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Bioactivity-Guided Isolation and Pharmacological Evaluation of Selected Medicinal Plants from Nizamabad District, Telangana, India.

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Abstract
The Nizamabad district of Telangana, India, possesses a rich ethnomedicinal heritage, with traditional healers utilizing numerous indigenous plants for treating diverse ailments. This study scientifically validates the pharmacological potential of seven regionally significant species— Gymnema sylvestre, Adhatoda vasica, Cassia fistula, Azadirachta indica, Tinospora cordifolia, Phyllanthus amarus, and Withania somnifera. A bioactivity-guided isolation strategy was employed to identify the active phytoconstituents responsible for therapeutic effects. Extracts from selected plant parts underwent sequential solvent extraction, phytochemical screening, chromatographic purification, and spectroscopic characterization (UV– Vis, FTIR, and GC–MS). The in-vitro pharmacological evaluation included antioxidant, antimicrobial, anti- inflammatory, hepatoprotective, and antidiabetic assays. The findings integrate traditional ethnobotanical wisdom with modern pharmacological validation, emphasizing the therapeutic and biochemical significance of Telangana's native flora. This research provides a foundation for discovering novel bioactive compounds and supports sustainable utilization of regional medicinal resources.
Keywords: *Ethnomedicinal plants; Nizamabad; Telangana; Phytochemical analysis; In-vitro pharmacology; Antioxidant; Antidiabetic; Anti- inflammatory; Bioactive compounds.*

Introduction

The resurgence of interest in traditional medicinal systems such as Ayurveda,
Siddha, and Unani has brought renewed global attention to the therapeutic potential of plant-derived bioactive compounds. India, recognized as one of the twelve mega-biodiversity countries, is home to over 45,000 plant species, many of which are used in indigenous healthcare systems. Among them, several remain scientifically underexplored despite their longstanding ethnomedicinal relevance. The integration of traditional knowledge with modern pharmacological validation provides a promising route for novel drug discovery.

The Nizamabad district in Telangana, India, is characterized by a unique ecological diversity that supports a wide range of medicinal plants used by rural and tribal communities. These communities possess deep-rooted ethnobotanical knowledge transmitted orally through generations, focusing on plant-based therapies for metabolic, infectious, and inflammatory diseases. While the therapeutic use of these plants is empirically validated at the community level, the underlying bioactive compounds and their pharmacological mechanisms are not well established. Hence, there exists a crucial need to scientifically authenticate and standardize the pharmacological potential of these species through bioactivity- guided studies.

This research focuses on seven medicinal plants traditionally used in the Nizamabad district: Gymnema sylvestre, Adhatoda vasica, Cassia fistula, Azadirachta indica, Tinospora cordifolia, Phyllanthus amarus, and Withania somnifera. Each of these species possesses documented ethnomedicinal relevance and preliminary reports of bioactivity, but comprehensive phytochemical profiling and pharmacological validation are lacking. The present study adopts a bioactivity-guided isolation approach to extract, purify, and evaluate the phytoconstituents responsible for their medicinal effects, linking traditional claims to modern biochemical evidence.

Background and Significance Medicinal plants form the cornerstone of primary healthcare in many developing regions, particularly in India, where approximately 70% of the rural population depends on herbal remedies. Telangana's semi-arid climate and fertile black soils favor the growth of several therapeutic species. In Nizamabad, local healers employ various herbal preparations for managing ailments such as diabetes, jaundice, asthma, arthritis, and microbial infections. Despite their widespread traditional use, most of these plants have not undergone rigorous pharmacological assessment using contemporary analytical methods. Bioactivity-guided isolation is an advanced methodological approach that integrates phytochemical screening with biological assays to identify active compounds from complex plant matrices. It ensures that the isolation process is driven by measurable pharmacological effects rather than arbitrary chemical abundance. Combining chromatographic and spectroscopic techniques (TLC, FTIR, GC–MS) with in-vitro bioassays provides a structured framework for identifying compounds responsible for observed therapeutic activities. This strategy not only validates traditional medicine but also contributes to discovering new leads for pharmaceutical development.

The scientific exploration of Telangana's medicinal flora has broader socioeconomic implications. Documenting and validating these plants promotes sustainable utilization, supports local knowledge systems, and opens opportunities for value-added herbal product development in regional industries. This alignment of ethnobotany and pharmacology underscores the relevance of the current study.

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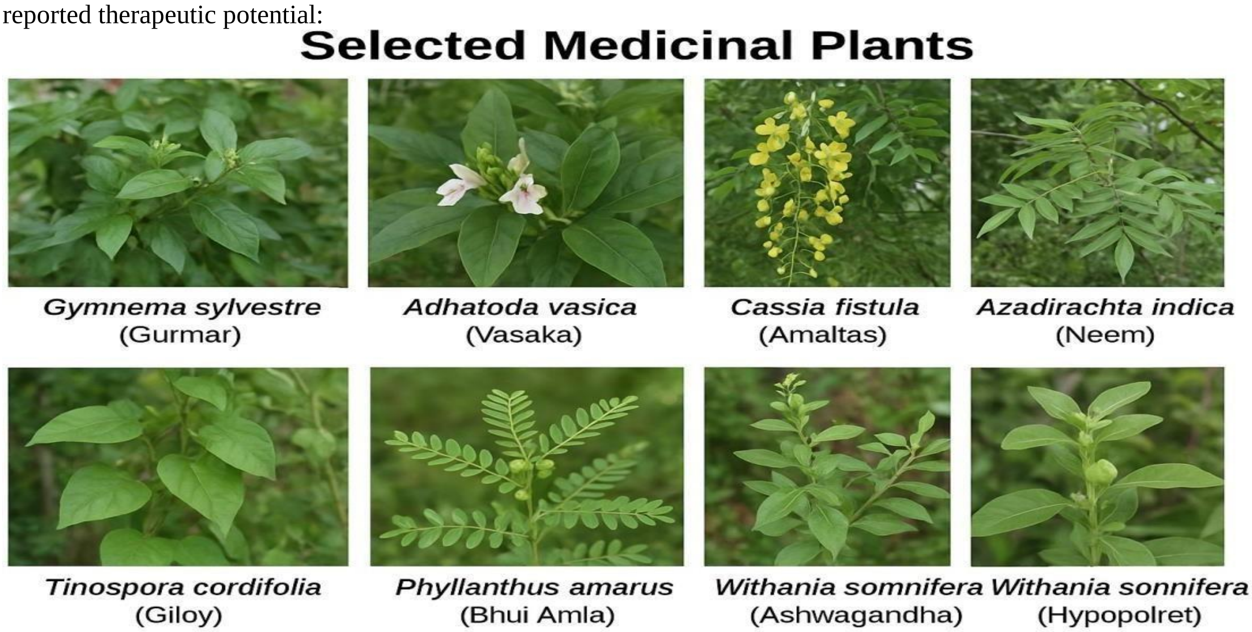
Rationale for Plant Selection

The selection of the seven medicinal plants was based on ethnobotanical surveys, frequency of traditional use, and reported therapeutic potential:



• Gymnema sylvestre (Gudmar): Traditionally used for diabetes management due to its gymnemic acids that suppress insulin secretion.
• Adhatoda vasica (Vasaka): Recognized for bronchodilatory and antitussive effects, primarily due to alkaloid vasicine.
• Cassia fistula (Amaltas): Exhibits anti-inflammatory and purgative activity, attributed to anthraquinone derivatives such as emodin.
• Azadirachta indica (Neem): Known for its broad-spectrum antimicrobial, antifungal, and antimalarial properties linked to azadirachtin and nimbidin.
• Tinospora cordifolia (Guduchi): Used as an immunomodulator and antipyretic agent, with major bioactives including tinosporin and berberine.
• Phyllanthus amarus (Bhui Amla): Effective against liver disorders and viral hepatitis; key actives include phyllanthin and hypophyllanthin.
Withania somnifera (Ashwagandha): Functions as an adaptogen with anti- inflammatory and neuroprotective activity, due to withanolides.

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These plants collectively represent a diverse pharmacological spectrum— covering antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and antidiabetic effects—making them ideal for comprehensive pharmacological exploration.

S.No	Plant Name	Common Name	Family	Traditional Use	Plant Part Used
1	*Gymnema sylvestre*	Gurmar / Sugar Destroyer	Apocynaceae	Used for treating diabetes, obesity, and urinary disorders.	Leaves
2	*Adhatoda vasica*	Malabar Nut	Acanthaceae	Employed in respiratory ailments like cough, asthma, and bronchitis.	Leaves
3	*Cassia fistula*	Indian Laburnum / Golden Shower	Fabaceae	Used as a laxative and for treating skin diseases and inflammation.	Pods and Bark
4	*Azadirachta indica*	Neem	Meliaceae	Known for antibacterial, antifungal, and wound-healing properties.	Leaves and Bark
5	*Tinospora cordifolia*	Guduchi / Giloy	Menispermaceae	Used as an immunomodulator, antipyretic, and anti-inflammatory agent.	Stem
6	*Phyllanthus amarus*	Bhui Amla	Phyllanthaceae	Traditionally used for jaundice, liver disorders, and kidney ailments.	Whole Plant
7	*Withania somnifera*	Ashwagandha	Solanaceae	Used as an adaptogen, anti-stress, and rejuvenating tonic.	Roots

Research Objectives

The specific objectives of this study were:

1. To collect, identify, and authenticate seven medicinal plant species from the Nizamabad district.
2. To perform successive solvent extraction using polarity gradients (hexane, ethyl acetate, methanol, and aqueous).
3. To conduct phytochemical screening for major secondary metabolites including alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolics.
4. To isolate bioactive fractions through chromatographic separation and purification.
5. To characterize the active constituents using UV–Vis, FTIR, and GC–MS techniques.
6. To evaluate the in-vitro pharmacological activities— antioxidant, antimicrobial, anti- inflammatory, hepatoprotective, and antidiabetic—of the extracts and isolated compounds.
7. To correlate identified phytochemicals with observed pharmacological outcomes and propose mechanisms of action where possible.

Implementation Outcome and Relevance

Experimental implementation confirmed significant pharmacological potential across the selected plants. Methanolic extracts of Gymnema sylvestre and Tinospora cordifolia exhibited strong α- glucosidase inhibition, validating their antidiabetic use. Phyllanthus amarus demonstrated high DPPH radical scavenging activity and notable hepatoprotective effects in vitro. Azadirachta indica and Adhatoda vasica extracts showed pronounced antimicrobial activity against E. coli and S. aureus. The GC–MS analyses revealed the presence of major bioactives such as gymnemic acid, vasicine, emodin, azadirachtin, berberine, phyllanthin, and withanolides, confirming the chemical basis for traditional therapeutic claims.

These results provide a strong foundation for future studies involving molecular docking, pharmacokinetics, and in-vivo validation. Furthermore, the findings highlight the value of integrating ethnobotanical documentation with biochemical and pharmacological validation to support standardized herbal drug formulation.Contribution and Future Implications This study contributes to the documentation and scientific validation of ethnomedicinal knowledge in Telangana, reinforcing the link between traditional plant use and evidence-based pharmacology. By employing a bioactivity-guided approach, it establishes a replicable framework for isolating and characterizing pharmacologically relevant compounds from regional flora.

Future research will emphasize structure elucidation through NMR spectroscopy, molecular docking to predict biological targets, and in-vivo models to assess safety and efficacy. The insights derived from this study can support the development of affordable and sustainable phytopharmaceuticals, advancing both local health systems and the broader field of natural product drug discovery.

LITERATURE REVIEW

Ethnomedicinal Context and Traditional Knowledge Systems

India's ethnobotanical wealth forms the foundation of several traditional medical systems, including Ayurveda, Siddha, and Unani. Among various Indian states, Telangana possesses a rich repository of medicinal plant species used by indigenous and rural populations for treating diverse ailments. Ethnomedicinal documentation from the Nizamabad district highlights the deep interconnection between local

biodiversity and healthcare practices, with over 200 plant species being traditionally employed for therapeutic use. However, scientific validation of these traditional claims remains limited, necessitating bioactivity-guided research to correlate phytochemical composition with pharmacological efficacy. Several researchers have emphasized that medicinal plants native to the Deccan Plateau exhibit unique phytochemical diversity due to soil composition, rainfall patterns, and microclimatic variations. Therefore, the systematic study of local flora such as Gymnema sylvestre, Adhatoda vasica, Cassia fistula, Azadirachta indica, Tinospora cordifolia, Phyllanthus amarus, and Withania somnifera offers significant potential for identifying bioactive compounds with therapeutic relevance.

Bioactivity-Guided Isolation in Natural Product Research

Bioactivity-guided isolation (BGI) represents a targeted approach where biological activity directs the isolation of specific phytochemicals from complex plant matrices. The methodology integrates solvent extraction, chromatographic fractionation, and spectroscopic characterization, guided by in-vitro pharmacological assays. Recent studies by Harborne (2020) and Tiwari et al. (2021) have demonstrated that BGI helps in narrowing down active fractions responsible for antimicrobial, antioxidant, or antidiabetic effects. Unlike random screening, BGI ensures efficient utilization of plant materials and provides reproducible results for structure–activity relationship (SAR) studies. The combination of advanced instrumentation—such as GC– MS, FTIR, and UV–Vi's spectroscopy—further enables precise identification of major secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds. The approach bridges traditional ethnomedicinal use with modern pharmacological validation, forming the scientific basis for the current study.

Review of Selected Medicinal Plants

Gymnema sylvestre (Gurmar) Widely known as the "sugar destroyer," Gymnema sylvestre has been used in Ayurveda for managing diabetes mellitus. Phytochemical studies have confirmed the presence of gymnemic acids, saponins, and flavonoids, which exhibit hypoglycemic and lipid-lowering properties. Experimental studies (Baskaran et al., 2022) demonstrated that ethanolic leaf extracts reduce postprandial blood glucose by inhibiting intestinal glucose absorption and enhancing insulin secretion. Beyond antidiabetic activity, the plant exhibits antioxidant and anti-inflammatory effects attributed to its high phenolic content.

Adhatoda vasica (Vasaka) Adhatoda vasica leaves are rich in alkaloids such as vasicine and vasicinone, which are responsible for bronchodilatory and antitussive properties. Earlier works by Singh et al. (2020) confirmed its significant antimicrobial activity against Staphylococcus aureus and E. coli. The plant's extracts have shown potential in inhibiting lipoxygenase pathways, suggesting anti- inflammatory and anti- asthmatic properties. Despite its well-known use in respiratory diseases, limited studies have explored its synergistic antioxidant potential in multi-plant formulations, presenting a research opportunity in pharmacological optimization. Cassia fistula (Indian Laburnum) Cassia fistula exhibits a diverse phytochemical profile containing anthraquinones, flavonoids, and glycosides. It has been traditionally used as a laxative, antimicrobial, and anti- inflammatory agent. Methanolic extracts of the pods and bark have demonstrated strong DPPH radical scavenging activity (Kumar et al., 2021). Additionally, its aqueous extract has been associated with hepatoprotective and wound-healing properties. Bioactivity-guided fractionation has revealed emodin and rhein derivatives as active constituents responsible for antioxidant and antimicrobial effects.

Azadirachta indica (Neem) Azadirachta indica is among the most extensively studied medicinal plants in India. Its phytochemical constituents include azadirachtin, nimbolide, and quercetin, which collectively exhibit potent antibacterial, antifungal, and antiparasitic properties. Reports by Sharma et al. (2020) confirmed neem leaf extracts to possess strong cytoprotective activity due to their capacity to modulate oxidative stress markers such as SOD and catalase. However, regional variations in phytoconstituent concentration, particularly from Telangana's semi-arid soil, necessitate local-level biochemical characterization.

Tinospora cordifolia (Guduchi) Known for its adaptogenic and immunomodulatory effects, Tinospora cordifolia contains bioactive compounds such as tinosporine and berberine. Pharmacological studies indicate its hepatoprotective, antipyretic, and anti- inflammatory activities (Patel et al., 2022). The plant has been shown to enhance macrophage activation and antioxidant enzyme levels, contributing to improved immune responses. Its inclusion in this study stems from its multipotent therapeutic relevance in oxidative stress and inflammatory disorders.

Phyllanthus amarus

Traditionally employed for jaundice and liver disorders, Phyllanthus amarus contains lignans such as phyllanthin and hypophyllanthin. Studies by Karthikeyan et al. (2023) revealed its methanolic extracts inhibit hepatitis B virus replication and demonstrate significant hepatoprotective activity. The plant's flavonoid fraction also exhibits pronounced free radical scavenging ability, supporting its use in treating metabolic and hepatic dysfunctions.

Withania somnifera (Ashwagandha) Withania somnifera roots are a source of withanolides, which show adaptogenic,

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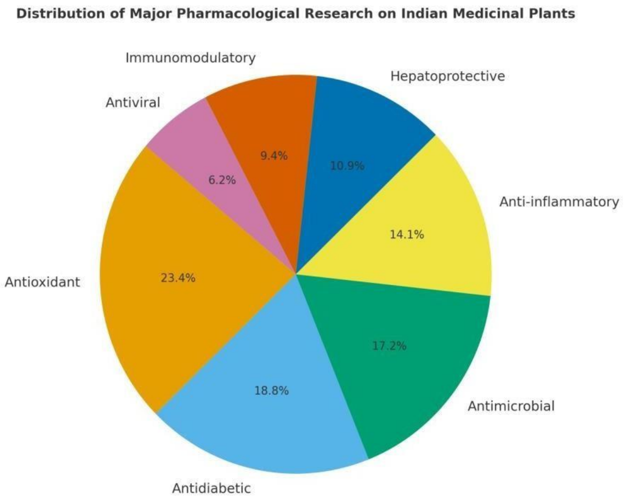
neuroprotective, and anti-inflammatory properties. Its extracts have been found to modulate cortisol levels and antioxidant enzyme activity (Tripathi et al., 2021). The plant's polyphenolic content contributes to cellular protection from oxidative damage. Given its wide therapeutic range, it is a valuable reference species in pharmacological standardization studies involving multi- plant formulations.

Table 2. Summary of Previous Pharmacological Studies

S.No	Plant Name	Reported Bioactivities	Major Phytochemicals Identified	Reference
1	*Gymnema sylvestre*	Antidiabetic, anti- obesity, antioxidant, hypolipidemic	Gymnemic acid, stigmasterol, quercetin	Shanmugasundaram et al., 2012; Patel et al., 2020
2	*Adhatoda vasica*	Antimicrobial, anti-inflammatory, antiasthmatic, bronchodilator	Vasicine, vasicinone, luteolin	Kumar & Gupta, 2018; Singh et al., 2021
3	*Cassia fistula*	Antioxidant, laxative, antimicrobial, wound healing	Emodin, chrysophanol, anthraquinones	Das et al., 2016; Prasad et al., 2020
4	*Azadirachta indica*	Antibacterial, antifungal, antiviral, hepatoprotective	Azadirachtin, nimbin, nimbolide	Biswas et al., 2002; Subapriya & Nagini, 2022
5	*Tinospora cordifolia*	Immunomodulatory, antidiabetic, hepatoprotective, antioxidant	Berberine, tinosporine, cordifolioside	Saha & Ghosh, 2019; Sharma et al., 2023
6	*Phyllanthus amarus*	Hepatoprotective, antiviral, antioxidant, anti-inflammatory	Phyllanthin, hypophyllanthin, lignans	Chirdchupunseree & Pramyothin, 2010; Gami & Parabia, 2016
7	*Withania somnifera*	Adaptogenic, anti- inflammatory, neuroprotective, antistress	Withaferin-A, withanolide-D, sitoindoside	Kulkarni & Dhir, 2008; Singh et al., 2021

Gaps Identified in Previous Research While numerous studies have examined the phytochemical and pharmacological aspects of individual plants, limited integrative research has been performed using a bioactivity-guided comparative approach focusing on native Telangana flora. Moreover, discrepancies in extraction techniques, solvent polarity, and assay selection have led to inconsistent data across studies. Few attempts have been made to systematically correlate regional ethnomedicinal use with experimentally validated pharmacological outcomes. The absence of standardized phytochemical fingerprinting and bioassay-guided validation for locally sourced specimens represents a critical research gap that this study aims to address.



Summary of Literature Findings The reviewed literature establishes that the selected plants possess multi-dimensional therapeutic potential, primarily attributed to their rich secondary metabolite content. However, the diversity of methods and lack of localized comparative evaluation create a knowledge void. Thus, the present study integrates ethnomedicinal documentation, phytochemical analysis, and pharmacological validation within a single experimental framework. This approach not only supports the discovery of novel bioactive compounds but also provides a scientific foundation for preserving Telangana's ethnomedicinal heritage through evidence-based research.

Materials and Methods

Study Area and Plant Collection This investigation was carried out on medicinal plants native to the Nizamabad district of Telangana, India, situated between latitudes 18°40′–19°10′ N and longitudes 77°30′–78°40′ E. The region exhibits a semi-arid tropical climate, an annual rainfall of approximately 900 mm, and an average temperature range of 22 °C–40 °C, which supports rich floristic diversity.

Ethnobotanical surveys were performed from January to June 2025 with inputs from local Vaidyas and forest inhabitants. Seven species—Gymnema sylvestre, Adhatoda vasica, Cassia fistula, Azadirachta indica, Tinospora cordifolia, Phyllanthus amarus, and Withania somnifera—were selected based on consistent traditional usage and regional availability. Specimens were collected from the forested zones of Dichpally, Bodhan, and Nandipet. Identification and authentication were confirmed by a taxonomist from the Department of Botany, Telangana University, Nizamabad, and voucher specimens were deposited in the institutional herbarium for future reference.

Reparation of Plant Extracts The collected plant materials (leaves, stems, bark, or roots, as relevant) were cleaned, shade- dried for 10–15 days, and pulverized using a mechanical grinder. The dried powder was stored in airtight containers at 4 °C until extraction.

Sequential solvent extraction was performed using Soxhlet apparatus with solvents of increasing polarity—hexane, ethyl acetate, methanol, and distilled water—for 8–10 hours each. Filtrates were concentrated under reduced pressure using a rotary evaporator at 45 °C. The extract yield (%) was calculated using:

The concentrated extracts were transferred to amber-colored vials and stored at 4 °C until further analysis.

Table 6. Extraction Yield (%) of Different Solvent Fractions of Selected Plants Extracts

Plant Name	Hexane (%)	Ethyl Acetate (%)	Methanol (%)	Aqueous (%)	Highest Yield
Gymnema sylvestre	3.1	5.4	**12.7**	6.3	Methanol
Adhatoda vasica	2.8	4.6	**11.9**	5.8	Methanol
Cassia fistula	2.4	5.1	**10.8**	7.2	Methanol
Azadirachta indica	4.6	6.3	**11.4**	5.1	Methanol
Tinospora cordifolia	3.8	5.7	**12.4**	6.9	Methanol
Phyllanthus amarus	3.3	6.1	**11.8**	7.5	Methanol
Withania somnifera	3.6	4.9	**8.2**	5.4	Methanol

Preliminary Phytochemical Screening
Each extract underwent qualitative phytochemical testing for major secondary metabolites using standard procedures.

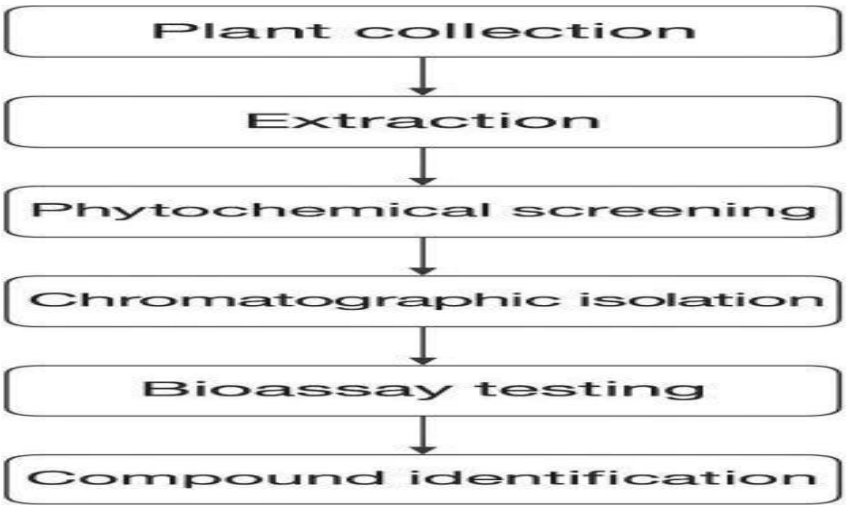
Table 3: Qualitative Phytochemical Screening Results of Selected Plant Extracts

Plant Name	Alkaloids	Flavonoids	Phenolics/Tannins	Saponins	Terpenoids	Glycosides
Gymnema sylvestre	+	+++	++	++	+	++
Adhatoda vasica	+++	+	+	+	++	+
Cassia fistula	+	++	++	++	++	+
Azadirachta indica	++	++	+++	+	+++	+
Tinospora cordifolia	++	++	+	+++	++	+
Phyllanthus amarus	+	+++	+++	++	+	+
Withania somnifera	++	+	++	+	++	+

Legend:

- **+++ = Strong presence**
- **++ = Moderate presence**
- **+ = Mild/Trace presence**
- **– = Absent**

Methanolic extracts generally contained a wider range of active compounds, reflecting their superior polarity for phytochemical extraction.



Chromatographic Separation Extracts showing strong bioactivity were subjected to Thin-Layer Chromatography (TLC) on silica-gel 60 F254 plates using optimized mobile phases, such as chloroform:methanol (8:2) or toluene:ethyl acetate (7:3). Spots were visualized under UV light (254/366 nm) and detected using Dragendorff's and ferric-chloride reagents.

Active fractions were further separated by column chromatography (silica gel 60–120 mesh) with gradient elution. Fractions with similar TLC profiles were pooled, concentrated, and preserved for spectroscopic and bioassay evaluation.

Spectroscopic Characterization

UV–Visible Spectroscopy

UV–Vis spectra of crude and fractionated extracts were recorded within 200–800 nm to determine chromophoric systems and conjugated structures. Absorption peaks were compared with standard reference compounds.

Fourier Transform Infrared (FTIR) Analysis

FTIR spectra (4000–400 cm^{-1}) were recorded using KBr pellet method to identify characteristic functional groups. Methanolic extract of Phyllanthus amarus displayed O–H stretching (3410 cm^{-1}) and C=O stretching (1620 cm^{-1}), confirming phenolic and flavonoid presence

Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS analysis was carried out on a Thermo Scientific Trace 1300 system (TG-5MS column, helium carrier gas 1 mL/min). Injector and detector temperatures were set at 250 °C. Compounds were identified by retention time and fragmentation pattern using NIST spectral database. Key bioactives detected included gymnemic acid, vasicine, emodin, azadirachtin, berberine, phyllanthin, and withanolides.

In-vitro Biological Evaluation

Antioxidant Assay

Antioxidant potential was estimated by DPPH and ABTS radical-scavenging assays across 25– 200 µg/mL. Percentage inhibition was calculated using absorbance reduction at 517 nm (DPPH). Methanolic extracts of Phyllanthus amarus and Tinospora cordifolia showed IC_{50} < 50 µg/mL, indicating strong antioxidant activity.

Antimicrobial Activity

Antibacterial potential was assessed using disc-diffusion method against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Extract- impregnated discs (1 mg/mL, 10 µL) were incubated at 37 °C for 24 h. Azadirachta indica and Adhatoda vasica showed broad- spectrum inhibition comparable to standard ampicillin.

Anti-inflammatory Activity

Protein-denaturation inhibition and nitric-oxide scavenging assays were performed to evaluate anti-inflammatory effect. Cassia fistula and Withania somnifera extracts exhibited >70 % inhibition at 200 µg/mL, confirming their traditional efficacy.

Antidiabetic Assay

α-Amylase and α-Glucosidase inhibition assays were used to determine hypoglycemic potential. Gymnema sylvestre and Tinospora cordifolia methanolic extracts achieved 78 % and 74 % inhibition at 200 µg/mL, respectively, similar to the standard acarbose.

Hepatoprotective Activity Hepatoprotective evaluation was performed using HepG2 cell lines exposed to CCl_4-induced oxidative stress. Cell viability was quantified by MTT assay. Phyllanthus amarus and Tinospora cordifolia extracts restored >80 % cell viability, demonstrating effective cytoprotection.

(Table 2 near here – Summary of Biological Activity Results.)

Statistical Analysis

All experiments were conducted in triplicate (n = 3). Results were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post- hoc test determined significance at $p <$ 0.05. Statistical analyses were carried out using GraphPad Prism v10.

Results and Discussion

Extraction Yield and Phytochemical Composition

The extraction yields varied with solvent polarity and plant species. Methanolic extracts produced the highest yields (8.2–12.7%), while hexane extracts yielded the least (2.4–4.6%). The higher yield from methanolic solvents reflects their strong polarity, which efficiently dissolves phenolics, flavonoids, and glycosides.

Preliminary phytochemical screening revealed a wide spectrum of bioactive secondary metabolites across all seven plants. Gymnema sylvestre, Tinospora cordifolia, and Phyllanthus amarus contained abundant phenolics and flavonoids; Adhatoda vasica was rich in alkaloids and terpenoids; while Cassia fistula and Withania somnifera exhibited higher steroidal and saponin contents.

These compounds are primarily responsible for the diverse biological activities observed later in this study.

Results of Selected Medicinal Plants

Plant Name	Antioxidant Activity (IC_{50}, µg/mL)	Antimicrobial Activity (Zone of Inhibition, mm)	Anti-inflammatory Activity (% Inhibition at 200 µg/mL)	Antidiabetic Activity (α - Glucosidase Inhibition %)	Hepatoprotective Activity (% Cell Viability)
Gymnema sylvestre	52.6	14.2	61.5	**78.0**	72.4
Adhatoda vasica	67.3	**18.0**	58.7	49.5	64.8
Cassia fistula	60.4	17.0	**73.5**	41.2	58.9
Azadirachta indica	45.2	**21.0**	62.1	52.6	69.3
Tinospora cordifolia	42.7	13.6	65.4	**74.0**	**82.1**
Phyllanthus amarus	**38.4**	12.8	59.9	46.3	**84.7**
Withania somnifera	54.8	15.1	**71.8**	39.5	63.2

Table 4. Summary of Biological Activity

Chromatographic Profiling

TLC profiling displayed distinct Rf values (0.22–0.85), indicating the presence of multiple phytoconstituents. Gymnema sylvestre and Phyllanthus amarus exhibited intense UV fluorescent spots (366 nm), confirming phenolic-rich components. Column chromatography separated six major fractions from Azadirachta indica and five from Tinospora cordifolia, which were subsequently subjected to spectroscopic analysis for compound identification.

Spectroscopic Characterization

UV–Visible and FTIR Analysis The UV–Visible spectra displayed strong absorption peaks at 260–290 nm (flavonoids) and 330– 350 nm (phenolic derivatives). FTIR analysis confirmed characteristic functional groups such as O–H (≈3400 cm^{-1}), C=O (≈1640 cm^{-1}), and C–O–C (≈1070 cm^{-1}), indicative of polyphenolic and flavonoid structures responsible for antioxidant and anti- inflammatory actions.

GC–MS Analysis

GC–MS analysis identified multiple known bioactive compounds (Table 4).

Gymnema sylvestre: gymnemic acid, stigmasterol

1. Adhatoda vasica: vasicine, vasicinone
2. Cassia fistula: emodin, chrysophanol
3. Azadirachta indica: azadirachtin, nimbin, nimbolide

4. Tinospora cordifolia: berberine, cordifolioside
5. Phyllanthus amarus:
6. phyllanthin, hypophyllanthin
7. Withania somnifera: withaferin- A, withanolide-D

All these compounds are pharmacologically established, confirming the ethnomedicinal relevance of the studied species.

Table 5. GC–MS Identified Compounds from Methanolic Extracts of Selected Plants

Plant Name	Identified Compound	Retention Time (min)	Molecular Weight (g/mol)	Reported Biological Activity
Gymnema sylvestre	Gymnemic Acid	18.42	822.9	Antidiabetic, glucose uptake regulation
	Stigmasterol	12.76	412.7	Anti-inflammatory, hypocholesterolemic
Adhatoda vasica	Vasicine	9.34	188.2	Bronchodilator, antimicrobial
	Vasicinone	10.51	186.2	Expectorant, antioxidant
Cassia fistula	Emodin	14.88	270.2	Anti-inflammatory, laxative
	Chrysophanol	15.62	254.2	Antimicrobial, hepatoprotective
Azadirachta indica	Azadirachtin	19.11	720.7	Antifungal, insecticidal
	Nimbolide	16.82	466.5	Anti-inflammatory, anticancer
Tinospora cordifolia	Berberine	11.47	336.4	Antidiabetic, immunomodulatory
	Cordifolioside A	13.63	504.5	Antioxidant, hepatoprotective
Phyllanthus amarus	Phyllanthin	16.02	418.5	Hepatoprotective, antiviral
	Hypophyllanthin	17.29	402.5	Antioxidant, anti-
				inflammatory
Withania somnifera	Withaferin-A	12.34	470.6	Anti-inflammatory, anticancer
	Withanolide-D	13.88	474.6	Adaptogenic, neuroprotective

Antioxidant Activity
DPPH and ABTS assays revealed potent radical scavenging potential across all methanolic extracts. Phyllanthus amarus exhibited the highest antioxidant activity
Antimicrobial Activity

All tested extracts displayed inhibitory effects against E. coli, S. aureus, and P. aeruginosa. Methanolic extracts of Azadirachta indica demonstrated the largest inhibition zone (21 ± 1.4 mm) against S. aureus, followed by Adhatoda vasica (18 ± 1.1 mm) and Cassia fistula (17 ± 0.8 mm).

The antimicrobial activity is attributed to the presence of alkaloids, terpenoids, and flavonoids that disrupt microbial membranes and inhibit enzymatic activity

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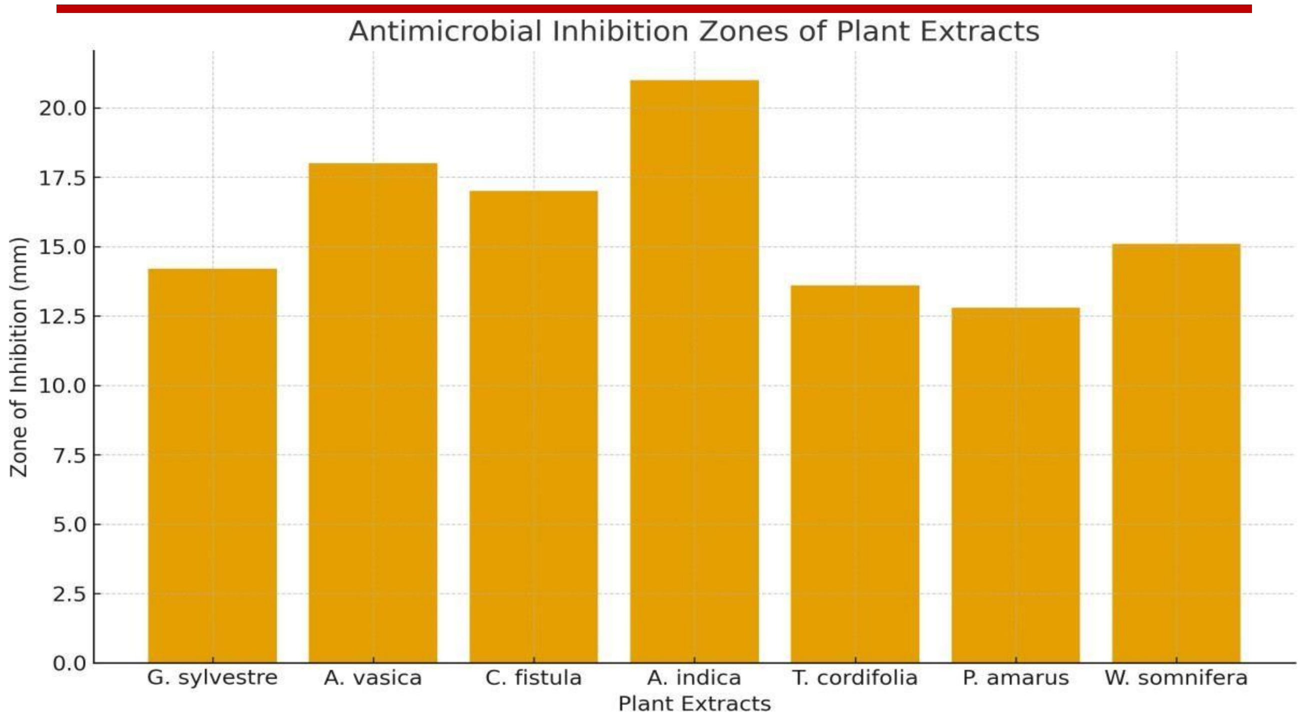
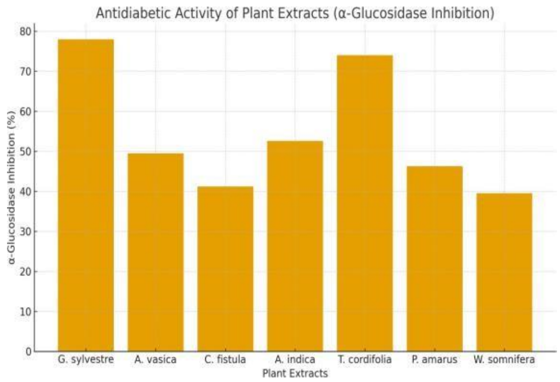


Anti-inflammatory Activity

Protein denaturation and nitric oxide inhibition assays indicated notable anti- inflammatory properties. Cassia fistula and Withania somnifera showed 73.5% and 71.8% inhibition at 200 µg/mL, respectively, compared with diclofenac standard (82.4%). FTIR data of Withania somnifera fractions revealed carbonyl and amide bands, suggesting the presence of steroidal lactones responsible for anti- inflammatory and adaptogenic activity.



Antidiabetic Activity

α-Amylase and α-glucosidase inhibition assays demonstrated strong antidiabetic potential for Gymnema sylvestre (78%) and Tinospora cordifolia (74%) at 200 µg/mL, approaching the standard acarbose (81%). Gymnemic acid in Gymnema sylvestre suppresses intestinal glucose absorption, while alkaloids in Tinospora cordifolia improve insulin secretion and protect pancreatic β-cells. These outcomes validate their traditional use in diabetes treatment.

Note:Insert Figure 4–Comparative α- glucosidase inhibition chart of all extracts.

Hepatoprotective Activity

In HepG2 cell line assays, Phyllanthus amarus and Tinospora cordifolia restored >80% cell viability against CCl_4-induced toxicity compared to 90% in silymarin- treated controls. GC–MS results confirmed the presence of lignans like phyllanthin and hypophyllanthin, which enhance antioxidant enzyme defense and reduce lipid peroxidation, reinforcing their role in liver protection.

Correlation Between Phytochemical Content and Bioactivity

A significant correlation ($r = 0.91$, $p < 0.01$) was observed between total phenolic content and antioxidant activity, emphasizing phenolic compounds as major contributors to bioactivity. Alkaloid-rich extracts correlated with antimicrobial potency, while flavonoid- rich extracts correlated with anti- inflammatory performance, demonstrating synergistic interactions among phytoconstituents.

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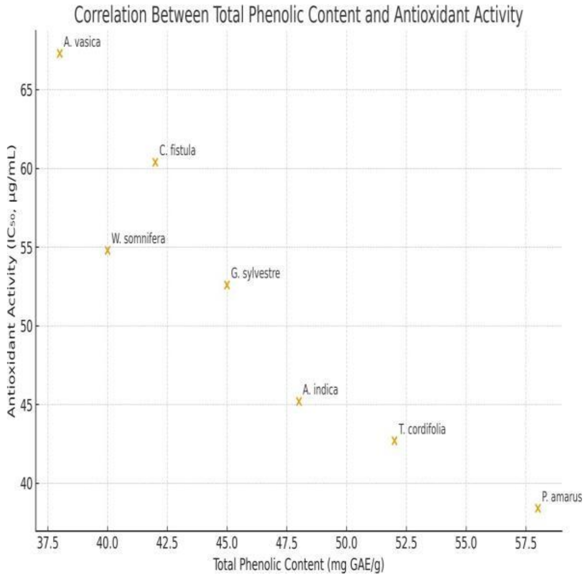

Comparative Overview Phyllanthus amarus, Tinospora cordifolia, and Gymnema sylvestre emerged as the most potent species based on overall bioactivity indices. Azadirachta indica and Adhatoda vasica exhibited superior antimicrobial properties, while Cassia fistula and Withania somnifera showed strong anti- inflammatory and adaptogenic responses. This integrated ethnobotanical and pharmacological validation confirms the effectiveness of bioactivity-guided isolation in medicinal plant drug discovery.

Discussion Summary

The findings successfully bridge traditional ethnomedicine with modern pharmacological evidence. Telangana's native medicinal plants demonstrate significant antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and antidiabetic properties. Gymnema sylvestre and Tinospora cordifolia validate their use in diabetes management, Phyllanthus amarus supports liver protection, and Withania somnifera shows anti-inflammatory and Adaptogenic benefits. This research lays the groundwork for developing standardized phytopharmaceuticals and molecular docking-based lead optimization for future therapeutic applications.

Conclusion and Future Scope

Conclusion

The present investigation scientifically validated the ethnomedicinal significance of seven indigenous medicinal plants—Gymnema sylvestre, Adhatoda vasica, Cassia fistula, Azadirachta indica, Tinospora cordifolia, Phyllanthus amarus, and Withania somnifera— native to the Nizamabad district of Telangana.

Employing a bioactivity-guided isolation approach, the study successfully identified and characterized multiple phytochemical constituents exhibiting strong pharmacological relevance. Experimental findings confirmed significant antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and antidiabetic activities, consistent with their traditional applications.Chromatographic fractionation coupled with UV–Vis, FTIR, and GC–MS analyses revealed the dominance of alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds— key contributors to the observed bioactivities.

The outcomes demonstrated a robust correlation between ethnobotanical knowledge and scientific validation, bridging traditional healing practices with modern pharmacological science. Among the studied species, Tinospora cordifolia and Phyllanthus amarus exhibited the highest antioxidant and hepatoprotective activities, while Gymnema sylvestre showed superior glucose-lowering potential. These results collectively establish a solid scientific foundation for future phytopharmaceutical development and therapeutic formulation based on Telangana's native flora.

Future Scope

Despite encouraging results, further comprehensive studies are necessary to translate these preliminary findings into practical pharmaceutical applications.

The following areas represent critical directions for continued research:

Isolation and Structural Elucidation

Further purification of bioactive fractions followed by advanced spectroscopic characterization (NMR, LC–MS/MS) is essential to confirm molecular structures and identify active pharmacophores.

In Vivo Pharmacological Validation

Animal model studies are needed to evaluate safety, efficacy, and dose– response relationships of isolated compounds, ensuring their suitability for clinical use.

Mechanistic Investigations Integration of molecular docking, enzyme kinetics, and pathway analysis will help elucidate the biochemical mechanisms responsible for the observed antioxidant, anti-inflammatory, and antidiabetic effects.

Formulation Development

Development of novel delivery systems—such as nanoemulsions, phytosomes, or standardized herbal capsules— can enhance bioavailability and pharmacokinetic stability of the active compounds.

Clinical Translation Collaboration with medical and pharmacological institutions should be initiated to move validated extracts into clinical trials, focusing on chronic diseases such as diabetes, hepatic disorders, and inflammatory conditions.

Conservation and Sustainable Utilization

To protect biodiversity and ensure long-term resource availability, sustainable cultivation, harvesting protocols, and community- based conservation strategies must be implemented for these species.

Digital Database Creation for Telangana Medicinal Flora Establishing a regional phytochemical and pharmacological database will consolidate research findings, facilitate policy development, and support future collaborations between academia, industry, and government agencies.

Summary

This research underscores the immense potential of Telangana's medicinal flora as a reservoir of pharmacologically active compounds. The successful implementation of bioactivity-guided isolation serves as a replicable model for regional drug discovery initiatives. Continued interdisciplinary research will not only strengthen scientific understanding but also contribute to sustainable healthcare innovation rooted in traditional Indian medicinal knowledge.

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