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A Study on Qualitative Phytochemical Analysis of Selected Medicinal Plants from Jharkhand

Deepa Sinha[1], Veermani Kumar[2], Kamal Kant Patra[3], Asha Mishra[4], Keshamma E[5*]

[1]Ph.D Scholar, Department of Botany, YBN University, Ranchi, Jharkhand, India

[2]Ph.D. Supervisor, Assistant Professor, Department of Botany, YBN University,
Ranchi, Jharkhand, India

[3]Associate Professor, Department of Botany, YBN University, Ranchi, Jharkhand, India

[4]Assistant Professor, Department of Biotechnology, YBN University,
Ranchi, Jharkhand, India

[5]Ph.D Co-Supervisor, Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

Corresponding Author:

Dr. Keshamma E

Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru-560 001, Karnataka, India.

Email ID : keshamma76@gmail.com

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ABSTRACT

Medicinal plants remain a primary source of healthcare in rural and tribal communities where access to modern medical facilities is limited. Tribal communities rely heavily on forests for their livelihoods and cultural practices. The Jharkhand, located in eastern India is one such state. Hence in the current we aimed for qualitative analysis of aqueous (aq.) and ethanolic extracts of four selected medicinal plants from Jharkhand state viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba, and Vigna umbellata. Results showed that Vicia faba exhibited the maximum extractive potential in both aq. and ethanolic solvents, highlighting its rich phytochemical content. The phytochemical profile of aq. plant extracts indicates that all four species of plants are rich in alkaloids, phenolics, flavonoids, saponins, and proteins, while terpenoids were unique to Vicia faba. The ethanolic plant extracts had shown a rich presence of alkaloids, phenolic compounds, flavonoids, and saponins across all species, while terpenoids had been exclusive to Vicia faba. The occurrence of proteins had been limited to Vicia faba and Vigna umbellata. In conclusion, the uniform presence of multiple bioactive compounds in selected medicinal plants from Jharkhand state viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba, and Vigna umbellata underlines their potential for pharmacological and nutraceutical applications..

Keywords: *Jharkhand, Medicinal plants, Phytochemicals, Alkaloids, Flavonoids, Saponins*

1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years.[1] Chemicals derived from plants have recently attracted a lot of attention due to their wide variety of medicinal applications. Medicinal plants are collection of species that accrue variety of active ingredients that are effective in treating a range of human and animal ailments.[2] Most drugs in the past i.e., allopathic, ayurvedic and homeopathic medicines were made from plants. Medicinal plants have secondary metabolites, and these secondary metabolites are the main source of medicinal products with therapeutic effects.[3] Furthermore, dietary phytochemicals are considered as an effective tool to cure various human physiological disorders.[4]

The modern pharmacopoeia still includes at least 25% of medications originated from plants, as well as numerous synthetic equivalents based on prototype chemicals extracted from plants. The growing expenses of prescription pharmaceuticals in the maintenance of human health and wellbeing, as well as the bioprospecting of novel plant-derived drugs, have fueled interest in medicinal plants as a re-emerging health assistance.[5] The increased acknowledgment of therapeutic plants is attributable to a variety of factors, including growing confidence in herbal treatment.[6] In addition, a growing dependence on the usage of plants for medicinal purposes in industrialized countries may be traced back to the extraction and production of

medications and chemotherapy drugs from these plants, as well as traditional herbal treatments.[7]

Plants' therapeutic capabilities might be based on the antioxidant, antimicrobial, and antipyretic actions of their phytochemicals.[8,9] As stated by the World Health Organization, plants with medicinal properties are the best source of a wide range of medications. As a result, such plants should be studied in order to properly determine their qualities, safety, and efficacy.[10] India is among of the nations that heavily relies on herbal medicine to suit its healthcare demands.[11]

Medicinal plants remain a primary source of healthcare in rural and tribal communities where access to modern medical facilities is limited.[12] Jharkhand, located in eastern India, is one such state, with approximately 26%–28% of its population belonging to Scheduled Tribes.[13] These tribal communities rely heavily on forests for their livelihoods and cultural practices. Forests provide essential resources such as food, fodder, fuel, and building materials, along with medicinal plants that play a central role in traditional healthcare systems.[14] Collaborative work on ethnobotanicals, ethnomedical, ethnopharmacological, and phytochemicals is crucial to attaining research progress in the field of medicinal plants.[15] With these viewpoints, present study was conducted with the main objectives screen for the presence of phytochemical in selected medicinal plants from Jharkhand.

2. MATERIALS AND METHODS

Collection and Identification of Plant

Samples of selected lesser-known medicinal plants from Jharkhand state *viz. Dolichos trilobus L., Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* were collected (Figure 1). Care was taken to collect a representative part of the plant (leaves) to facilitate accurate identification. The specimens were then pressed and preserved for later identification. The collected plant samples were identified using standard taxonomic procedures with the help of floras, monographs, and expert consultation at a recognized herbarium.

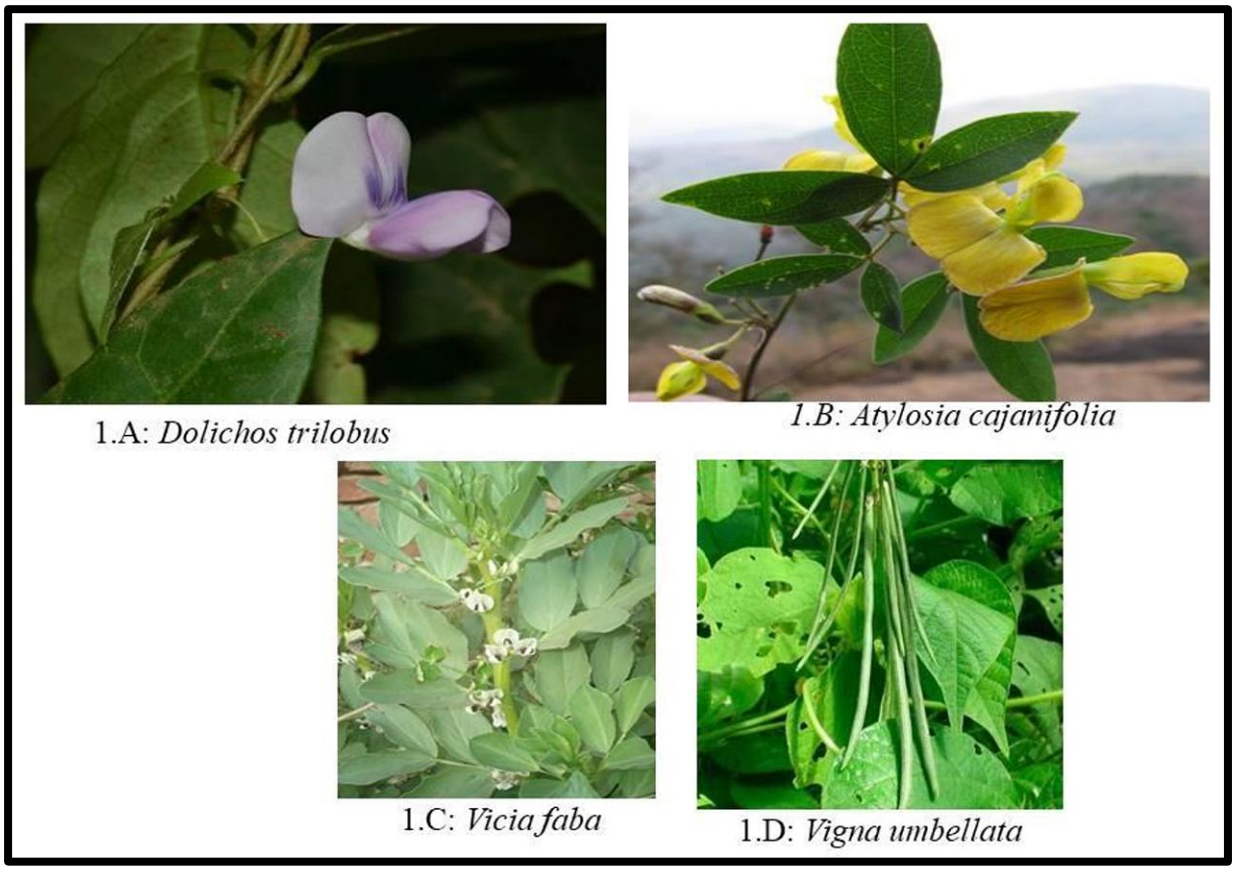

Figure 1. Selected lesser-known medicinal plants from Jharkhand state: *Dolichos trilobus* L. (1A), *Atylosia cajanifolia* (1B), *Vicia faba* (1C), and *Vigna umbellata* (1D)

Sample Preparation

Leaves were collected by hand using clean scissors and gloves to minimize contamination. After collection, leaves were placed in sterile, labeled polythene bags lined with moist tissue paper to maintain freshness during transport to the laboratory. Leaves were first rinsed under running tap water in the laboratory to remove dust, soil particles, and insect residues. This

was followed by a rinse with distilled water to eliminate any remaining contaminants. In some cases, a mild sterilization step using 0.1% sodium hypochlorite solution was employed. Cleaned leaves were spread on blotting papers and air-dried under shade to remove surface moisture before further processing. After complete drying, leaves were ground into fine powder using a sterile mechanical grinder. The powdered samples were sieved through a mesh (60–80 µm) to obtain uniform particle size. The powdered leaves were stored in airtight amber glass bottles. Bottles were kept in desiccators with silica gel at room temperature until further experimental analysis.

Extraction

The leaves of *Dolichos trilobus L., Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* were subjected to both aqueous (*aq.*) and ethanolic extraction techniques to obtain crude extracts. The *aq.* extracts of the selected plant leaves had been prepared using the maceration method. A standard ratio of 1:10 (w/v) i.e., 100g of powdered leaf material macerated in 1000 ml of distilled water had been maintained for uniformity across species. The powdered material was placed in sterile glass conical flasks, and freshly boiled and cooled distilled water was added. The flasks were sealed with parafilm to prevent contamination and kept at room temperature (25 ± 2°C) for a period of 72 hours. During this period, the macerated mixture was stirred intermittently with a sterile glass rod to facilitate maximum solubilization of bioactive constituents. After completion of the maceration period, the mixture was filtered through a double layer of muslin cloth followed by filtration through Whatman No. 1 filter paper to remove coarse plant debris. The filtrates obtained were further clarified by centrifugation at 5000rpm for 10 minutes, ensuring removal of suspended particles. The *aq.* extracts were collected in pre-labeled sterile amber-colored bottles and stored in refrigerator at -20°C.

For ethanol-soluble phytochemicals, extraction had been performed using the Soxhlet extraction apparatus, a widely accepted method for exhaustive extraction of plant metabolites. Approximately 50g of powdered leaves of each species was accurately weighed and placed in a cellulose thimble inside the Soxhlet extractor. Analytical grade ethanol (95%) was used as solvent, and a solvent-to-sample ratio of 200ml per 50g was maintained. The system was heated on a heating mantle, allowing ethanol vapors to rise, condense, and repeatedly wash over the powdered leaf material. Each extraction cycle took approximately 30 minutes, and the process was continued for 8–10 hours, until the siphon tube of the Soxhlet ran clear, indicating exhaustive extraction. The ethanolic extracts were collected and concentrated immediately after extraction, and stored in pre-labeled sterile amber-colored bottles in refrigerator at -20°C.

The percentage yield (extractive value) was calculated by dividing the weight of the dried extract by the initial weight of the leaf powder and multiplying by 100.

Phytochemical Screening

The leaves of *Dolichos trilobus* L., *Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* had been subjected to a series of qualitative chemical tests in order to identify the classes of bioactive compounds present. These assays were performed using standard protocols recommended by Sofora,[16] Trease and Evans[17] and Harborne.[18]

Alkaloids

1g of powdered leaf material was extracted with 10ml of 1% hydrochloric acid by heating on a water bath for 10 minutes. The extract was cooled and filtered.

Mayer's Test: 2 ml of filtrate was treated with a few drops of Mayer's reagent. Formation of a creamy white precipitate indicated the presence of alkaloids.

Wagner's Test: Another 2 ml of filtrate was treated with Wagner's reagent. Formation of a reddish-brown precipitate confirmed alkaloids.

Phenolic compounds

2 ml of *aq.* or ethanolic plant extracts were diluted with 5ml of distilled water. A few drops of 5% Ferric chloride solution were added. Development of a deep blue, green, or black coloration indicated the presence of phenolic compounds.

Flavonoids

Alkaline reagent test: 2 ml of plant extracts were treated with 2ml of 2% NaOH solution. An intense yellow color was observed, which disappeared on addition of dilute HCl, confirming flavonoids.

Shinoda test: To 2ml of plant extracts, a few magnesium turnings were added. A few drops of concentrated HCl were carefully added. Development of a red or pink coloration indicated the presence of flavonoids.

Terpenoids

5ml of plant extracts were mixed with 2ml of chloroform. To this solution, 3ml of concentrated H_2SO_4 was added carefully along the side of the test tube to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Saponins

5ml of plant extracts were vigorously shaken in a graduated cylinder for 15 minutes. The formation of a stable, persistent froth of 1–2cm height indicated the presence of saponins. The froth was tested for stability by adding a few drops of olive oil and reshaking, which resulted in the formation of an emulsion.

Proteins

Biuret test: 2ml of plant extracts were treated with 1ml of 10% NaOH solution. A few drops of 0.5% $CuSO_4$ solution were added. Formation of a violet or purple coloration indicated proteins.

Lowry's method: 1ml of plant extract were mixed with 5ml of alkaline copper reagent (2% Na_2CO_3 in 0.1N NaOH + 0.5% $CuSO_4$ + 1% potassium sodium tartrate). After 10 minutes, 0.5ml of Folin–Ciocalteu reagent (1N, diluted 1:1) was added. The mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 750nm using a spectrophotometer. Bovine Serum Albumin (BSA) was used as a standard for calibration.

3. RESULT

The results of extractive values of selected plant extracts from Jharkhand were represented in Table 1. Results depicted that among all the studied *aq.* plant extracts, *Vicia faba* produced the highest aqueous extractive value (22.1%) followed by *Atylosia cajanifolia* (18.9%), *Vigna umbellata* (17.8%), and *Dolichos trilobus* (15.5%).

With respect to ethanolic extraction, the maximum extractive yield was again obtained from *Vicia faba* (16.5%), followed by *Atylosia cajanifolia* (14.6%) and *Vigna umbellata* (13.0%). The lowest ethanolic extractive value was recorded for *Dolichos trilobus* (11.2%).

Table 1. The extractive value of selected plant extracts from Jharkhand

Plants	***Aq.* Extracts (%)**	**Ethanolic Extracts (%)**
Dolichos trilobus	15.50	11.20
Atylosia cajanifolia	18.90	14.60
Vicia faba	22.10	16.50
Vigna umbellata	17.80	13.00

Values were expressed as percentages of the dry weight of the plant material

The results of phytochemical screening of *aq.* plant extracts from Jharkhand *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* revealed the presence of major bioactive groups, with some variation across species. Alkaloids were consistently present in all four plant species as confirmed by both Mayer's and Wagner's tests. Phenolic compounds were detected in all plant species using the Ferric Chloride test. Flavonoids, as indicated by both the alkaline reagent and Shinoda tests, were universally present in all four plant species. Terpenoids were found exclusively in *Vicia faba* as confirmed through the Salkowski reaction test. Saponins were detected in all four *aq.* plant extracts as confirmed by the Froth test. Proteins, as tested by both the Biuret and Lowry's methods, were present in all four *aq.* plant extracts (Table 2).

Table 2. Phytochemical screening of *aq.* plant extracts from Jharkhand

Phytochemicals	***Dolichos trilobus***	***Atylosia cajanifolia***	***Vicia faba***	***Vigna umbellata***
Alkaloids				
Mayer's test	+	+	+	+
Wagner's test	+	+	+	+
Phenolic compounds				
Ferric chloride test	+	+	+	+

Flavonoids				
Alkaline reagent test	+	+	+	+
Shinoda test	+	+	+	+
Terpenoids				
Salkowski reaction test	-	-	+	-
Saponins				
Froth test	+	+	+	+
Proteins				
Biuret test	+	+	+	+
Lowry's test	+	+	+	+

(+ Sign: Present); (- Sign: Absent)

The results of phytochemical screening of ethanolic plant extracts from Jharkhand *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* inferred that alkaloids had been detected in all four plant species through Mayer's and Wagner's tests, indicating their consistent occurrence across the studied legumes. Phenolic compounds had been present in all plant extracts, as confirmed by the Ferric chloride test, suggesting that the ethanolic solvent efficiently extracted antioxidant compounds. Flavonoids had been universally observed in all four plant species using both the alkaline reagent and Shinoda tests, which reflected their widespread distribution and possible pharmacological relevance. Terpenoids had been found only in *Vicia faba*, as indicated by the Salkowski reaction, while the other three species lacked detectable levels. This uniqueness pointed toward a species-specific phytochemical profile. Saponins had been consistently detected in all four plant species as confirmed through the Froth test. Proteins had been observed only in *Vicia faba* and *Vigna umbellata*, whereas *Dolichos trilobus* L. and *Atylosia cajanifolia* tested negative for proteins. This selective presence highlighted interspecies differences in protein solubility or extractability under ethanolic conditions (Table 3).

Table 3. Phytochemical screening of ethanolic plant extracts from Jharkhand

Phytochemicals	***Dolichos trilobus***	***Atylosia cajanifolia***	***Vicia faba***	***Vigna umbellata***
Alkaloids				
Mayer's test	+	+	+	+
Wagner's test	+	+	+	+
Phenolic compounds				
Ferric chloride test	+	+	+	+
Flavonoids				
Alkaline reagent test	+	+	+	+
Shinoda test	+	+	+	+

Terpenoids				
Salkowski reaction test	-	-	+	-
Saponins				
Froth test	+	+	+	+
Proteins				
Biuret test	-	-	+	-
Lowry's test	-	-	+	-

(+ Sign: Present); (- Sign: Absent)

4. DISCUSSION

Indian fora accounts for about 45,000 plant species out of which several thousands have pharmacological significance.[19] The repository of bioactive phytochemicals present in plants is unique, complex, taxonomically distinct and results in the beneficial medicinal effects of plants.[20] The focus point of some of the studies was on the single solvents on multiple plants.[21-23] Moreover, medicinal plants remain a primary source of healthcare in rural and tribal communities where access to modern medical facilities is limited.[12] Jharkhand, located in eastern India, is one such state, with approximately 26%–28% of its population belonging to Scheduled Tribes.[13] These tribal communities rely heavily on forests for their livelihoods and cultural practices.[14] Hence the present study was carried out with the main purpose of qualitative analysis of *aq*. and ethanolic extracts of four selected medicinal plants from Jharkhand state *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata.*

The trend of extractive values of *aq.* plant extracts of *Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* indicated that the majority of the phytoconstituents in *Vicia faba* were predominantly water soluble, possibly due to the presence of polar compounds such as carbohydrates, glycosides, tannins, and phenolic compounds. Comparatively lower *aq.* extractive values of *Dolichos trilobus* suggested a reduced abundance of highly polar constituents in its leaf matrix. The observations with respect to ethanolic extraction confirmed that ethanol, being a semi-polar solvent, had selectively extracted constituents such as flavonoids, alkaloids, terpenoids, and some phenolics that were moderately soluble in alcohol.

Our phytochemical analysis on *aq.* plant extracts, revealed the presence of a wide array of phytochemicals in the aqueous extracts of the selected plants, confirming their potential for therapeutic applications. Alkaloids and saponins were detected in all four plant species. The presence of alkaloids, known for their potent pharmacological effects, and saponins, which are recognized for their detergent properties and potential in reducing cholesterol, validated their use in traditional medicine. The tests for phenolic compounds and flavonoids also yielded positive results across all four plants. This was a significant finding, as both are powerful antioxidants.[2,24] Phenolic compounds are known for their ability to neutralize free radicals, while flavonoids, a type of polyphenol, have been widely studied for their anti-inflammatory and antiviral properties. Their widespread presence explained the traditional use of these plants for general health and wellness. Furthermore, Phytochemicals provide plants with their colour, flavor, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites.[25]

However, a key differentiation was observed with terpenoids in *aq.* plant extracts. The Salkowski reaction for terpenoids was positive only in the *aq.* extract of *Vicia faba*. This suggested that *Vicia faba* possessed a unique phytochemical profile compared to the other three plants, which could account for specific therapeutic properties attributed to it in traditional folklore. The complete absence of terpenoids in the other three species was an important finding for future research. Finally, the Biuret test and Lowry's method for proteins were positive in all the four *aq.* plant extracts. This indicated the presence of proteins and amino acids, which are the fundamental building blocks of life and contribute to the nutritional value of the plants. The combined presence of these various compounds underscores the complex biochemical nature of these lesser-known plants and their potential as a source for novel drugs.

Our analysis of the ethanolic extracts of the selected plants' leaves revealed several key findings regarding their phytochemical composition: (i) Similar to the aqueous extracts, alkaloids, phenolic compounds, and flavonoids were consistently detected in the ethanolic extracts of all four plant species. This indicated that these compounds were soluble in both polar (water) and semi-polar (ethanol) solvents. The presence of these compounds is significant, as they are known to contribute to a plant's therapeutic properties, including antioxidant and anti-inflammatory effects, and (ii) A notable

difference between the *aq.* and ethanolic plant extracts was the distribution of specific compounds. Our study found terpenoids exclusively in the ethanolic extract of *Vicia faba*. This suggested that the terpenoids in *Vicia faba* were not water-soluble but readily dissolved in ethanol, highlighting the importance of the extraction method for obtaining specific phytochemicals. Furthermore, proteins were also detected exclusively in the ethanolic extract of *Vicia faba*. This was a surprising and important finding, as proteins are generally more soluble in water. The presence of protein in this extract might indicate the presence of certain lipid-soluble proteins or a complex with other compounds that enhanced their solubility in ethanol.

5. CONCLUSION

In conclusion, the phytochemical profile indicates that all four plant species *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* are rich in alkaloids, phenolics, flavonoids, and saponins. While terpenoids were unique to *Vicia faba*. The uniform presence of multiple bioactive compounds underlines their potential for pharmacological and nutraceutical applications.

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