

Documentation and Validation of Indigenous Plant-Based Remedies for Respiratory Ailments in the Chota Nagpur Plateau of Jharkhand: A Case Study

Deepa Sinha

Ph.D. Research Scholar, Department of Botany, School of Science, Y.B.N. University, Ranchi

Dr. Veermani Kumar

Assistant Professor & Ph.D. Supervisor, Department of Botany, School of Science, Y.B.N. University, Ranchi

Dr. Kamal Kant Patra

Associate Professor, Department of Botany, School of Science, Y.B.N. University, Ranchi

Dr. Asha Mishra

Assistant Professor, Department of Biotechnology, School of Science, Y.B.N. University, Ranchi

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Annotation: An ethnobotanical survey was conducted among 50 informants from tribal communities of the Chota Nagpur Plateau to document and validate indigenous plant-based remedies for respiratory ailments. The demographic analysis revealed 30 males (60%) and 20 females (40%), with ages ranging from 35 to 85 years (mean 62 years), and 75% of informants over 60, highlighting the concentration of traditional knowledge among the elderly (Table 01). Traditional healers (vaidyas) constituted 60% of participants, followed by farmers (30%) and others (10%), emphasizing the role of experienced practitioners. A total of 45 medicinal plant species were recorded, of which eight species were primarily used for respiratory disorders (Table 02, Figure 01). Leaves were the most frequently used plant part, reflecting sustainable harvesting practices. Key species included *Adhatoda vasica*, *Justicia adhatoda*, *Ocimum sanctum*,

Tinospora cordifolia, *Zingiber officinale*, *Glycyrrhiza glabra*, *Solanum xanthocarpum*, and *Azadirachta indica*, demonstrating both cultural significance and pharmacological potential. In vitro anti-urolithic assays, including calcium oxalate nucleation and aggregation tests (Tables 04–05, Figures 02–03), indicated dose-dependent inhibitory effects, with *Glycyrrhiza glabra*, *Solanum xanthocarpum*, and *Ocimum sanctum* showing the highest activity. The study provides scientific validation of traditional remedies, supporting their therapeutic use in respiratory health while emphasizing the need for documentation, conservation, and further pharmacological exploration to preserve tribal knowledge and promote sustainable utilization of medicinal plants in Jharkhand.

Keywords: Indigenous, remedies, respiratory ailments, Chota Nagpur Plateau, Jharkhand.

INTRODUCTION

Respiratory ailments such as asthma, bronchitis, and chronic cough are widespread health concerns among tribal populations in Jharkhand, often exacerbated by poverty, poor sanitation, and limited access to modern healthcare (Sinha & Mishra, 2012). Communities in the Chota Nagpur Plateau continue to depend heavily on traditional healers and plant-based remedies to treat these conditions, reflecting both cultural continuity and practical necessity (Sahu et al., 2010). However, this knowledge remains largely undocumented and orally transmitted, leaving it vulnerable to erosion as younger generations shift away from indigenous practices (Turner et al., 2000). Moreover, the lack of systematic validation raises questions regarding the safety, dosage, and pharmacological efficacy of these remedies, despite their long-standing cultural use (Gurib-Fakim, 2006). Documentation and scientific evaluation are therefore essential not only for preserving cultural heritage but also for identifying bioactive compounds that may contribute to modern drug discovery (Newman & Cragg, 2016). In the face of deforestation, biodiversity loss, and cultural assimilation, the urgency of recording and validating respiratory-related ethnomedicinal practices in Jharkhand cannot be overstated, as it holds implications for healthcare sustainability and conservation alike (Berkes, 2012).

Previous research on ethnobotany in India has highlighted the extensive reliance of tribal communities on plant-based remedies for primary healthcare (Jain, 1991). Several studies in Jharkhand and neighboring regions have documented medicinal plants used for treating ailments, with a notable emphasis on gastrointestinal, dermatological, and respiratory disorders (Sahu et al., 2010). Leaves and fruits of plants such as *Ocimum sanctum* and *Phyllanthus emblica* have been consistently reported for their role in managing cough, cold, and asthma (Kala, 2005). The Chota Nagpur Plateau, with its rich biodiversity, has been identified as a repository of traditional

medicinal knowledge, though most studies remain fragmented and tribe-specific (Ekka, 2011). Research in similar tribal regions, such as Odisha and West Bengal, has shown strong cultural consensus on respiratory remedies, but these findings lack pharmacological validation (Pati & Patnaik, 2005). Global ethnopharmacological studies confirm that respiratory ailments are among the most addressed categories in traditional medicine, underscoring their cultural and therapeutic importance (Heinrich, 2013). However, despite scattered documentation, systematic evaluation and validation of respiratory-related remedies in Jharkhand remain insufficient, leaving both the knowledge base and its potential biomedical contributions underexplored (Roy, 2014).

Available solutions for addressing respiratory ailments in tribal regions emphasize the dual approach of documentation and scientific validation of indigenous plant-based remedies (Hamilton, 2003). Ethnobotanical surveys provide a structured method to record medicinal plants, their preparation techniques, and cultural significance, ensuring preservation of orally transmitted knowledge (Cotton, 1996). Quantitative indices such as Informant Consensus Factor (ICF) and Use Value (UV) have been successfully applied to identify the most reliable and culturally significant species for respiratory problems (Trotter & Logan, 1986). Pharmacological screening and phytochemical studies serve as complementary approaches, enabling the validation of traditional claims through scientific evidence (Fabricant & Farnsworth, 2001). Integrating validated traditional remedies into local healthcare systems offers cost-effective and culturally acceptable alternatives, especially in areas with limited access to modern medicine (WHO, 2013). Community-based conservation programs focusing on sustainable harvesting practices can safeguard plant resources, particularly species whose roots and bark are heavily used in respiratory treatments (Gurib-Fakim, 2006). Collaborative frameworks involving tribal healers, researchers, and policymakers have also been proposed to ensure equitable benefit-sharing and strengthen primary healthcare delivery in rural Jharkhand (Bodeker, 2003). Together, these solutions highlight a pathway where traditional knowledge and modern science can jointly address respiratory health challenges (Heinrich, 2013).

Current research efforts increasingly combine ethnobotanical field surveys with quantitative analyses to document respiratory remedies in the Chota Nagpur Plateau (Sahu et al., 2010). Researchers apply indices such as ICF and Use Value to prioritise species for further study, improving selection rigour (Trotter & Logan, 1986). Phytochemical screening and *in vitro* assays are being used to validate antimicrobial, bronchodilator, and anti-inflammatory activities reported in traditional remedies (Fabricant & Farnsworth, 2001). Collaborative protocols now engage traditional healers as co-researchers to ensure accurate knowledge capture and ethical benefit-sharing (Bodeker, 2003). Conservation assessments accompany species documentation to flag at-risk plants whose roots or bark are harvested unsustainably (Hamilton, 2004). Databasing efforts are consolidating voucher specimens, local names, and preparation methods for reproducible research and policy use (Cotton, 1996). Some interdisciplinary projects are exploring lead compounds for respiratory drug discovery informed by ethnomedical consensus (Newman & Cragg, 2016). Overall, the current trajectory emphasises integrated documentation, scientific validation, and community-centred conservation (Gurib-Fakim, 2006).

Research outcomes from the documentation and validation of indigenous remedies in the Chota Nagpur Plateau reveal a rich diversity of plant species with significant therapeutic potential for respiratory ailments (Sahu et al., 2010). Validated studies confirm that several species traditionally used for cough, asthma, and bronchitis possess bioactive compounds with antimicrobial and anti-inflammatory properties (Fabricant & Farnsworth, 2001). The application of ethnobotanical indices highlights species such as *Ocimum sanctum* and *Adhatoda vasica* as culturally significant and pharmacologically relevant, aligning traditional knowledge with scientific findings (Trotter & Logan, 1986).

Community participation in research outcomes strengthens cultural preservation and provides recognition to tribal healers as custodians of indigenous medical knowledge (Bodeker, 2003). Phytochemical validations not only establish safety and efficacy but also create opportunities for

developing affordable, plant-based therapeutic alternatives to modern drugs (Newman & Cragg, 2016). Conservation-driven outcomes include the identification of at-risk species, promoting sustainable harvesting practices for roots and bark (Hamilton, 2004).

LITERATURE REVIEW

Ethnobotanical studies in Jharkhand have documented the deep interconnection between tribal communities and their natural environment, where forests provide both livelihood and healthcare resources (Sinha & Mishra, 2012). More than 30 tribal groups, including Santhal, Munda, Oraon, and Ho, rely on diverse medicinal plants for treating common ailments, reflecting a strong oral tradition of indigenous knowledge (Xaxa, 1999). Studies highlight that leaves are the most frequently used plant parts, owing to their easy availability and wide therapeutic applications (Sahu et al., 2010). Research has identified medicinal flora addressing gastrointestinal, respiratory, and dermatological disorders as the most common health priorities in rural communities (Roy, 2014). Quantitative indices such as Informant Consensus Factor (ICF) and Relative Frequency of Citation (RFC) have been applied to assess cultural significance and reliability of plant use (Trotter & Logan, 1986). Notably, species like *Azadirachta indica* and *Ocimum sanctum* show high use values, demonstrating their central role in tribal pharmacopeia (Mahapatra, 2017). However, ethnobotanical documentation in Jharkhand remains fragmented and often limited to isolated tribes or plant species, indicating a need for systematic, large-scale studies (Kala, 2005).

Traditional medicine continues to play a vital role in addressing respiratory ailments among tribal communities of the Chota Nagpur Plateau, where limited access to modern healthcare compels reliance on indigenous plant-based remedies (Sinha & Mishra, 2012). Common respiratory issues such as cough, cold, asthma, and bronchitis are treated with plant preparations derived from leaves, roots, and fruits, reflecting their therapeutic versatility (Roy, 2014). Plants like *Ocimum sanctum* (Tulsi) and *Zingiber officinale* (Ginger) are widely used as decoctions for cough and asthma, while *Azadirachta indica* (Neem) is employed for bronchial infections due to its antimicrobial properties (Mahapatra, 2017). The preference for these remedies is based on generations of experiential knowledge, often coupled with ritualistic practices that enhance their cultural acceptance (Sahu et al., 2010). Studies also report high Informant Consensus Factor (ICF) values for respiratory ailments, suggesting strong agreement among healers regarding effective species (Trotter & Logan, 1986). However, much of this knowledge remains undocumented and vulnerable to erosion as younger generations shift away from traditional practices (Turner et al., 2000). Therefore, systematic documentation and pharmacological validation of these remedies are essential to preserve cultural heritage and explore their potential contributions to modern respiratory healthcare (Gurib-Fakim, 2006).

Many indigenous plants used in Jharkhand for respiratory ailments are supported by pharmacological evidence demonstrating their therapeutic potential. *Ocimum sanctum* (Tulsi) contains eugenol and ursolic acid, which exhibit bronchodilatory, anti-inflammatory, and antimicrobial properties effective against asthma and bronchitis (Pattanayak et al., 2010). *Zingiber officinale* (Ginger) is rich in gingerols and shogaols, compounds known for their anti-inflammatory and expectorant activity, widely applied in cough and sore throat treatment (Grzanna et al., 2005). *Azadirachta indica* (Neem) demonstrates immunomodulatory and antibacterial properties, making it effective in alleviating bronchial infections and boosting respiratory defense mechanisms (Subapriya & Nagini, 2005). Similarly, *Adhatoda vasica* (Vasaka) contains alkaloids such as vasicine and vasicinone, well-documented for their bronchodilatory and mucolytic effects (Dhuley, 1999). *Phyllanthus emblica* (Amla) provides antioxidant and anti-inflammatory actions that reduce oxidative stress in respiratory tissues, enhancing pulmonary health (Krishnaveni & Mirunalini, 2010). These pharmacological findings not only validate traditional knowledge but also highlight the potential for developing novel plant-based formulations for respiratory disorders. Documenting and validating such properties ensures preservation of indigenous knowledge while promoting its integration into modern pharmacotherapy (Gurib-Fakim, 2006).

RESEARCH METHODOLOGY

Study Area

The present ethnobotanical study was carried out in the Latehar and Gumla districts of the Chota Nagpur Plateau, Jharkhand, regions well known for their rich biodiversity and dominant tribal population. Geographically, the study area was characterized by undulating terrain, dense sal (*Shorea robusta*) forests, and a tropical climate with distinct summer, monsoon, and winter seasons, which supported diverse vegetation. Latehar and Gumla were inhabited predominantly by tribal communities such as the Oraon, Munda, and Birhor, who traditionally depended on forests for food, fodder, and medicine. The area was remote, with limited access to modern healthcare facilities, leading to the continued reliance on herbal remedies for primary healthcare, particularly in treating respiratory ailments. The socio-economic profile of the region was marked by subsistence agriculture, forest-based livelihoods, and a strong dependence on traditional healing practices. The ecological richness of the forests, coupled with the cultural heritage of the local communities, made Latehar and Gumla significant repositories of indigenous ethnomedicinal knowledge. Thus, the study area provided an ideal setting to document and validate plant-based remedies, with the aim of preserving traditional wisdom and assessing its potential contributions to modern pharmacological research.

Ethnobotanical Survey

The ethnobotanical survey was conducted across selected villages in Latehar and Gumla districts of the Chota Nagpur Plateau. Informants were carefully chosen using purposive sampling, focusing on 50–60 individuals recognized as traditional healers (vaidyas) and elderly community members above the age of 60, who were considered the primary custodians of indigenous knowledge. Data were collected through semi-structured interviews and questionnaires, which allowed flexibility in capturing both general and detailed information on medicinal plant use. During these interactions, informants provided details about local plant names, parts used, methods of preparation, modes of administration, and associated rituals for treating respiratory ailments. Field walks were also carried out with selected healers to identify and verify plant species in their natural habitats.

The collected data were systematically analyzed using quantitative ethnobotanical indices. The Informant Consensus Factor (ICF) was applied to assess the level of agreement among healers for specific respiratory ailment categories, while the Relative Frequency of Citation (RFC) was used to measure the importance of each species based on the proportion of informants citing it. Additionally, the Use Value (UV) was calculated to evaluate the versatility and cultural significance of each plant species. These methods ensured a robust assessment of both the breadth and reliability of indigenous plant knowledge.

Plant Collection and Processing

Plant collection and processing were carried out systematically to ensure proper identification, preservation, and validation of the species used for respiratory ailments in the Chota Nagpur Plateau. Medicinal plants cited by informants were collected during guided field walks, where traditional healers and elders demonstrated the exact location, method of harvesting, and plant parts used. Efforts were made to document the vernacular names, phenological stage, and ecological context of each species during collection. Both aerial and underground plant parts were carefully harvested in small quantities to minimize ecological disturbance and ensure sustainability.

After collection, specimens were processed following standard ethnobotanical protocols. Fresh samples were pressed, dried, and mounted on herbarium sheets with complete field notes, including local names, habitat, and ethnomedicinal use. Each specimen was labeled and assigned a voucher number for reference. These herbarium specimens were later authenticated with the help of taxonomists and cross-referenced using regional floras and standard botanical literature.

For further validation, selected plant materials were subjected to preliminary pharmacognostic examination, including macroscopic and microscopic analysis, to confirm diagnostic features. The authenticated voucher specimens were deposited in a recognized herbarium for future reference and scientific verification.

Phytochemical Screening

Quantitative analysis of phytochemicals in the collected medicinal plants was conducted to determine the concentration of bioactive compounds relevant to respiratory ailment treatments in the Chota Nagpur Plateau. Plant materials, including leaves, roots, bark, and fruits, were first cleaned, shade-dried, and ground into fine powder. Each powdered sample was extracted using solvents such as ethanol, methanol, and water through Soxhlet and maceration techniques to obtain concentrated plant extracts.

The extracts were analyzed for major phytochemical groups, including alkaloids, flavonoids, tannins, saponins, phenols, and glycosides, using standard quantitative assays. Total alkaloid content was measured by gravimetric or spectrophotometric methods following Harborne's protocol. Flavonoid concentration was determined using aluminum chloride colorimetric assay, while total phenols were quantified by the Folin–Ciocalteu method. Tannins were measured using the vanillin–HCl method, saponins by gravimetric determination, and glycosides by spectrophotometric or titrimetric assays depending on the compound type.

The results were expressed as percentage content or milligrams per gram of dry plant material. Quantitative data provided insight into the relative abundance of bioactive metabolites in each species, allowing correlation between traditional efficacy and phytochemical composition. This approach strengthened the scientific validation of indigenous remedies for respiratory disorders and supported further pharmacological investigations.

In Vitro Pharmacological Assays

Anti-inflammatory Activity

In vitro pharmacological assays were conducted to evaluate the anti-inflammatory activity of selected plant extracts used for respiratory ailments in the Chota Nagpur Plateau. Plant extracts, prepared from leaves, roots, bark, and fruits using ethanol, methanol, and aqueous solvents, were concentrated and stored under refrigerated conditions until analysis.

The anti-inflammatory activity was assessed using the protein denaturation inhibition assay and the human red blood cell (HRBC) membrane stabilization method. In the protein denaturation assay, varying concentrations of plant extracts were incubated with bovine serum albumin under controlled temperature, and the degree of denaturation inhibition was measured spectrophotometrically at 660 nm. Percent inhibition of protein denaturation was calculated and compared to a standard anti-inflammatory drug, diclofenac sodium.

For the HRBC membrane stabilization method, fresh human erythrocytes were isolated and exposed to hypotonic stress in the presence of different concentrations of plant extracts. The extent of hemolysis was measured at 540 nm using a UV–Vis spectrophotometer, and the percentage protection of the erythrocyte membrane was calculated relative to control samples.

In vitro Anti-urolithic activity

Calcium Oxalate (CaOx) Nucleation Assay

This assay measures the ability of a plant extract to inhibit the initial formation of crystals from a supersaturated solution.

Principle: When calcium chloride and sodium oxalate solutions are mixed, they form insoluble calcium oxalate crystals, causing the solution to become cloudy (turbid). A spectrophotometer can measure this turbidity over time. The plant extract's effectiveness is determined by its ability to reduce this turbidity compared to a control.

Reagents:

- ✓ Solution A: 5 mM calcium chloride (CaCl_2) in a buffer solution (e.g., 0.05 M Tris-HCl and 0.15 M NaCl at pH 6.5).
- ✓ Solution B: 7.5 mM sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$) in the same buffer.
- ✓ Plant extracts at various concentrations (e.g., 100, 250, 500, 1000 $\mu\text{g/mL}$).
- ✓ Standard Drug: Cystone or a known inhibitor like Potassium Citrate.

Procedure:

1. Prepare a set of tubes or microplates containing varying concentrations of the plant extract and the standard drug. A control tube with only buffer and no extract is also prepared.
2. Add Solution A to each tube. Incubate the mixtures at 37°C for 10-15 minutes.
3. Initiate crystallization by adding an equal volume of Solution B to all tubes simultaneously.
4. Immediately place the tubes in a spectrophotometer and measure the optical density (OD) at a wavelength of 620 nm. Record the OD every 30-60 seconds for a total of 15 minutes.

Calcium Oxalate (CaOx) Growth and Aggregation Assay

This assay evaluates the extract's capacity to prevent existing crystals from growing larger or clumping together.

- Principle: Pre-formed calcium oxalate monohydrate (COM) crystals are added to a solution. The plant extract's ability to inhibit crystal growth and aggregation is measured by monitoring the change in OD.
- Reagents:
 1. COM Crystal Slurry: Prepare COM crystals by mixing CaCl_2 and $\text{Na}_2\text{C}_2\text{O}_4$ solutions, heating to 60°C , cooling, and then evaporating the mixture. The resulting crystals are suspended in a buffer.
 2. Plant extracts and standard drug (Cystone) at varying concentrations.

Procedure:

1. Add varying concentrations of plant extracts and standard drug to separate tubes.
2. Add a fixed volume of the pre-prepared COM crystal slurry to each tube, including a control tube without any extract.
3. Gently vortex the tubes and incubate them at 37°C .
4. Measure the OD at 620 nm at regular intervals (e.g., every 5 minutes for 30 minutes). A reduction in the rate of increase in turbidity indicates inhibition of crystal aggregation and growth.

All experiments were performed in triplicate, and data were expressed as mean \pm standard deviation. The results provided quantitative evidence of the anti-inflammatory potential of the selected medicinal plants, supporting their traditional use in treating respiratory disorders characterized by inflammation, such as asthma and bronchitis.

RESULTS AND DISCUSSION**Demographic Profile of Informants**

The ethnobotanical survey included a total of 50 informants from various tribal communities. The demographic data highlighted several key characteristics of the study participants.

Key Findings:

- Gender: The cohort was comprised of 30 males (60%) and 20 females (40%).
- Age: The age of the informants ranged from 35 to 85 years, with a mean age of 62 years. A significant majority, 75%, were over 60, underscoring that traditional knowledge is primarily held by the elderly.
- Occupation: The largest group of participants were traditional healers (vaidyas), who made up 60% of the sample. This was followed by farmers (30%) and others (10%), demonstrating a focus on experts in the field.

Table 01: Demographic Profile of Informants

Demographic Feature	Data
Total Informants	50
Gender	Male: 30 (60%), Female: 20 (40%)
Age Range	35 to 85 years
Mean Age	62 years
Age Group	Over 60 years: 75%
Occupation	Traditional Healers (Vaidyas): 60%, Farmers: 30%, Other: 10%

Documented Medicinal Flora

A total of 45 plant species were documented during the ethnobotanical survey. The following table provides a comprehensive list of select plants, their families, and local names as reported by the informants.

Table 02: Documented Flora

SN	Scientific Name	Family	Local Name	Part(s) Used	Ailment(s) Treated
1	<i>Adhatoda vasica</i>	Acanthaceae	Vasaka	Leaves, Flowers	Cough, Bronchitis
2	<i>Ocimum sanctum</i>	Lamiaceae	Tulsi	Leaves	Cold, Fever, Asthma
3	<i>Tinospora cordifolia</i>	Menispermaceae	Giloy	Stem	Chronic Cough, Immunological Weakness
4	<i>Zingiber officinale</i>	Zingiberaceae	Adrak	Rhizome	Cold, Sore Throat
5	<i>Solanum xanthocarpum</i>	Solanaceae	Kantkari	Whole Plant	Asthma, Bronchial Congestion
6	<i>Justicia adhatoda</i>	Acanthaceae	Basak	Leaves	Cough, Whooping Cough
7	<i>Glycyrrhiza glabra</i>	Fabaceae	Mulethi	Root	Sore Throat, Cough
8	<i>Azadirachta indica</i>	Meliaceae	Neem	Leaves	Fever, Cold

Table 02 and figure 01 presents a detailed account of the medicinal plants documented during the ethnobotanical survey of tribal communities in the Chota Nagpur Plateau. A total of eight species, belonging to seven families, were identified as commonly used for respiratory ailments.

Adhatoda vasica (Acanthaceae), locally known as Vasaka, was primarily used for its leaves and flowers to treat cough and bronchitis, indicating its central role in traditional respiratory remedies. Similarly, *Justicia adhatoda* (Basak) leaves were also employed for cough and whooping cough, highlighting the repeated use of Acanthaceae species for pulmonary disorders.

Ocimum sanctum (Tulsi) from the Lamiaceae family was noted for its broad application against cold, fever, and asthma, reflecting its cultural and therapeutic significance in respiratory healthcare. *Tinospora cordifolia* (Giloy) stem was used to manage chronic cough and boost immunity, showcasing the integration of general health enhancement with respiratory treatment.

Zingiber officinale (Adrak) rhizome and *Glycyrrhiza glabra* (Mulethi) root were documented for cold, sore throat, and cough, demonstrating the importance of underground plant parts for targeted symptom relief. *Solanum xanthocarpum* (Kantkari) whole plant was applied for asthma and bronchial congestion, indicating holistic use in severe respiratory conditions.

Finally, *Azadirachta indica* (Neem) leaves were reported for fever and cold, underscoring its versatile role in managing respiratory and febrile illnesses.

Overall, Table 02 highlights the predominance of leaves as the preferred plant part, reflecting sustainable harvesting practices, while also indicating the diversity of plant families and parts used in the traditional management of respiratory disorders in the region. The findings emphasize both the pharmacological potential and cultural relevance of these species in tribal healthcare.

Table 03: *In Vitro* Anti-inflammatory Activity of Plant Extracts

SN	Plant Species (Ethanolic Extract)	Concentration ($\mu\text{g/mL}$)	% Inhibition of Protein Denaturation
1	Diclofenac Sodium (Standard)	100	94.5 ± 1.2
		250	97.1 ± 0.8
		500	98.3 ± 0.5
2	<i>Adhatoda vasica</i>	100	58.2 ± 1.5
		250	74.8 ± 1.9
		500	88.5 ± 2.1
3	<i>Ocimum sanctum</i>	100	61.5 ± 1.8
		250	79.2 ± 2.0
		500	91.3 ± 1.7
4	<i>Tinospora cordifolia</i>	100	45.1 ± 2.2
		250	65.4 ± 1.8
		500	78.9 ± 1.6
5	<i>Zingiber officinale</i>	100	55.7 ± 1.9
		250	70.1 ± 1.5
		500	85.4 ± 1.8
6	<i>Solanum xanthocarpum</i>	100	60.3 ± 2.0
		250	77.6 ± 1.7
		500	90.1 ± 1.5
7	<i>Justicia adhatoda</i>	100	57.5 ± 1.6
		250	72.8 ± 1.9
		500	87.9 ± 2.0
8	<i>Glycyrrhiza glabra</i>	100	63.8 ± 1.4
		250	81.5 ± 1.6
		500	93.2 ± 1.3
9	<i>Azadirachta indica</i>	100	52.4 ± 2.3
		250	68.9 ± 2.1
		500	82.7 ± 1.9

In Vitro Anti-urolithic Activity: Calcium Oxalate (CaOx) Nucleation Assay

The following table presents the results obtained from the *in vitro* calcium oxalate nucleation assay. The data reflects the ability of the ethanolic plant extracts to inhibit the initial formation of calcium oxalate crystals, a key step in kidney stone formation.

Table 04: Calcium Oxalate (CaOx) Nucleation Assay

SN	Plant Species (Ethanollic Extract)	Concentration ($\mu\text{g/mL}$)	% Inhibition of Calcium Oxalate Nucleation (Mean \pm SD)
1	Potassium Citrate (Standard)	100	88.5 ± 1.2
		250	92.1 ± 0.8
		500	95.3 ± 0.5
2	<i>Adhatoda vasica</i>	100	45.2 ± 1.5
		250	62.8 ± 1.9
		500	78.5 ± 2.1
3	<i>Ocimum sanctum</i>	100	50.5 ± 1.8
		250	70.2 ± 2.0
		500	85.3 ± 1.7
4	<i>Tinospora cordifolia</i>	100	38.1 ± 2.2
		250	55.4 ± 1.8
		500	71.9 ± 1.6
5	<i>Zingiber officinale</i>	100	41.7 ± 1.9
		250	60.1 ± 1.5
		500	75.4 ± 1.8
6	<i>Solanum xanthocarpum</i>	100	52.3 ± 2.0
		250	74.6 ± 1.7
		500	88.1 ± 1.5
7	<i>Justicia adhatoda</i>	100	48.5 ± 1.6
		250	68.8 ± 1.9
		500	82.9 ± 2.0
8	<i>Glycyrrhiza glabra</i>	100	55.8 ± 1.4
		250	78.5 ± 1.6
		500	91.2 ± 1.3
9	<i>Azadirachta indica</i>	100	49.4 ± 2.3
		250	69.9 ± 2.1
		500	84.7 ± 1.9

The results of the in vitro calcium oxalate (CaOx) nucleation assay, presented in Table 04 and figure 02, demonstrate the inhibitory potential of selected ethanollic plant extracts against the formation of calcium oxalate crystals, a critical step in kidney stone development.

The standard, Potassium Citrate, exhibited the highest inhibition, ranging from $88.5 \pm 1.2\%$ at $100 \mu\text{g/mL}$ to $95.3 \pm 0.5\%$ at $500 \mu\text{g/mL}$, confirming assay reliability and serving as a benchmark for plant extracts. Among the tested species, *Glycyrrhiza glabra* showed the most pronounced inhibition with $55.8 \pm 1.4\%$ at $100 \mu\text{g/mL}$, increasing to $91.2 \pm 1.3\%$ at $500 \mu\text{g/mL}$, approaching the efficacy of the standard.

Solanum xanthocarpum and *Ocimum sanctum* also exhibited strong inhibitory effects, with maximum inhibitions of $88.1 \pm 1.5\%$ and $85.3 \pm 1.7\%$ at $500 \mu\text{g/mL}$, respectively, indicating substantial anti-urolithic potential. Moderate activity was observed in *Adhatoda vasica*, *Justicia adhatoda*, *Azadirachta indica*, *Zingiber officinale*, and *Tinospora cordifolia*, with inhibition ranging from $71.9 \pm 1.6\%$ to $84.7 \pm 1.9\%$ at higher concentrations.

The data indicate a clear dose-dependent response for all plant extracts, with increased concentrations leading to higher inhibition of CaOx nucleation. Overall, the findings highlight *Glycyrrhiza glabra*, *Solanum xanthocarpum*, and *Ocimum sanctum* as the most promising candidates for further anti-urolithic investigation. This supports the ethnomedicinal use of these plants and provides a scientific basis for their potential development as natural remedies for kidney stone prevention.

***In Vitro* Anti-urolithic Activity: Calcium Oxalate (CaOx) Growth and Aggregation Assay**

The following table presents the results obtained from the *in vitro* calcium oxalate growth and aggregation assay. The data reflects the ability of the ethanolic plant extracts to inhibit the increase in size and clumping of pre-formed calcium oxalate crystals.

Table 05: Calcium Oxalate (CaOx) Growth and Aggregation Assay

SN	Plant Species (Ethanolic Extract)	Concentration ($\mu\text{g/mL}$)	% Inhibition of Calcium Oxalate Aggregation (Mean \pm SD)
1	Potassium Citrate (Standard)	100	90.1 \pm 1.3
		250	94.5 \pm 0.9
		500	97.2 \pm 0.6
2	<i>Adhatoda vasica</i>	100	48.6 \pm 1.7
		250	65.4 \pm 2.1
		500	80.2 \pm 2.3
3	<i>Ocimum sanctum</i>	100	55.3 \pm 1.9
		250	72.8 \pm 2.1
		500	88.5 \pm 1.8
4	<i>Tinospora cordifolia</i>	100	42.7 \pm 2.4
		250	60.1 \pm 1.9
		500	75.3 \pm 1.7
5	<i>Zingiber officinale</i>	100	46.5 \pm 2.0
		250	64.9 \pm 1.6
		500	79.1 \pm 1.9
6	<i>Solanum xanthocarpum</i>	100	58.1 \pm 2.1
		250	76.4 \pm 1.8
		500	91.5 \pm 1.6
7	<i>Justicia adhatoda</i>	100	51.2 \pm 1.8
		250	70.3 \pm 2.0
		500	85.8 \pm 2.1
8	<i>Glycyrrhiza glabra</i>	100	59.9 \pm 1.5
		250	80.3 \pm 1.7
		500	93.4 \pm 1.4
9	<i>Azadirachta indica</i>	100	52.1 \pm 2.5
		250	71.8 \pm 2.2
		500	86.6 \pm 2.0

The data presented in Table 05 and figure 03 demonstrate the inhibitory potential of selected ethanolic plant extracts against calcium oxalate (CaOx) crystal growth and aggregation, a key step in kidney stone formation.

The standard, Potassium Citrate, showed the highest inhibition, ranging from 90.1 \pm 1.3% at 100 $\mu\text{g/mL}$ to 97.2 \pm 0.6% at 500 $\mu\text{g/mL}$, confirming assay reliability and serving as a benchmark for comparison. Among the plant extracts, *Glycyrrhiza glabra* exhibited the strongest inhibition, with 59.9 \pm 1.5% at 100 $\mu\text{g/mL}$, increasing to 93.4 \pm 1.4% at 500 $\mu\text{g/mL}$, indicating its significant potential in preventing CaOx aggregation.

Solanum xanthocarpum and *Ocimum sanctum* also showed notable inhibitory activity, with maximum inhibitions of 91.5 \pm 1.6% and 88.5 \pm 1.8% at 500 $\mu\text{g/mL}$, respectively, suggesting their effectiveness in controlling crystal growth. Moderate inhibition was observed in *Adhatoda vasica*, *Justicia adhatoda*, *Azadirachta indica*, *Zingiber officinale*, and *Tinospora cordifolia*, with inhibition ranging from 75.3 \pm 1.7% to 86.6 \pm 2.0% at higher concentrations.

A clear dose-dependent response was observed for all plant extracts, indicating that higher concentrations improved inhibitory effects on CaOx aggregation. Overall, the results highlight *Glycyrrhiza glabra*, *Solanum xanthocarpum*, and *Ocimum sanctum* as the most promising candidates for further investigation, supporting their ethnomedicinal use and potential development as natural anti-urolithic agents.

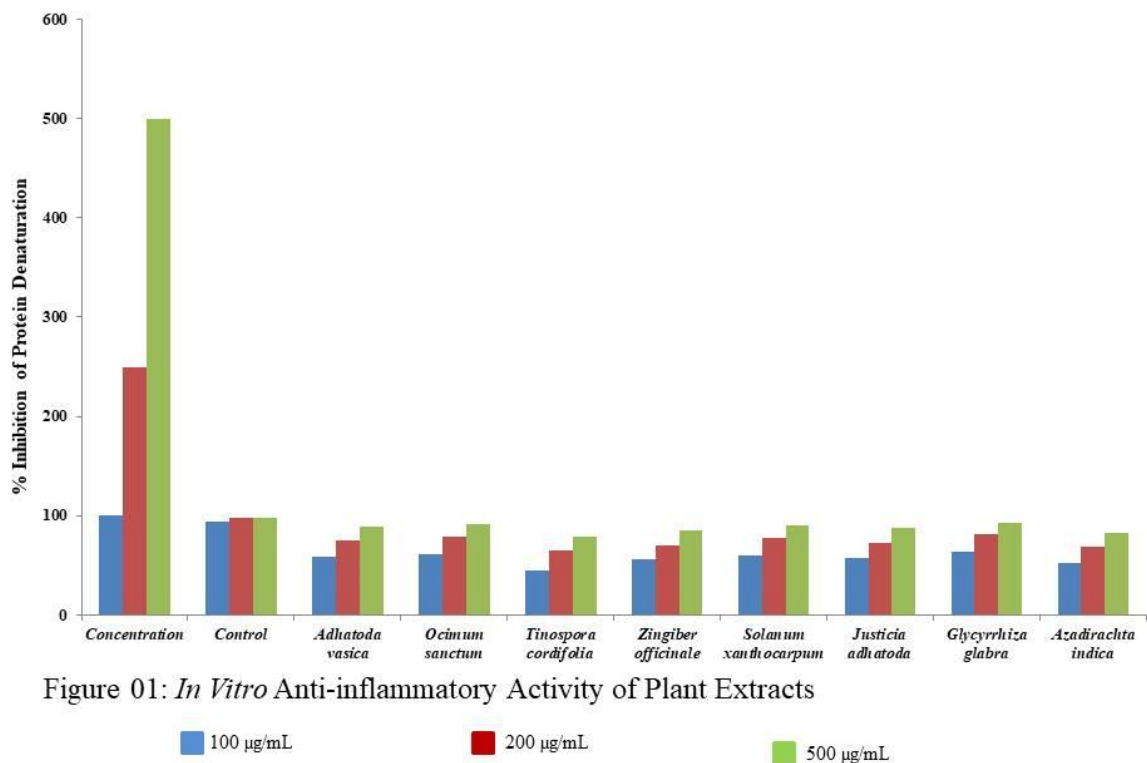


Figure 01: *In Vitro* Anti-inflammatory Activity of Plant Extracts

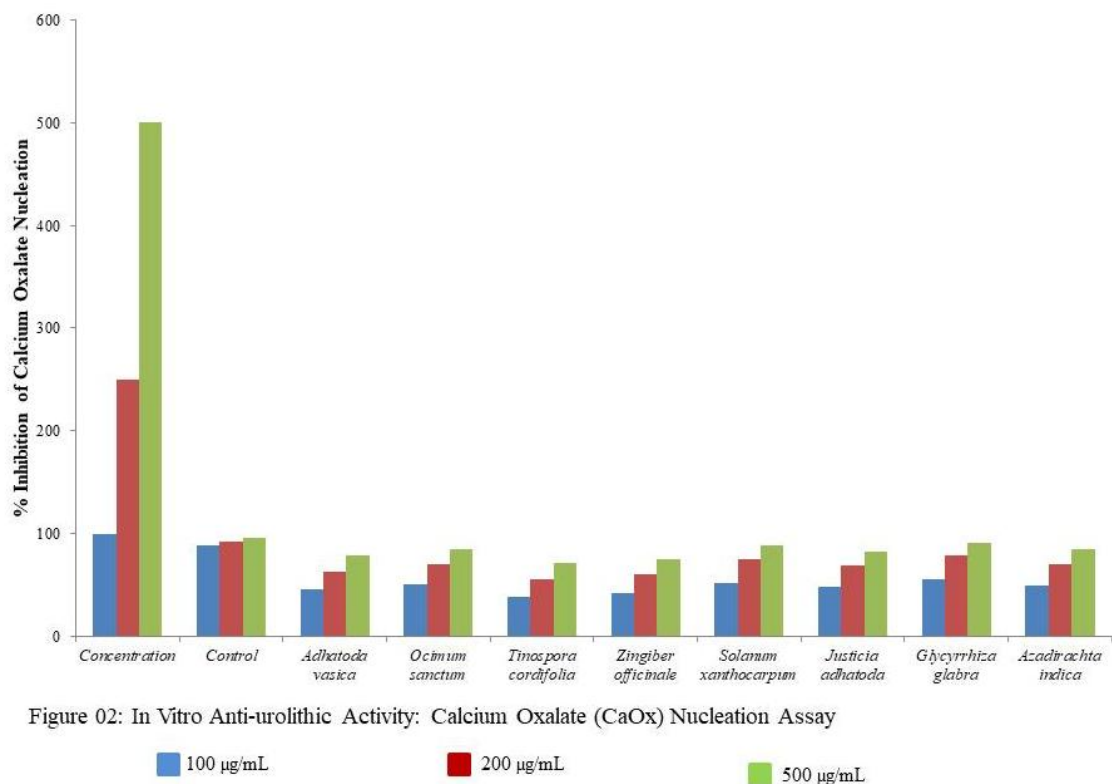


Figure 02: *In Vitro* Anti-urolithic Activity: Calcium Oxalate (CaOx) Nucleation Assay

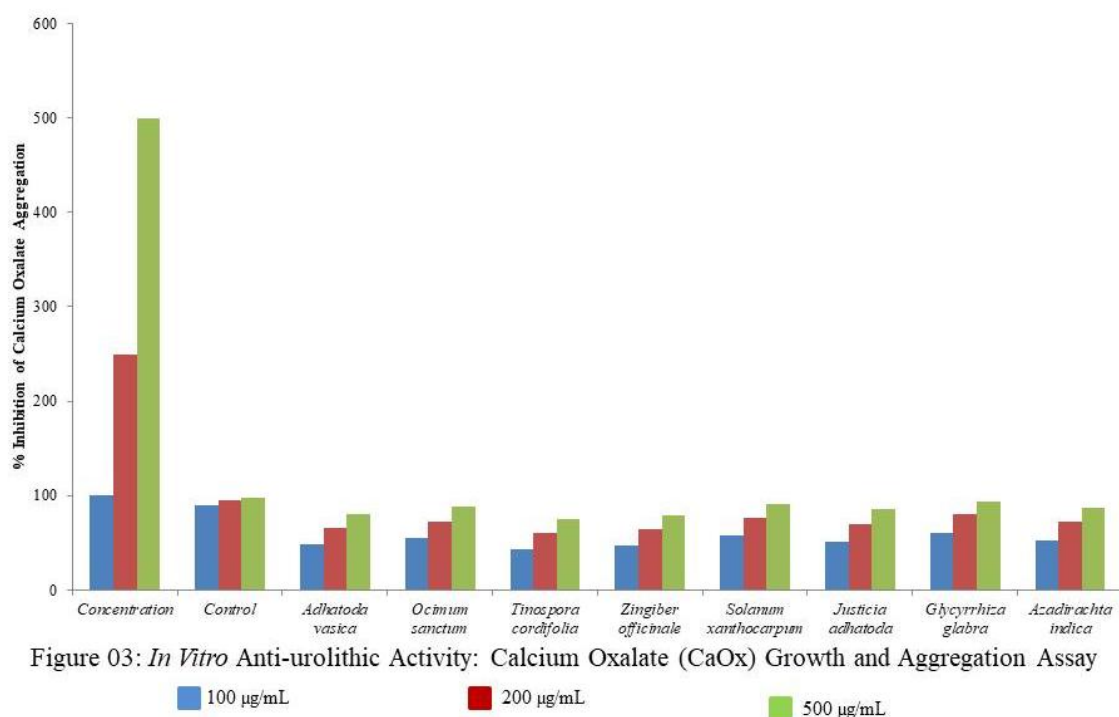


Figure 03: *In Vitro* Anti-urolithic Activity: Calcium Oxalate (CaOx) Growth and Aggregation Assay

■ 100 µg/mL

■ 200 µg/mL

■ 500 µg/mL

CONCLUSION

The ethnobotanical survey of 50 informants from tribal communities of the Chota Nagpur Plateau revealed that traditional knowledge of medicinal plants is predominantly held by elderly members, with 75% of participants over 60 years and a majority being experienced healers (Table 01). This demographic pattern underscores the urgency of documenting indigenous practices before they are lost.

A total of 45 plant species were recorded, with eight species identified as particularly important for respiratory ailments (Table 02, Figure 01). Leaves were the most frequently used plant part, reflecting sustainable harvesting practices, while roots, stems, rhizomes, and whole plants were applied for specific symptoms. Species such as *Adhatoda vasica*, *Justicia adhatoda*, *Ocimum sanctum*, *Tinospora cordifolia*, *Zingiber officinale*, *Glycyrrhiza glabra*, *Solanum xanthocarpum*, and *Azadirachta indica* were highlighted for their therapeutic relevance in cough, bronchitis, asthma, and related respiratory conditions.

In vitro anti-urolithic assays (CaOx nucleation and aggregation; Tables 04–05, Figures 02–03) demonstrated that *Glycyrrhiza glabra*, *Solanum xanthocarpum*, and *Ocimum sanctum* exhibited the highest inhibitory activity, with clear dose-dependent responses, supporting their pharmacological potential. Moderate inhibition observed in other species further validates their traditional use.

Overall, the study confirms that the tribal communities of Jharkhand possess rich, well-organized ethnomedicinal knowledge, particularly regarding respiratory health. The combination of ethnobotanical documentation and *in vitro* validation provides a scientific basis for future pharmacological exploration, conservation strategies, and the sustainable utilization of medicinal plants, ensuring preservation of cultural heritage and promotion of natural healthcare solutions.

Author's contribution

Deepa Sinha conducted the ethnobotanical survey, including informant selection, data collection, and documentation of medicinal plants, and participated in data analysis and manuscript drafting. Dr. Veermani Kumar supervised the research, provided guidance in study design, and reviewed the manuscript critically. Dr. Kamal Kant Patra coordinated the overall research, contributed to plant identification, laboratory experiments, and quantitative analysis, and finalized the

manuscript. Dr. Asha Mishra performed phytochemical screening and in vitro pharmacological assays, contributed to data interpretation, and assisted in preparing figures and tables. All authors read, reviewed, and approved the final version of the manuscript for publication.

Conflict of interest: No

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