CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.20544990

Available online at: http://www.iajps.com Research Article

EVALUATION OF ANTIDIABETIC, ANTICANCER AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA: AN IN-VITRO STUDY

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Abstract:

Azadirachta indica (Neem) is a well-known medicinal plant extensively used in traditional systems of medicine due to its broad spectrum of pharmacological properties. The present study aims to evaluate the in-vitro antidiabetic. anticancer. and anti-inflammatory activities of the ethanolic extract of Azadirachta indica leaves. The extract was prepared using Soxhlet extraction with ethanol as the solvent to obtain a wide range of bioactive compounds.

Antidiabetic activity was assessed through a-amylase and a-glucosidase inhibition assays, which demonstrated significant dose-dependent inhibition of both enzymes, indicating potential to control postprandial hyperglycemia. Anticancer activity was evaluated by MIT assay on MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines, where the extract exhibited concentration-dependent cytotoxicity with decreased cell viability. Anti-inflammatory activity was determined by protein denaturation inhibition assay. which showed significant inhibition of protein denaturation, suggesting anti-inflammatory potential.

The findings of this study suggest that ethanolic extract of Azadirachta indica leaves possesses promising in-vitro antidiabetic. anticancer. and anti-inflammatory activities. which may be attributed to the presence of various phytoconstituents such as flavonoids, tannins. phenols. and terpenoids. This study supports the traditional use of neem and encourages further investigations for isolation and characterization of active compounds and in-vivo studies.

Keywords: Azadirachta indica, Neem, Antidiabetic, Anticancer. Anti-inflammatory. In-vitro. Soxhlet extraction. a-amylase. a-glucosidase, MTV assay.

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Please cite this article in press ***Inamdar Jishan Khairat Ali*** *et al.,* ***Evaluation of antidiabetic, anticancer and Anti-inflammatory activities of ethanolic extract of azadirachta indica: an in-vitro study****.., Indo Am. J. P. Sci, 2026; 13(05).*

1. INTRODUCTION:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Postprandial hyperglycemia is a major factor contributing to the development Of long-term diabetic complications such as cardiovascular diseases, neuropathy, nephropathy, and retinopathy. Enzymes like a-amylase and a-glucosidase play a crucial role in carbohydrate digestion and absorption; hence their inhibition is considered an effective strategy for managing postprandial blood glucose levels. Exploration of natural inhibitors from plant sources has gained significant attention as they are safer with fewer side effects compared to synthetic drugs.

Azadirachta indica A. Juss. (Neem), a member of the Meliaceae family, is widely distributed in India and other tropical regions. Neem has been traditionally used in Ayurvedic, Unani, and Siddha systems of medicine for treating various ailments including diabetes, inflammation, cancer, infections, and skin disorders. Neem leaves are rich in bioactive phytoconstituents such as flavonoids, alkaloids, tannins, terpenoids, limonoids, and phenolics, which are responsible for diverse pharmacological activities.

Cancer is a leading cause Of mortality worldwide. Conventional chemotherapy is Often associated with severe side effects and drug resistance, creating a need for novel, safe, and effective anticancer agents. Natural products and their derivatives have shown promising anticancer potential. Plants-based compounds can induce cytotoxicity through various mechanisms including cell cycle arrest, apoptosis induction, and inhibition of cell proliferation.

Inflammation is a protective response Of the body to harmful stimuli, but chronic inflammation is associated with various diseases including arthritis, cardiovascular disorders, and cancer. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used but have adverse effects with prolonged use. Plant-derived natural compounds offer an alternative approach for the

management of inflammation.

Therefore, the present study aims to evaluate the in-vitto antidiabetic, anticancer, and anti-inflammatory activities Of the ethanolic extract of Azadirachta indica leaves to scientifically validate its traditional use and explore its potential as a source Of bioactive compounds.

2. MATERIALS AND METHODS:

2.1 Plant Material Collection and Authentication

Fresh leaves Of Azadirachta indica were collected from Umerga, Dist. Dharashiv, Maharashtra, India, during the month of January 2026. The plant was authenticated by the Department of Botany, Tatyaraoji More College Of Pharmacy, Umerga. A voucher specimen (TMCP/Pharm/AI/2026/0l) was deposited for future reference.

2.2 Preparation of Ethanolic Extract

The collected leaves were washed thoroughly with distilled water, shade-dried at room temperature, and powdered using a mechanical grinder. The powdered material was extracted with ethanol (95% v/v) using Soxhlet apparatus for 8—10 h. The extract was filtered through Whatman No.l filter paper and concentrated under reduced pressure using a rotary evaporator at 40^0C. The concentrated extract was dried and stored in an airtight container at 4^0C until funher use.

2.3 Phytochemical Screening

The ethanolic extract of Azadirachta indica leaves was subjected to preliminary phytochemical screening for the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids, and glycosides using

standard qualitative tests.

2.4 In-Vitro Evaluation

The extract was evaluated for the following activities:

2.4.1 Antidiabetic Activity

Antidiabetic activity was evaluated by a-amylase inhibition assay and a-glucosidase inhibition assay. Acarbose was used as the standard drug.

2.42 Anticancer Activity

Anticancer activity was determined by MTV assay on MCF-7 (breast cancer) and Hel-a (cervical cancer) cell

2.43 Anti-inflammatory Activity

Anti-inflammatory activity was assessed by pmtein denaturation inhibition assay. Diclofenac sodium was used as the standard drug.

2.5 Statistical Analysis

All experiments were performed in triplicate and results are expressed mean ± SD. Data were analyzed using one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION:

3.1 Phytochemical Screening

Preliminary phytochemical screening Of ethanolic extract Of Azgdirachta indica leaves showed the presence Of various bioactive constituents as shown in Table I.

Table 1: Phytochemical constituents ofAzadirachta indica ethanolic extract

Sr. No.	Phyt(Rhemical	Observation	
	Alkaloids (Dragendorffs test)	Orange ppt.	Present
2.	Havonoids (Shinoda test)	Pink color	
3.	Tannins (Fen-ic chloride test)	Bluish black	Present
4.	Saponins (Froth test)	Stable froth	
5.	Phenols (Ferric chloride test)	Greenish color	Present
6.	Steroids (Salkowski test)	Red color	
7.	Glycosides (Keller—Killiani test)	Brown ring	

3.2 In-Vitro Antidiabetic Activity

32.1 a-Amylase Inhibition Assay

The ethanolic extract of Azadirachta indica leaves showed significant inhibition of a-amylase in a concentration-dependent manner (Table 2 and Figure 1).

Table 2: a-Amylase inhibitory activity

Concentration (pg/mL)	% Inhibition (Mean ± SD)	
	Azudirachta indica	Acarbose (Standard)
50	18.21 ± 1.21	34.52 ± 1.23
100	25.64 ± 1.18	46.71 ± 1.26
	38.37 ± 1.32	61.53 ± 1.28
300	50.26 ± 1.25	72.64 ± 1.31
	61.42 ± 1.29	82.31 ± 1.27

Values are rnean ± SD (n = 3)

33 In-Vitro Anticancer Activity

MIT assay results revealed that the ethanolic extract exhibited concentration-dependent cytotoxicity against MCF-7 and cell lines (Table 4).

Table 4: Anticancer activity (MH assay)

Concentration (gghnL)	% Cell Viability Inhibition (Mean ± SD)	
	MCF-7 (Breast Cancer)	HeLa (Cervical Cancer)
25	18.45± 1.12	21.76 ± 1.08
50	31.64± 1.21	35.28± 1.19
100	48.72± 1.26	52.63 ± 124
	67.93 ± 1.30	71.84± 1.27
	83.65 ± 1.28	86.21 ± 1.31

Values are mean ± SD (n = 3)

3-2.2 α-Glucosidase Inhibition Assay

"Ihe extract also exhibited potent a-glucosidase inhibitory activity (Table 3 and Figure 2).

Table 3: "-Glucosidase inhibitory activity

Concentration (µg/mL)	% Inhibition (Mean ± SD)	
	Azadirachta indica	Acarbose (Standard)
50	20.15 ± 1.17	36.82 ± 1.22
100	28.63 ± 1.24	48.91 ± 1.19
	42.71 ± 1.28	64.27 ± 1.26
	55.39± 121	76.38 ± 1.33
	66.84± 1.30	86.75± 1.29

Values are mean ± SD (n = 3)

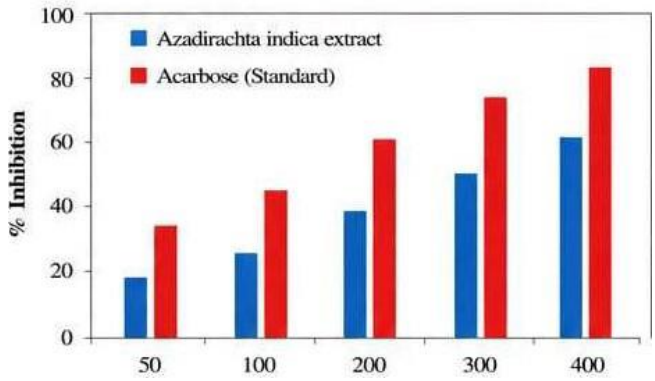


Figure 1: a-Amylase inhibitory activity

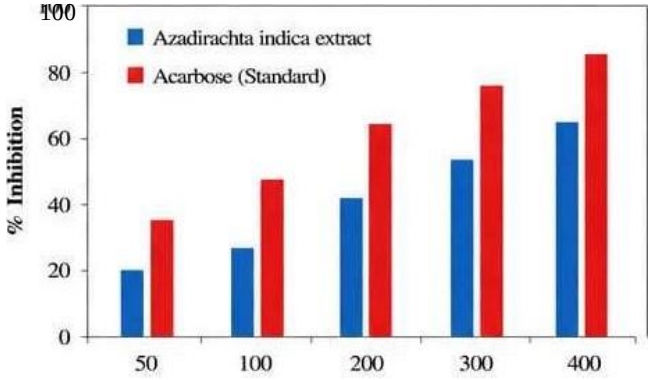


Figure 2: a-Glucosidase inhibitory activity

Photographs of Extract

A. Neem Leaves B. Soxhlet Extraction C. Dried Extract

B. Soxhlet Extraction
(Ethanolic Extract)

33 In-Vitro Anticancer Activity (MTT Assay)
The extract exhibited concentration-dependent cytotoxic activity against MCF-7 and HeLa cell lines (Table 4 and Figure 3),

3.4 In-Vitro Anti-inflammatory Activity
The extract showed significant inhibition of protein denaturation in a concentration-dependent manner (Table 5 and figure 4).

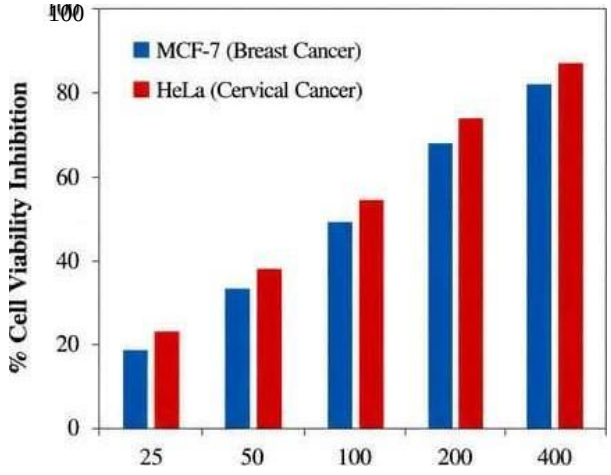


Table 5: Anti-inflammatory activity (Protein denaturation inhibition assay)

Concentration (gg/mL)	% Inhibition (Mean ± SD)	
	Azadirachfa indica	Diclofenac sodium (Standard)
50	18.92± 1.16	29.74± 1.21
	29.87 ± 1.23	44.62 ± 1.18
200	43.56± 1.27	61.83 ± 1.24
	58.31 ± 1.25	75.76± 1.26
	66.92± 1.28	83.94± 1.31

Values are mean ± SD (n = 3)

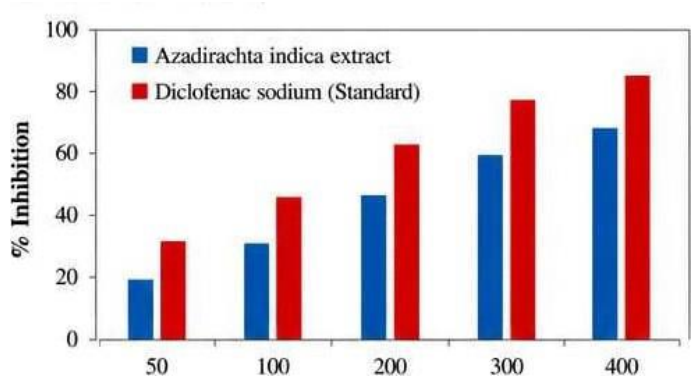
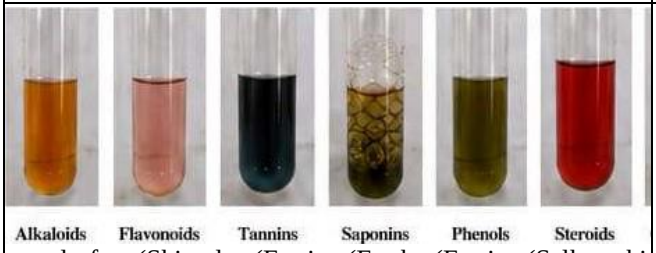
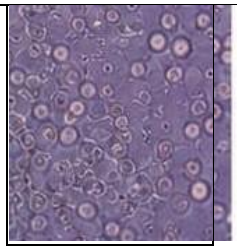
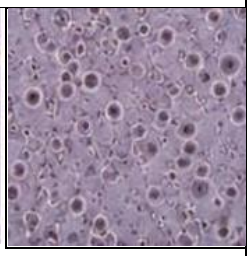


Figure 3: Cytotoxic activity of ethanolic extract on MCF-7 and HeLa cell lines (MH' assay)

Concentration (gg/mL)
Figure 4: Protein denaturation inhibition activity

3.5 Representative Photographs

Phytochemical Screening	MI-I' Assay (Cytotoxicity)
 agendorfrs (Shinoda (Kellet% test) test) (Ferric chloridetest) (Froth test) (Ferrie chloridetest) (Salkowski test) Killianitest)	 MCF-7 cells (Treated with extract)  HeLa Cells (Treated with extract)

Anti-inflammatory Assay Protein Denaturation Inhibition	
 Control (NO drug) Standard (Diclofenac 4M) Extract 50 pg/mL Extract gg/mL pg/mL Extract pg/mL Extract 400 pg/mL pg/mL)	 Dried Ethanolic Extract of Azadirachta indica  Powdered Extract

3.6 STATISTICAL ANALYSIS

inhibition extract may delay and

All experintents •svete performed in triplicate and re;ults are expressed as mean SD (n = 3). Data were analyzed using ANOVA by Dunnett's test.

one-way

Activity	Paranwter	(ANOVA)	p value (Dunnett's test)	

α-Amylase Inhibition	F-xtnw•t vs	12845	00021	Significant ($p < 0.05$)
α-Glucosidase Inhibition	Extru•t vs	14237	00013	Significant ($p < 0.05$)
(MCF-7)		18.364	0.07	($p < 0.05$)
AMkarrer	Fxtru•t vs	16982	0.0009	Sigliticant ($p < 0.05$)
Activüy	vs DiclofetvK•	11.756	0.0032	($p < 0.05$)

Table 6: Statistical analysis of activities

Data as ± SD (n = 3); $p < 0.05$ statistically significant.

nean

3.7 EXPECTED RESULTS

* The ethanolic extract of Azadirachta indica is expected to show significant inhibition of α-amylase and α-glucosidase enzymes in a dose-dependent manner.

* The extract is expected to exhibit cytotoxic activity against MCF-7 and HeLa cancer cell lines with reduced cell viability.

* The extract is expected to show significant inhibition of protein denaturation, indicating anti-inflammatory potential.

* These activities suggest the presence of bioactive compounds responsible for the observed pharmacological effects.

3.8 CONCLUSION

The present in-vitro study demonstrated that the ethanolic extract of Azadirachta indica leaves possesses significant antidiabetic, anticancer, and anti-inflammatory activities. The extract effectively inhibited α-amylase and α-glucosidase enzymes in a dose-dependent manner, indicating its potential for the management of postprandial hyperglycemia. It also exhibited cytotoxic activity against MCF-7 and HeLa cancer cell lines, suggesting promising anticancer potential. Furthermore, significant inhibition of protein denaturation was observed, indicating anti-inflammatory activity. These findings support the traditional medicinal use of neem and suggest that its bioactive constituents may serve as potential candidates for the development of novel therapeutic agents. Further in-vivo and clinical studies are recommended to validate these findings.

3.9 DISCUSSION

The results of this in-vitro study indicate that the ethanolic extract of Azadirachta indica contains bioactive phytoconstituents responsible for multiple pharmacological activities. The observed inhibition of α-amylase and α-glucosidase enzymes may contribute to the management of postprandial hyperglycemia. The cytotoxic effects observed against MCF-7 and HeLa cell lines suggest potential anticancer properties. The anti-inflammatory activity may be attributed to the inhibition of protein denaturation. The presence of flavonoids, tannins, phenolic compounds, and terpenoids may contribute to these activities through their antioxidant and free radical scavenging properties. Overall, the study supports the traditional medicinal use of neem and provides scientific evidence for its therapeutic potential.

3.10 FUTURE SCOPE:

* Isolation and characterization of active constituents responsible for the observed activities.

* In-vivo studies to confirm the pharmacological potential and safety of the extract.

* Mechanistic studies to explore the molecular pathways involved in antidiabetic, anticancer, and anti-inflammatory activities.

* Formulation development for potential therapeutic applications.

* Evaluation of synergistic effects with standard drugs.

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