was used for extraction, and all reagents were of standard laboratory grade. For antidiabetic studies, α-amylase, α-glucosidase, soluble starch, DNS reagent, and p-nitrophenyl-α-D-glucopyranoside were used, with Acarbose as the reference standard. For anticancer evaluation, MCF-7 cells were cultured in DMEM supplemented with fetal bovine serum and antibiotics. The MTT assay was used to assess cytotoxicity. Dialysis membrane tubing and glucose solution were used for the glucose diffusion assay.

Preparation of Plant Extract

Red leaves of *Terminalia catappa* were collected, authenticated, and washed to remove impurities. The leaves were shade-dried at room temperature and then pulverized into coarse powder. The powdered material was subjected to Soxhlet extraction using ethanol as the solvent until complete extraction was achieved. The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The resulting semi-solid mass was dried, weighed, and stored in airtight containers at low temperature for further analysis.[9-16]

Preliminary Phytochemical Screening

The ethanolic extract was screened for major phytoconstituents using standard qualitative methods. The presence of alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, terpenoids, proteins, and amino acids was determined based on characteristic color changes or precipitate formation upon treatment with specific reagents. These tests provided a preliminary profile of bioactive compounds present in the extract.[17-20]

In-vitro Antidiabetic study

Importance of α-Amylase Enzyme in the Body

α-Amylase plays a crucial role in the digestion of dietary carbohydrates in humans. The process begins in the oral cavity, where salivary amylase initiates the breakdown of complex starch molecules into smaller oligosaccharides. This process continues in the small intestine, where pancreatic α-amylase further hydrolyzes these intermediates into simpler sugars such as maltose, maltotriose, and other short-chain carbohydrates. These products are subsequently converted into glucose, which is absorbed into the bloodstream.[21-28]

In diabetic conditions, rapid digestion and absorption of carbohydrates can lead to elevated postprandial blood glucose levels. Therefore, inhibition of α-amylase activity is considered an effective strategy to delay carbohydrate digestion and reduce postprandial hyperglycemia.

Current therapeutic approaches for diabetes management include enhancing insulin sensitivity using agents such as biguanides and thiazolidinediones, stimulating insulin secretion through sulfonylureas, and reducing glucose absorption by inhibiting carbohydrate-digesting enzymes using drugs like Acarbose and Miglitol. These enzyme inhibitors help in controlling blood glucose levels by slowing down the conversion of complex carbohydrates into absorbable sugars.

Alpha-amylase inhibition activity

The inhibitory effect of the ethanolic extract of *Terminalia catappa* red leaves on α-amylase activity was evaluated using a standard in vitro method. This assay is based on the ability of the extract to suppress enzymatic hydrolysis of starch, thereby reducing glucose release.[25-32]

Briefly, a reaction mixture containing α-amylase enzyme, phosphate buffer (pH ~6.9), and varying concentrations of the plant extract (20–100 µg/mL) was pre-incubated at 37°C. After incubation, soluble starch solution was added as the substrate and the mixture was further incubated to allow enzymatic digestion. The reaction was terminated by adding dinitrosalicylic acid (DNS) reagent, followed by heating to develop color. The absorbance of the resulting solution was measured spectrophotometrically, and acarbose was used as the standard reference drug. All experiments were carried out in triplicate, along with a control lacking the extract. The percentage inhibition of α-amylase activity was calculated using the following equation:

$$\textbf{Percentage inhibition (\%)} = A_{control} - A_{extract} / A_{control} \times 100$$

Alpha-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the ethanolic extract of *Terminalia catappa* red leaves was determined using a substrate-based colorimetric method. This assay evaluates the ability of the extract to inhibit the breakdown of disaccharides into glucose, thereby contributing to glycemic control. In this method, the reaction mixture consisted of α-glucosidase enzyme, phosphate buffer (pH ~6.9), and different concentrations of the extract (20–100 µg/mL). The mixture was incubated at 37°C for a specific duration. Subsequently, p-nitrophenyl-α-D-glucopyranoside (pNPG) was added as a substrate and further incubated. The enzymatic reaction leads to the release of p-nitrophenol, which produces a yellow color measurable at 405 nm. The absorbance was recorded using a microplate reader. Acarbose was used as the positive control, and all assays were performed in triplicate with appropriate blanks.[33-36]

The percentage inhibition of α-glucosidase activity was calculated using the following formula:

$$\textbf{Percentage inhibition (\%)} = A_{control} - A_{extract} / A_{control} \times 100$$

Where,

$A_{control}$= Absorbance of control at 405 nm

$A_{extract}$ = Absorbance of extract at 405 nm

Glucose Diffusion Assay (Dialysis Membrane Model)

The effect of the ethanolic extract on glucose movement was evaluated using a dialysis membrane model, which simulates intestinal glucose diffusion. Dialysis tubing was pre-treated by soaking in distilled water to remove preservatives and ensure membrane activation.

A known concentration of glucose solution was prepared and mixed with the plant extract at different concentrations. This mixture was transferred into the dialysis bag, which was then securely sealed. The dialysis bag was immersed in a beaker containing distilled water and maintained at room temperature with gentle stirring.[37-46]

At predetermined time intervals, samples were withdrawn from the external solution, and glucose concentration was estimated using a suitable method such as glucose oxidase-peroxidase assay. A control setup without plant extract was maintained for comparison.

The ability of the extract to retard glucose diffusion across the membrane was evaluated by comparing glucose concentrations in the external medium. A decrease in glucose diffusion indicates potential antidiabetic activity by limiting glucose absorption.

$$\boldsymbol{Glucose\ diffusion\ inhibition}\ (\%) = \frac{\boldsymbol{Gcontrol - Gsample}}{\boldsymbol{Gcontrol}} \times \mathbf{100}$$

Where:

$G_{control}$ = Glucose concentration in control

G_{sample} = Glucose concentration in presence of extract

***In Vitro* Anticancer Activity**

Cell Line and Culture Conditions

The anticancer potential of the ethanolic extract of *Terminalia catappa* red leaves was evaluated using the MCF-7 cell line. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with high glucose, 10% fetal bovine serum (FBS), and 1% antibiotic–antimycotic solution. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2.[47-49]

MTT Cytotoxicity Assay

The cytotoxic activity of the plant extract was determined using the MTT assay based on mitochondrial reduction of MTT into insoluble formazan crystals by viable cells. The cells were seeded in 96-well plates at a density of approximately 1×10^4 cells per well and allowed to adhere for 24 hours under standard incubation conditions.

After incubation, the cells were treated with different concentrations of the extract (10–100 µg/mL). A control group containing cells treated with a small amount of DMSO (vehicle control) was also maintained. All treatments were performed in triplicate. The plates were further incubated for 24 hours at 37°C in a CO_2 incubator.[42-49]

Following treatment, the culture medium was carefully removed, and MTT reagent (5 mg/mL in

PBS) was added to each well. The plates were incubated for an additional 4 hours to allow the formation of purple formazan crystals by metabolically active cells. After incubation, the supernatant was discarded, and dimethyl sulfoxide (DMSO) was added to dissolve the formed crystals.

The absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability and cytotoxicity was calculated relative to the control.

$$\textbf{Cell viability}\ (\%) = \frac{\boldsymbol{Asample}}{\boldsymbol{Acontrol}} \times \mathbf{100}$$

Acridine Orange/Ethidium Bromide (AO/EB) Apoptosis Assay

The induction of apoptosis by the ethanolic extract of *Terminalia catappa* red leaves was evaluated using Acridine Orange/Ethidium Bromide (AO/EB) dual staining method. This assay differentiates viable, early apoptotic, late apoptotic, and necrotic cells based on membrane integrity and nuclear morphology.

The MCF-7 cells were seeded in culture plates and incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2 to allow cell attachment. After incubation, the cells were treated with different concentrations of the plant extract (e.g., IC_{50} concentration and lower doses) and further incubated for 24 hours. Untreated cells served as the control.

Following treatment, the cells were washed gently with phosphate-buffered saline (PBS) to remove residual media. A freshly prepared mixture of acridine orange and ethidium bromide (1:1 ratio, typically 100 µg/mL each) was added to the cells. A small volume of stained cell suspension was placed on a clean glass slide and covered with a coverslip.[50-57]

The stained cells were immediately observed under a fluorescence microscope. Viable cells appeared uniformly green, early apoptotic cells showed green fluorescence with nuclear condensation, late apoptotic cells exhibited orange to red fluorescence with fragmented nuclei, and necrotic cells appeared uniformly red. Apoptotic cells were quantified by counting cells in different fields, and the percentage of apoptosis was calculated.

$$\textbf{Apoptotic cells}\ (\%) = \frac{\textbf{Number of apoptotic cells}}{\boldsymbol{Total\ number\ of\ cells}} \times \mathbf{100}$$

RESULTS AND DISCUSSIONS:

Extract Yield and Characteristics

The ethanolic extraction of *Terminalia catappa* red leaves produced a dark green, semi-solid mass with a characteristic odor. The percentage yield of the extract was found to be in the moderate range, indicating efficient extraction of phytoconstituents using ethanol. The use of ethanol as a solvent facilitated the recovery of both polar and moderately non-polar compounds, which are often associated with biological activities such as antidiabetic and anticancer effects.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extract revealed the presence of multiple classes of bioactive compounds. The preliminary phytochemical investigation confirmed that the ethanolic extract of *Terminalia catappa* red leaves contains a diverse range of secondary metabolites. The presence of flavonoids and phenolic compounds suggests strong antioxidant potential, which plays a crucial role in both antidiabetic and anticancer activities by reducing oxidative stress. Alkaloids and glycosides are known for their pharmacological significance, including enzyme inhibition and modulation of metabolic pathways, which may contribute to antidiabetic effects. Saponins and terpenoids have been reported to exhibit cytotoxic and antiproliferative activities, supporting the potential anticancer properties of the extract. The detection of multiple bioactive constituents indicates that the therapeutic effects of the extract may be attributed to a synergistic interaction among these compounds. These findings provide a scientific basis for further quantitative analysis and in vitro biological evaluation of the extract.

Table 1: Preliminary Phytochemical Screening of Ethanolic Extract of *Terminalia catappa* Red Leaves

Phytoconstituent	Test Performed	Result
Alkaloids	Dragendorff's / Mayer's	+
Flavonoids	Shinoda Test	+
Tannins	Ferric Chloride Test	+
Phenolic Compounds	Ferric Chloride Test	+
Saponins	Foam Test	+
Glycosides	Keller–Killiani Test	+
Terpenoids	Salkowski Test	+
Proteins	Biuret Test	+
Amino Acids	Ninhydrin Test	+

(+): Present; (–): Absent

***In Vitro* Antidiabetic Studies**

α-Amylase Inhibitory Activity of Ethanolic Extract of *Terminalia catappa* Red Leaves (TCRLE)

The ethanolic extract of *Terminalia catappa* red leaves exhibited significant inhibitory activity against the α-amylase enzyme in a concentration-dependent manner. The inhibitory potential of the extract was compared with the standard drug Acarbose, and the results are summarized in Table 2.

Table 2: α-Amylase Inhibitory Activity of *Terminalia catappa* Red Leaf Extract (TCRLE)

Sr. No.	Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Acarbose)
1	20	18.75 ± 0.82	22.10 ± 0.64
2	40	31.25 ± 1.10	36.85 ± 0.92
3	60	40.00 ± 0.95	48.30 ± 1.15
4	80	50.00 ± 1.20	58.42 ± 0.88
5	100	60.00 ± 1.05	70.25 ± 1.02
6	IC_{50} (µg/mL)	72.40 ± 1.32	55.80 ± 1.18

Values are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test ($p < 0.05$ considered significant).

TCRLE - *Terminalia catappa* Red Leaf Extract

The results clearly demonstrate that the ethanolic extract of *Terminalia catappa* red leaves possesses notable α-amylase inhibitory activity. The inhibition increased progressively with increasing concentration, confirming a dose-dependent effect. At the highest tested concentration (100 µg/mL), the extract exhibited 60.00% inhibition, which, although lower than the standard acarbose (70.25%), indicates substantial enzyme inhibitory potential.

The IC_{50} value of the extract was found to be higher than that of acarbose, suggesting comparatively lower potency; however, the observed activity remains pharmacologically relevant. The inhibitory effect may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, and tannins, which are known to interfere with carbohydrate-digesting enzymes.

α-Amylase inhibitors play a crucial role in managing postprandial hyperglycemia by delaying the breakdown of starch into glucose. By reducing glucose release and absorption, these inhibitors help maintain better glycemic control. The findings of the present study suggest that *Terminalia catappa* red leaf extract can act as a natural source of α-amylase inhibitors.

Although the extract exhibited slightly lower activity compared to acarbose, its plant-based origin and potential for fewer side effects make it a promising candidate for further investigation. These results support the potential application of the extract in the development of alternative or adjunct therapies for diabetes management.

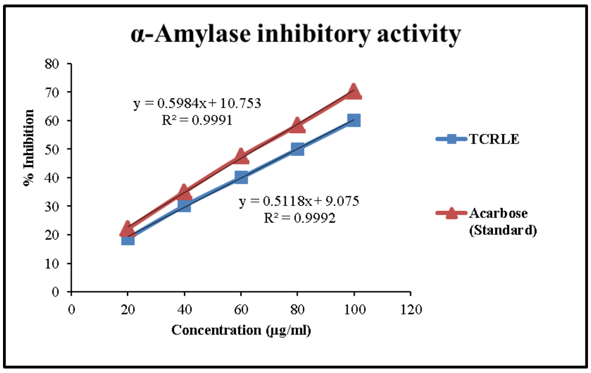

Figure 1: Percentage inhibition of TCRLE α -Amylase inhibitory activity at various concentrations.

α-Glucosidase Inhibitory Activity of Ethanolic Extract of *Terminalia catappa* Red Leaves

The inhibitory activity of the ethanolic extract of *Terminalia catappa* red leaves against α-glucosidase enzyme was evaluated and compared with the standard drug Acarbose. The results demonstrated a concentration-dependent increase in enzyme inhibition, as presented in table 3.

Table 3: α-Glucosidase Inhibitory Activity of *Terminalia catappa* Red Leaf Extract (TCRLE)

Sr. No.	Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Acarbose)
1	20	22.10 ± 0.74	30.25 ± 0.68
2	40	34.50 ± 1.20	42.80 ± 1.05
3	60	45.75 ± 1.05	53.60 ± 0.92
4	80	55.20 ± 0.88	63.40 ± 0.80
5	100	65.50 ± 0.95	72.80 ± 1.10
6	IC_{50} (µg/mL)	70.85 ± 1.45	58.30 ± 1.22

Values are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test ($p < 0.05$).

The ethanolic extract of *Terminalia catappa* red leaves exhibited significant inhibitory activity against α-glucosidase enzyme, with inhibition increasing in a dose-dependent manner. At a concentration of 100 µg/mL, the extract showed 65.50% inhibition, which was slightly lower than the standard acarbose (72.80%), indicating moderate but noteworthy activity.

The IC_{50} value of the extract was found to be higher than that of acarbose, suggesting that the standard drug possesses greater potency. However, the extract still demonstrated considerable inhibitory activity, supporting its potential as a natural antidiabetic agent.

α-Glucosidase is a key enzyme responsible for the final step in carbohydrate digestion, converting oligosaccharides into absorbable glucose. Inhibition of this enzyme delays glucose absorption in the intestine, thereby reducing postprandial blood glucose levels. The observed activity of the extract may be attributed to the presence of phytoconstituents such as flavonoids, phenolics, and tannins, which are known to interact with digestive enzymes and modulate their activity.

Overall, the findings indicate that *Terminalia catappa* red leaf extract possesses promising α-glucosidase inhibitory potential. Although its activity is comparatively lower than acarbose, the natural origin and possible reduced side effects make it a valuable candidate for further pharmacological and clinical investigations.

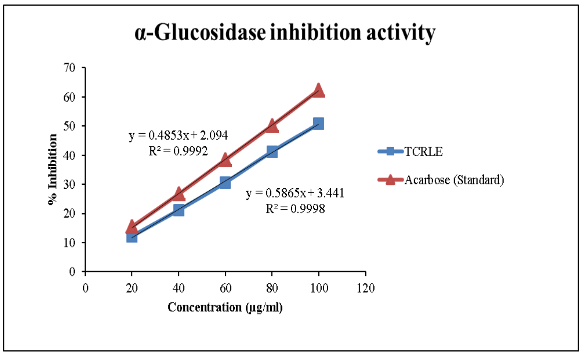

Figure 2: Percentage inhibition of TCRLE α-glucosidase inhibitory activity at various concentrations

Glucose Diffusion Assay (Dialysis Membrane Model)

The effect of the ethanolic extract of *Terminalia catappa* red leaves on glucose diffusion was investigated using a dialysis membrane model. The experiment was performed in triplicate, and the results are expressed as mean ± standard deviation. The extract exhibited both time-dependent and concentration-dependent inhibition of glucose diffusion.

Table 4: Time-Dependent Glucose Diffusion (100 µg/mL Extract)

Time (min)	Control (mg/dL)	Sample 1	Sample 2	Sample 3	Mean (mg/dL)	% Inhibition
0	0	0	0	0	0	0.00
30	40	29	30	31	30.00 ± 0.98	25.00
60	75	54	55	56	55.00 ± 1.00	26.69
90	110	79	80	81	80.00 ± 0.95	27.24
120	140	99	100	101	100.00 ± 1.14	28.57

Table 5: Concentration-Dependent Glucose Diffusion at 120 min

Concentration (µg/mL)	Control (mg/dL)	Sample 1	Sample 2	Sample 3	Mean (mg/dL)	% Inhibition
20	140	120	118	119	119.00 ± 1.00	15.00
40	140	112	110	111	111.00 ± 1.00	20.71
60	140	105	104	103	104.00 ± 1.00	25.71
80	140	98	97	96	97.00 ± 1.00	30.71
100	140	100	99	101	100.00 ± 1.00	28.57

The results demonstrate that the ethanolic extract of *Terminalia catappa* red leaves effectively reduced glucose diffusion across the dialysis membrane. A time-dependent increase in inhibition was observed, with glucose diffusion inhibition rising from 25.00% at 30 min to 28.57% at 120 min for the highest concentration tested.

In addition, the extract exhibited a concentration-dependent effect, where increasing concentrations (20–80 µg/mL) led to a progressive reduction in glucose diffusion. The maximum inhibition (30.71%) was observed at 80 µg/mL, after which a slight plateau was noted at 100 µg/mL, possibly due to saturation effects or equilibrium in diffusion dynamics.

The observed inhibitory activity may be attributed to the presence of phytoconstituents such as flavonoids, tannins, and phenolic compounds. These compounds are known to interact with glucose molecules or alter membrane permeability and solution viscosity, thereby slowing glucose movement. This mechanism mimics delayed glucose absorption in the gastrointestinal tract, contributing to the control of postprandial hyperglycemia.

Overall, the findings suggest that *Terminalia catappa* red leaf extract possesses significant antidiabetic potential through the inhibition of glucose diffusion, complementing enzyme inhibition mechanisms such as α-amylase and α-glucosidase inhibition.

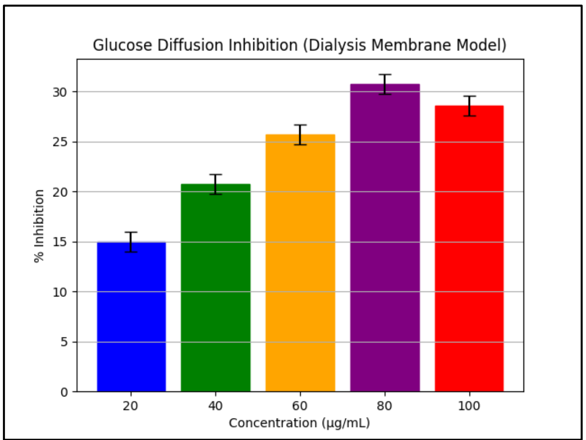

Figure 3: Effect of ethanolic extract of *Terminalia catappa* red leaves on glucose diffusion showing percentage inhibition at different concentrations *(mean ± SD, n = 3)*

***In Vitro* Anticancer Activity (MTT Assay)**

The cytotoxic activity of the ethanolic extract of *Terminalia catappa* red leaves was evaluated against MCF-7 using the MTT assay. The results demonstrated a concentration-dependent decrease in cell viability, indicating significant anticancer potential.

Table 6: Cytotoxic Activity and IC_{50} Determination

Concentration (µg/mL)	Cell Viability (%)	Cytotoxicity (%)
10	91.11 ± 1.20	8.89 ± 1.20
20	83.33 ± 1.05	16.67 ± 1.05
40	72.22 ± 0.95	27.78 ± 0.95
60	56.67 ± 1.10	43.33 ± 1.10
80	44.44 ± 0.88	55.56 ± 0.88
100	33.33 ± 0.92	66.67 ± 0.92
IC_{50} (µg/mL)	~72.5 ± 1.30	—

Values are expressed as mean ± standard deviation (n = 3).

The IC_{50} value (concentration required to inhibit 50% of cell viability) was determined from the dose–response curve plotted between concentration and percentage cell viability. The IC_{50} of the extract was found to be approximately 72.5 µg/mL, indicating moderate cytotoxic potency.

The graphical representation (concentration vs. cell viability) shows a clear sigmoidal dose–response relationship, confirming that the extract exerts a progressive inhibitory effect on cancer cell growth with increasing concentration.

The anticancer activity of the extract was compared with a standard chemotherapeutic agent, Doxorubicin. Typically, doxorubicin exhibits a much lower IC_{50} value (in the range of 1–10 µg/mL for MCF-7 cells), indicating higher potency compared to plant extracts. In contrast, the ethanolic extract of *Terminalia catappa* red leaves showed a higher IC_{50} value (~72.5 µg/mL), suggesting comparatively lower cytotoxic strength. However, plant extracts are generally considered safer and may exert their effects through multiple mechanisms, including induction of apoptosis, antioxidant activity, and inhibition of cellular proliferation pathways.

The results indicate that the extract possesses significant, concentration-dependent anticancer activity against MCF-7 cells. The decrease in cell viability with increasing concentration confirms its cytotoxic effect. The activity may be attributed to phytoconstituents such as flavonoids, phenolic compounds, and tannins, which are known to interfere with cancer cell survival and proliferation. Although the extract is less potent than doxorubicin, its natural origin and potential for reduced toxicity make it a promising candidate for further investigation. The moderate IC_{50} value suggests that the extract could be explored as an adjunct or complementary therapeutic agent.

Overall, the findings support the potential of *Terminalia catappa* red leaves as a source of bioactive compounds with anticancer properties, warranting further studies including mechanism-based assays and in vivo evaluations.

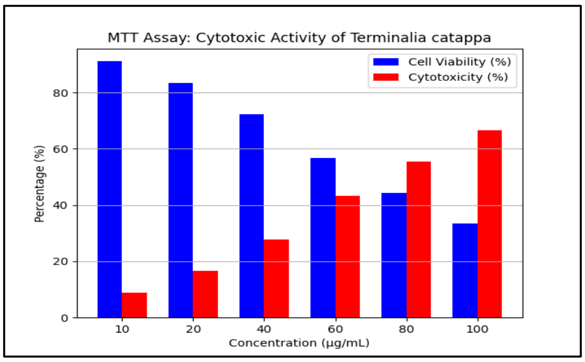

Figure 4: Cytotoxic activity of ethanolic extract of *Terminalia catappa* red leaves on MCF-7 cells showing percentage cell viability and cytotoxicity at different concentrations.

Acridine Orange/Ethidium Bromide (AO/EB) Apoptosis Assay

The apoptotic potential of the ethanolic extract of *Terminalia catappa* red leaves was assessed in MCF-7 using AO/EB dual staining. Cells were categorized as viable (green), early apoptotic (green with condensed nuclei), late apoptotic (orange/red with fragmented nuclei), and necrotic (uniform red). Data are expressed as mean ± SD (n = 3).

Table 7: Apoptotic Profile of MCF-7 Cells Treated with Extract

Concentration (µg/mL)	Live Cells (%)	Early Apoptosis (%)	Late Apoptosis (%)	Necrosis (%)
0 (Control)	95.00 ± 1.00	3.33 ± 0.58	1.00 ± 0.00	0.67 ± 0.58
25	80.00 ± 2.00	10.00 ± 1.00	5.67 ± 0.58	4.33 ± 0.58
50	66.00 ± 1.00	15.00 ± 1.00	11.67 ± 0.58	7.33 ± 1.15
75	45.33 ± 1.53	20.00 ± 1.00	24.67 ± 0.58	10.00 ± 0.00
100	30.33 ± 1.53	18.00 ± 1.00	35.00 ± 1.00	16.67 ± 0.58

The AO/EB staining results revealed a dose-dependent induction of apoptosis in MCF-7 cells treated with the extract. As the concentration increased, the percentage of viable cells decreased markedly from 95.00% (control) to 30.33% at 100 µg/mL, indicating strong cytotoxic action.

A progressive increase in early and late apoptotic populations was observed. Early apoptosis increased initially, followed by a substantial rise in late apoptotic cells at higher concentrations, suggesting progression of apoptotic events. The increase in late apoptosis (up to 35.00%) confirms effective induction of programmed cell death.

Necrotic cell populations remained comparatively lower than apoptotic cells, indicating that the extract primarily induces apoptosis rather than nonspecific necrosis. This is a desirable feature for anticancer agents, as apoptosis is a controlled and targeted mechanism of cell death.

The apoptotic activity may be attributed to phytoconstituents such as flavonoids, phenolics, and tannins, which are known to trigger apoptosis via oxidative stress, mitochondrial dysfunction, and activation of caspase pathways.

Overall, the AO/EB assay confirms that the ethanolic extract of *Terminalia catappa* red leaves induces apoptosis in cancer cells, supporting and strengthening the MTT assay findings.

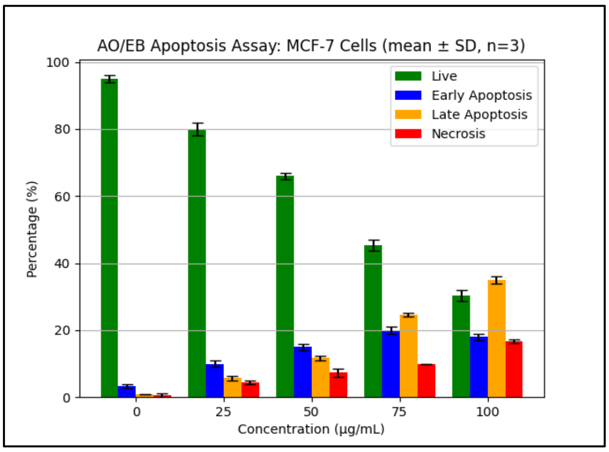

Figure 5: AO/EB staining showing percentage distribution of viable, early apoptotic, late apoptotic, and necrotic MCF-7 cells treated with *Terminalia catappa* red leaf extract *(mean ± SD, n = 3)*

CONCLUSION:

The present investigation demonstrates that the ethanolic extract of *Terminalia catappa* red leaves possesses significant *in vitro* antidiabetic and anticancer activities. The extract exhibited effective inhibition of key carbohydrate-digesting enzymes, α-amylase and α-glucosidase, along with a notable reduction in glucose diffusion, indicating its potential role in the management of postprandial hyperglycemia. In addition, the extract showed considerable cytotoxic activity against MCF-7 cells, as evidenced by reduced cell viability and induction of apoptosis confirmed through AO/EB staining. The observed biological activities can be attributed to the presence of diverse phytoconstituents, particularly flavonoids and phenolic compounds, which are known for their therapeutic potential. Although the extract demonstrated moderate potency compared to standard drugs, its natural origin and multi-targeted mechanisms make it a promising candidate for further investigation. Future studies focusing on isolation of active compounds, mechanistic pathways, and in vivo validation are recommended to establish its potential as a novel therapeutic agent.

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CONFLICT OF INTEREST:
The authors declare that there are no conflicts of interest regarding the publication of this research work.

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