



Phytochemical screening and qualitative analysis of selected ethnomedicinal plants from Yavatmal district, Maharashtra

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ABSTRACT

Ethnomedicinal plants serve as a vital source of bioactive secondary metabolites with proven therapeutic applications in traditional healthcare systems, including Ayurveda and folk medicine. This study evaluates the qualitative phytochemical composition and extraction yields of three prominent ethnomedicinal species *Azadirachta indica* (Neem), *Calotropis procera* (Rui/ Madar), and *Aegle marmelos* (Bel) collected from the Yavatmal district in the semi-arid Vidarbha region of Maharashtra, India. Shade-dried leaf samples were subjected to sequential Soxhlet extraction using solvents of increasing polarity petroleum ether, chloroform, methanol, and aqueous solvents. Standard qualitative biochemical colorimetric and precipitation assays revealed the presence of key phytoconstituents including alkaloids, flavonoids, tannins, saponins, terpenoids, and cardiac glycosides. Comparative analysis demonstrated that polar crude extracts yielded the highest extractive values, with methanol extracts exhibiting the broadest profile of secondary metabolites across all taxa. The high concentration of polyphenols and flavonoids in these regional specimens correlates with their documented traditional uses as antimicrobial, anti-inflammatory, immunomodulatory, and antipyretic agents. Overall, these findings substantiate the traditional therapeutic claims associated with these regional taxa and highlight their potential for bio-guided isolation, structural elucidation, and standardized drug formulation of active novel pharmaceuticals.

Keywords: Ethnobotany, Soxhlet Extraction, Phytochemical Screening, *Azadirachta indica*, *Calotropis procera*, *Aegle marmelos*, Yavatmal, Secondary Metabolites.

INTRODUCTION

Traditional medicine systems, including Ayurveda, Unani, and regional folk healing, rely heavily on plant-derived remedies. Ethnomedicinal plants synthesize primary metabolites necessary for growth alongside secondary metabolites such as alkaloids, flavonoids, phenolics, and terpenoids that mediate defense mechanisms and confer physiological activity in humans. Ethnobotany, defined as the interdisciplinary scientific evaluation of dynamic interactions between indigenous human populations and botanical diversity, serves as a fundamental discipline for deciphering socio-ecological reliance on plant resources (Martin,

1995; Chavan *et al.*, 2015). For centuries, phytotherapy and localized traditional ecological knowledge have formed the backbone of primary healthcare infrastructure among rural and tribal demographics worldwide (WHO, 2013). In socio-economically disadvantaged or geographically isolated regions where modern allopathic healthcare systems remain inaccessible, empirically derived herbal therapeutics represent a cost-effective, culturally congruent, and primary intervention strategy for disease management and health maintenance (Yirga, 2010; Mahalik *et al.*, 2015). Consequently, the systematic field documentation and scientific preservation of ethnobotanical methodologies are essential safeguards against the irreversible erosion of bio-cultural heritage and non-codified indigenous health traditions (Hamilton, 2004).

Within the Indian subcontinent, the state of Maharashtra represents a crucial phytogeographic locus characterized by extensive floristic diversity and endemic traditional medical systems (Patil, 2003). Despite this biological wealth, rapid socio-economic shifts threaten the oral transmission of indigenous healthcare knowledge. Addressing this critical gap, the present investigation was formulated to conduct a rigorous ethnobotanical assessment within the Yavatmal district of Maharashtra. The primary objective of this study is to systematically inventory, document, and analyse the therapeutic applications of localized flora utilized by resident tribal communities, thereby facilitating the conservation of ethnomedicinal resources for future pharmacological and conservation frameworks.

The semi-arid flora of the Yavatmal district supports a rich biodiversity of species long utilized by local communities to treat skin disorders, gastrointestinal distress, inflammation, and febrile conditions. Despite their documented ethnobotanical uses, systematic phytochemistry of specific micro-regional populations remains essential to standardize bio-activity profiles and account for chemo-geographic variations. This investigation presents a preliminary qualitative screening of the bioactive spectrum of three selected medicinal species collected from various regions of Yavatmal district of Maharashtra.

MATERIALS AND METHODS

Study Area:

Yavatmal district, spanning an area of 13,582 km², is characterized by a diverse geography and abundant natural resources. The region encompasses six major forest ranges: Yavatmal, Hiwri, Parwa, Ghatanji, Pandharkawda, and Wani. These forest landscapes are inhabited by indigenous tribal communities, notably the Gond and Kolam. Historically, these tribes have depended on local forest resources for their livelihoods and cultural practices, highlighting the ecological and socio-cultural importance of these six ranges. The dense forests, along with their rich flora and fauna, play a vital role in shaping the local environment and economy, demonstrating the deep interconnectedness between the indigenous communities and their surrounding environment (Figure 1).

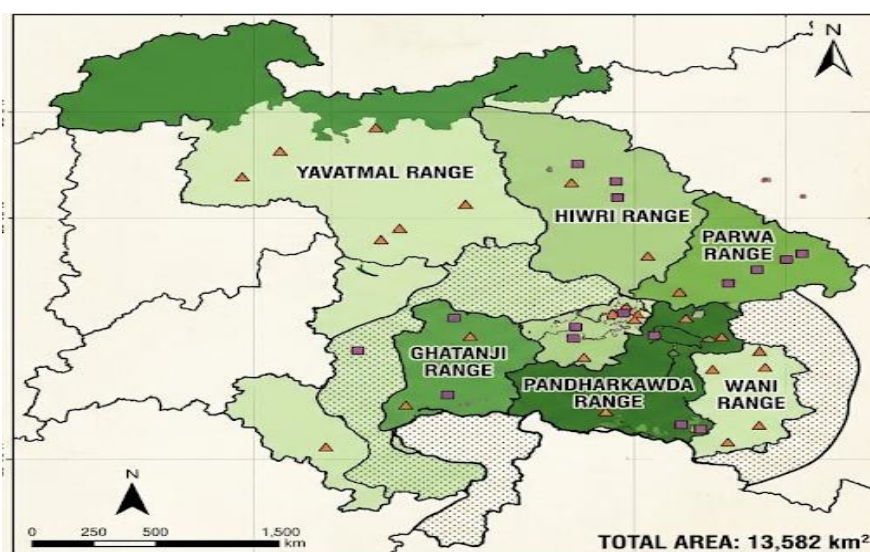


Figure 1: Study area

Data Collection:

From Oct 2025 to Jan 2026, an ethnobotanical survey was conducted across six major forest ranges Yavatmal, Hiwri, Parwa, Ghatanji, Pandharkawda, and Wani of Yavatmal district, Maharashtra, India. Traditional healers of varying age groups from the Gond and Kolam ethnic communities were selected as key informants. To document a comprehensive range of ailments and their corresponding remedies, semi-structured personal interviews were conducted with these traditional medicine practitioners. Additionally, guided field visits accompanied by local healers were undertaken to assist in plant identification and to observe their medicinal applications *in situ*. Ethnobotanical data were systematically recorded using a standardized questionnaire adapted from Jain (1987) and Masih (1990). The collected data included the local or common names of the plants, their growth habits, and the specific plant parts utilized in medicinal preparations. Furthermore, informants provided detailed accounts regarding the methods of remedy preparation, formulations, and modes of administration for treating specific health conditions.

Plant Material Collection and Preparation:

Fresh, disease-free leaves of the selected plant species were collected from the field region (forest ranges Yavatmal, Hiwri, Parwa, Ghatanji, Pandharkawda, and Wani of Yavatmal district, Maharashtra, India). The plant specimens were authenticated with the aid of regional flora references and deposited in an institutional herbarium. The leaves were thoroughly washed under running tap water, rinsed with distilled water to remove surface contaminants, and shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 10–14 days. The dried leaf samples were coarsely ground into a fine powder using a mechanical grinder and stored in airtight, dark glass containers prior to extraction.

Solvent Extraction:

Approximately 20 g of leaf powder was subjected to sequential extraction using solvents of increasing polarity (petroleum ether, chloroform, methanol, and aqueous) via a Soxhlet apparatus for 6 - 8 hours per solvent. Alternatively, cold maceration was performed by soaking the powder in methanol/ water (80:20) with intermittent stirring for 48 hours. The crude extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator. The residue was dried, weighed, and stored at 40°C .

Qualitative Phytochemical Screening:

Qualitative preliminary tests were performed on the crude leaf extracts using standard chemical methodologies to determine the presence or absence of major bioactive secondary metabolites:

Alkaloids Test: The extract was collected in a test tube and add 0.2 ml of diluted HCl. Then 1ml of Meyer's reagent was added. A yellowish coloration suggests the existence of alkaloids (Kancherla *et al.*, 2019).

Flavonoid Test: Two ml of 2% NaOH mixture was mixed with leaves extract, formation of concentrated yellow colour and this colour disappears when we add 2 drops of diluted acid to the mixture, this result indicates the flavonoid presence (Panchal and Parvez, 2019)

Steroids Test: Plant extract were taken in test tube, dissolved with chloroform (10 ml), and added equal amount of concentrated sulphuric acid to the test tube by the side. The upper layer of the solution was converted into red, and sulphuric acid layer showed yellow with green fluorescence. It shows the existence of steroids in the leaf extracts (Hossain *et al.*, 2013)

Terpenoid Test: Chloroform (2 ml) was mixed with 5ml of plant extract, and 3 ml of conc H_2SO_4 was carefully applied to create a layer. In order to show a positive result for terpenoids, a coloration of reddish brown was developed (Junaid and Patil, 2013)

Tannin Test: About 5 ml of the leaf extract was taken in a test tube, adding 2 ml of 5% of the solution FeCl_3 . A greenish-black precipitate suggests the presence of tannin (Nagaraju Kancherla *et al.*, 2019).

Glycosides Test: A small amount of extract was taken in a test tube, and add 1 ml of water and a few drops of aqueous NaOH; a yellowish coloration indicating the presence of glycosides (Ekeleme *et al.*, 2017)

Phenol Test: About 0.2 g of plant extract was weighted and treated with 5% ferric chloride and observed for the formation of deep blue color, which indicates the presence of phenol Hossain *et al.*, 2013).

RESULTS AND DISCUSSION

Ethnobotanical Field Survey and Documentation:

The field investigation conducted between October 2025 and January 2026 across six forest ranges of Yavatmal district (Yavatmal, Hiwri, Parwa, Ghatanji, Pandharkawda, and Wani) recorded indigenous medicinal knowledge preserved by Gond and Kolam traditional healers. The key species identified in the study area predominantly including *Calotropis procera*,

Azadirachta indica and *Aegle marmelos* form a cornerstone of the primary healthcare practices among these tribal populations. The ethnobotanical documentation showed that leaf formulations are predominantly prepared via fresh decoctions, pastes, or aqueous infusions. These modes of administration align with traditional ethnobotanical methodologies documented across the Deccan Plateau (Jain, 1987).

The extraction yields varied significantly according to solvent polarity, with methanol yielding the highest mass of crude extract across all three species (12.4 % to 14.20%). The highest extractive yield was observed in the aqueous extract of *A. indica* (14.20%), followed by the methanolic extract of *A. marmelos* (12.80%). The lowest yields were recorded in non-polar petroleum ether fractions (1.80% - 3.10%).

Table 1: Ethnobotanical profile and utilization pattern of selected flora in Yavatmal district

Plant Species / Family	Local Name	Growth Habit	Plant Part Used	Preparation Method & Mode of Administration	Reported Therapeutic Indications
<i>Azadirachta indica</i> A. Juss. (<i>Meliaceae</i>)	Kadunimb	Tree	Leaves	Fresh aqueous leaf paste applied topically; decoction taken orally	Skin disorders, wounds, eczema, malaria/febrile conditions
<i>Calotropis procera</i> (Ait.) R. Br. (<i>Apocynaceae</i>)	Rui / Mandar	Shrub	Leaves	Warm leaf paste with sesame oil applied topically; poultice	Joint inflammation, rheumatism, skin eruptions, boils
<i>Aegle marmelos</i> (L.)Corrêa (<i>Rutaceae</i>)	Bel	Tree	Leaves	Leaf juice mixed with honey/water; fresh leaf decoction	Gastrointestinal distress, diarrhea, dysentery, diabetes management

Table 2: Percentage extractive yields (% w/w) of leaf powders across solvents of varying polarity

Plant Species	Petroleum Ether (%)	Chloroform (%)	Methanol (%)	Aqueous (%)
<i>Azadirachta indica</i>	2.10	3.85	11.50	14.20
<i>Calotropis procera</i>	3.10	4.20	9.80	11.40
<i>Aegle marmelos</i>	1.80	3.15	12.80	10.60

Table 3: Qualitative phytochemical profile of leaf extracts across four solvent systems

Chemical Test	Target Class	<i>A. indica</i>				<i>C. procera</i>				<i>A. marmelos</i>			
		PE	C	M	Aq	PE	C	M	Aq	PE	C	M	Aq
Meyer's Test	Alkaloids	-	+	++	+	-	++	+++	+	-	+	++	+
Alkali (NaOH) Test	Flavonoid	-	-	+++	++	-	+	++	++	-	-	+++	+++
Salkowski / H ₂ SO ₄ Test	Steroids	++	+++	+	-	+++	+++	+	-	++	++	-	-
Chloroform / H ₂ SO ₄ Test	Terpenoids	+++	++	+	-	++	+++	++	-	+++	++	+	-
FeCl ₃ (5%) Test	Tannins	-	-	+++	+++	-	-	++	++	-	-	+++	+++
NaOH Aqueous Test	Glycosides	-	-	++	++	-	+	+++	++	-	-	++	+++
FeCl ₃ Deep Blue Test	Phenols	-	-	+++	+++	-	-	++	+++	-	-	+++	+++

(+) Presence, (++) Moderate concentration, (+++) High concentration, (-) Absence.

Solvent Abbreviations: PE = Petroleum Ether, C = Chloroform, M = Methanol, Aq = Aqueous

Solvation Dynamics and Phytoconstituent Extraction:

The differential solubility of bioactive molecules across the polar gradient validates the use of sequential solvent extraction. Non-polar solvents (petroleum ether) preferentially extracted lipophilic constituents such as phytosterols and terpenoids. Intermediate polarity solvents (chloroform) solubilized free alkaloids, aglycones, and low-polarity flavonoids. Highly polar solvents (methanol and water) successfully recovered phenolic compounds, condensed tannins, and glycosidic conjugates.

Physiological Implications of Detected Metabolites:

Alkaloids & Glycosides: The strong positive reaction for alkaloids in methanolic extracts (yellowish coloration with Mayer's reagent) supports the widespread ethnomedicinal use of local leaf decoctions as antipyretic and analgesic agents by Gond and Kolam practitioners (Kancherla et al., 2019). Plant alkaloids act directly on the central nervous system and vascular pathways to reduce febrile responses.

Phenolics & Flavonoids: Intense yellow coloration (disappearing upon acidification) and deep blue ferric chloride reactions confirm rich concentrations of flavonoids and polyphenols in the polar fractions. These compounds act as free-radical scavengers and metal chelators, providing a biochemical basis for the traditional topical application of leaf pastes to manage inflammatory skin disorders, speed wound healing, and alleviate gastrointestinal irritation (Panchal and Parvez, 2019).

Tannins: Greenish-black precipitates observed in methanol and aqueous fractions indicate significant concentrations of condensed tannins. Tannins precipitate mucosal proteins, forming a protective pellicle that exerts astringent, antimicrobial, and anti-diarrheal effects (Kancherla et al., 2019).

Steroids & Terpenoids: The distinct reddish-brown ring at the interface in Salkowski's test and red chloroform layering in petroleum ether extracts confirm the presence of lipophilic terpenoids and phytosterols (Hossain et al., 2013; Junaid and Patil, 2013). These non-polar constituents mediate plant defence and exhibit significant membrane-stabilizing and anti-inflammatory activities in human physiological systems.

CONCLUSION

The qualitative phytochemical analysis verifies that *Azadirachta indica*, *Calotropis procera*, and *Aegle marmelos* from the different regions of Yavatmal district are rich sources of diverse bioactive secondary metabolites. These results validate the traditional therapeutic usage of these plants by regional practitioners. Further quantitative estimation, high-performance liquid chromatography (HPLC) profiling, and quantitative pharmacological assays are recommended to isolate individual pure compounds for drug formulation.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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