



Qualitative Analysis of Alkaloids in *Rauvolfia micrantha* Hook. F. Leaf and Root Extracts Using Different Solvents

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Abstract

Alkaloids are one of the most important bioactive compounds present in medicinal plants, especially in the genus *Rauvolfia*, which is well known for its pharmacological properties. The present study was conducted to perform qualitative analysis of alkaloids in *Rauvolfia micrantha* Hook. F. leaf and root extracts using different solvents such as methanol, chloroform, ethyl acetate, hexane, and petroleum ether. Alkaloid detection was carried out using standard qualitative tests such as Mayer's test. The results revealed that methanol extract showed a strong presence of alkaloids in both leaf and root extracts, while chloroform and ethyl acetate extracts showed a moderate presence of alkaloids. Hexane and petroleum ether extracts showed negative results for alkaloids due to their nonpolar nature. The study confirms that solvent polarity plays a crucial role in alkaloid extraction, and methanol was found to be the most effective solvent for alkaloid extraction from *Rauvolfia micrantha*. The presence of alkaloids indicates that the plant has significant medicinal value and can be used for further pharmacological and pharmaceutical research.

Keywords: *Rauvolfia micrantha* HOOK. F., Alkaloids, Mayer's test, Solvent extraction, Apocynaceae, Phytochemical screening

1. Introduction

Alkaloids are nitrogen-containing secondary metabolites that are widely distributed in medicinal plants and are known for their significant physiological and pharmacological activities. Many alkaloids are used as important therapeutic agents in modern medicine for the treatment of hypertension, mental disorders,

cancer, pain, and various infectious diseases. Plants belonging to the family Apocynaceae, particularly the genus *Rauvolfia*, are well known for their rich source of indole alkaloids with high medicinal value.

Rauvolfia micrantha Hook.F. is an important medicinal plant recognized for its therapeutic properties, mainly due to the presence of bioactive alkaloids.

Qualitative phytochemical screening for alkaloids is an essential step in determining the medicinal importance and drug potential of plant species. Therefore, the present study was undertaken to analyze the presence of alkaloids in leaf and root extracts of *Rauvolfia micrantha* using different solvents and to identify the most suitable solvent for alkaloid extraction.

Medicinal plants have been considered a valuable natural resource for human health since ancient times and continue to play a vital role in disease prevention and treatment (Raval et al., 2012). It is estimated that about 80% of the global population, especially in developing countries, relies on plant-based medicines as their primary healthcare system (Preiyasamy et al., 2010). Different plant parts such as roots, leaves, bark, flowers, and seeds are used in the preparation of herbal medicines due to their diverse therapeutic properties.

Herbal medicines are increasingly used in both developed and developing countries because of their wide range of biological activities, cost-effectiveness, better safety margins, and fewer side effects (Goswami et al., 2013). The therapeutic potential of medicinal plants is mainly due to the presence of bioactive phytochemical constituents, particularly secondary metabolites such as alkaloids, which produce various physiological effects on the human body (Sheikh et al., 2013). Hence, phytochemical screening, especially alkaloid analysis, is an important step in identifying biologically active compounds present in medicinal plants.

Extraction is a crucial step in phytochemical studies, as it is the primary process for isolating bioactive compounds from plant materials. The efficiency of extraction largely depends on the type of solvent used, which influences the yield and quality of extracted compounds. An effective extraction method should provide a high yield of target compounds with maximum purity and concentration (Spigno et al., 2007).

The genus *Rauvolfia* belongs to the family Apocynaceae and consists of approximately 85

species distributed mainly in tropical regions, with about five species reported in India. Among these, *Rauvolfia micrantha* and *Rauvolfia hookeri* are endemic to the Western Ghats of South India (Hu et al., 2008; Ahmedulla and Nayar, 1986). Species of this genus are well known for the presence of indole alkaloids, which possess significant pharmacological properties.

Several important alkaloids such as reserpine, reserpiline, resinnamine, ajmaline, ajmalicine, rauwolfinine, serpentine, serpetinine, and yohimbine have been reported from different *Rauvolfia* species (Gao et al., 2012; Sahu, 1983). Among these compounds, reserpine is one of the most important alkaloids and is widely used in the treatment of hypertension, cardiovascular disorders, and nervous disorders. It also acts as a tranquilizer and has significant demand in the pharmaceutical industry (Weiss and Mann, 2000). Reserpine is considered one of the earliest plant-derived compounds to be introduced into modern medicine.

Due to overexploitation and extensive use, some *Rauvolfia* species, particularly *Rauvolfia serpentina*, have become endangered. Similarly, *Rauvolfia hookeri* is also considered an endangered species. This situation emphasizes the importance of studying other species such as *Rauvolfia micrantha*, which may serve as an alternative source of valuable alkaloids.

Therefore, the present study was carried out to evaluate the effect of different solvents on the qualitative detection of alkaloids in *Rauvolfia micrantha*. Identifying the most effective solvent for alkaloid extraction is important for improving extraction efficiency, increasing yield, and promoting sustainable utilization of medicinal plants for pharmaceutical applications.

Objectives of the Study

The present study was undertaken with the following objectives:

1. To qualitatively analyze the presence of alkaloids in leaf and root extracts of *Rauvolfia micrantha* Hook.F.

2. To evaluate the effect of different solvents on the extraction of alkaloids.
3. To identify the most suitable solvent for efficient alkaloid extraction.
4. To assess the potential of *Rauvolfia micrantha* as a source of pharmacologically important alkaloids.

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

Healthy plant materials of *Rauvolfia micrantha* such as roots, stems, and leaves were collected from natural habitats. The collected plant materials were thoroughly washed under running tap water to remove adhering soil particles, dust, and other contaminants. After washing, the plant materials were rinsed with distilled water to remove any remaining impurities and then allowed to drain.

The cleaned plant materials were cut into small pieces using a sterile knife to facilitate proper drying. The chopped plant parts were then shade dried at room temperature for several days until they became completely dry. Shade drying was preferred in order to prevent the degradation of heat-sensitive bioactive compounds, especially alkaloids. The plant materials were regularly checked to ensure proper drying and to prevent fungal contamination.

After complete drying, the plant materials (roots, stems, and leaves) were powdered separately using a mechanical grinder to obtain a fine powder. The powdered plant material was sieved to obtain uniform particle size and then stored in clean, dry, airtight containers. The samples were labeled properly and stored at ambient temperature until further use for extraction and phytochemical analysis.

2.2 Preparation of Plant Extracts

The powdered plant material was subjected to solvent extraction for the isolation of alkaloids.

Different solvents such as hexane, chloroform, methanol, hydro-alcohol (methanol: water mixture), and distilled water were used for extraction in order to study the effect of solvent polarity on alkaloid extraction.

About 10 grams of dried plant powder (leaf and root separately) was taken in a Soxhlet extraction apparatus and extracted with 100 ml of respective solvent for 6–8 hours. The extraction process was continued until the solvent in the siphon tube became colorless, indicating complete extraction of phytochemicals.

After extraction, the extracts were filtered using Whatman No. 1 filter paper to remove plant residues. The filtrates were then concentrated using a rotary vacuum evaporator to remove excess solvent and obtain concentrated extracts. The concentrated extracts were transferred into clean, labeled glass vials and stored in a refrigerator at 4°C for further qualitative analysis of alkaloids.

2.3 Qualitative Analysis of Alkaloids

The presence of alkaloids in the plant extracts was determined by using standard qualitative phytochemical tests. These tests are based on the formation of precipitates or color changes when alkaloids react with specific chemical reagents.

2.3.1 Mayer's Test

A small quantity of plant extract was dissolved in 2 ml of dilute hydrochloric acid and filtered. To the filtrate, a few drops of Mayer's reagent (potassium mercuric iodide solution) were added along the sides of the test tube. The formation of a creamy white precipitate indicated the presence of alkaloids.

2.3.2 Dragendorff's Test

About 2 ml of plant extract was taken in a test tube and a few drops of Dragendorff's reagent were added. The formation of an orange or reddish-brown precipitate indicated the presence of alkaloids.

2.3.3 Wagner’s Test

To 2 ml of plant extract, a few drops of Wagner’s reagent (iodine in potassium iodide solution) were added. The formation of a brown or reddish-brown precipitate confirmed the presence of alkaloids.

2.3.4 Hager’s Test

To the plant extract, a few drops of Hager’s reagent (saturated aqueous solution of picric acid)

were added. The appearance of a yellow precipitate indicated the presence of alkaloids.

2.4 Interpretation of Results

The presence of alkaloids in different solvent extracts of *Rauvolfia micrantha* leaf and root was determined based on the intensity of precipitate formation during qualitative tests. The results were recorded and interpreted as follows:

Table 1. Qualitative Analysis of Alkaloids in *Rauvolfia micrantha* Leaf and Root Extracts Using Different Solvents

| S. No | Solvent Used | Leaf Extract (Alkaloids) | Root Extract (Alkaloids) |
|-------|-----------------|--------------------------|--------------------------|
| 1 | Hexane | – | – |
| 2 | Chloroform | ++ | ++ |
| 3 | Methanol | +++ | +++ |
| 4 | Hydro-alcohol | ++ | +++ |
| 5 | Distilled Water | + | ++ |
| 6 | Petroleum Ether | – | – |
| 7 | Ethyl Acetate | + | ++ |

3. Results

The qualitative analysis of alkaloids in *Rauvolfia micrantha* leaf and root extracts showed variation depending on the solvent used for extraction.

Methanol extracts of both leaves and roots showed a strong positive result for alkaloids in Mayer’s, Dragendorff’s, and Wagner’s tests, indicating that methanol is highly effective in extracting alkaloids due to its polar nature. Chloroform and ethyl acetate extracts showed moderate presence of alkaloids, indicating that these solvents can extract some alkaloids but not as effectively as methanol. Hexane and petroleum ether extracts showed negative results for alkaloids because alkaloids are polar compounds and do not dissolve in non-polar solvents.

The results indicate that solvent polarity plays an important role in alkaloid extraction. Polar solvents like methanol are more efficient in extracting alkaloids compared to non-polar solvents like petroleum ether and hexane. Similar results were reported in other *Rauvolfia* species, where methanol extracts showed higher alkaloid content compared to other solvents.

Alkaloids present in *Rauvolfia* species are known for their medicinal properties such as antihypertensive, antipsychotic, anticancer, anti-inflammatory, and antimicrobial activities. Therefore, the presence of alkaloids in *Rauvolfia micrantha* confirms its medicinal importance and pharmacological potential.

The qualitative analysis of alkaloids in leaf and root extracts of *Rauvolfia micrantha* revealed significant variation depending on the solvent used for extraction (Table 1). The results clearly indicate that solvent polarity plays a crucial role in the extraction efficiency of alkaloids.

Among the solvents tested, **methanol extract showed a strong presence (+++) of alkaloids** in both leaf and root samples. This suggests that methanol is highly effective for extracting alkaloids due to its polar nature, which facilitates the dissolution of nitrogen-containing compounds. Similar findings have been reported in other studies on *Rauvolfia* species and medicinal plants, where methanol proved to be the most efficient solvent for alkaloid extraction (Spigno et al., 2007; Sheikh et al., 2013).

The **hydro-alcohol extract also showed a strong to moderate presence of alkaloids**, particularly in root samples (+++), indicating that a mixture of polar solvents enhances extraction efficiency. This may be due to the combined polarity of water and alcohol, which allows better penetration into plant tissues and improved solubilization of alkaloids. Comparable results have been reported in phytochemical studies where hydro-alcoholic solvents yielded higher concentrations of bioactive compounds (Goswami et al., 2013).

Chloroform extract exhibited moderate presence (++) of alkaloids in both leaf and root extracts. Although chloroform is a semi-polar solvent, it can extract certain alkaloids, particularly those with less polarity. Similar observations have been reported in studies on *Rauvolfia* species, where chloroform showed moderate efficiency in extracting alkaloids (Sahu, 1983).

The **ethyl acetate extract showed weak to moderate presence (+ to ++) of alkaloids**, indicating limited extraction efficiency. Ethyl acetate, being moderately polar, can dissolve some alkaloids but is less effective compared to methanol. This agrees with previous reports that solvent polarity significantly influences alkaloid

solubility and extraction efficiency (Spigno et al., 2007).

In contrast, **hexane and petroleum ether extracts showed absence (-) of alkaloids** in both leaf and root samples. These solvents are non-polar and are not suitable for extracting polar compounds such as alkaloids. Similar findings have been reported in earlier studies, where non-polar solvents failed to extract alkaloids effectively (Sheikh et al., 2013).

The **aqueous extract showed weak to moderate presence of alkaloids**, suggesting that water alone is less efficient than organic solvents like methanol. Although alkaloids are generally soluble in acidic aqueous solutions, pure water extraction may not be sufficient for maximum recovery.

Overall, the results confirm that **methanol is the most effective solvent for alkaloid extraction**, followed by hydro-alcohol and chloroform. The findings are consistent with earlier reports on *Rauvolfia* species, which are known for their rich content of indole alkaloids such as reserpine, ajmaline, and serpentine (Gao et al., 2012; Weiss and Mann, 2000).

The presence of alkaloids in *Rauvolfia micrantha* supports its medicinal importance, as alkaloids are known to possess various pharmacological properties such as antihypertensive, antipsychotic, anticancer, anti-inflammatory, and antimicrobial activities. These results highlight the potential of *Rauvolfia micrantha* as a valuable source of alkaloid-based therapeutic compounds and emphasize the importance of selecting appropriate solvents for efficient extraction.

4. Discussion

The present study was carried out to evaluate the effect of different solvents on the qualitative extraction of alkaloids from the leaf and root extracts of *Rauvolfia micrantha*. The results obtained from the qualitative alkaloid tests clearly

indicated that the type of solvent used plays a significant role in the extraction efficiency of alkaloids.

Among all the solvents used in the study, methanol extract showed a strong presence of alkaloids in both leaf and root extracts. This indicates that methanol is highly effective in extracting alkaloids from *Rauvolfia micrantha*. The high efficiency of methanol may be due to its polar nature, which allows it to dissolve a wide range of polar and semi-polar bioactive compounds, including alkaloids. Similar findings were reported by Spigno et al. (2007), who stated that polar solvents such as methanol are highly effective for extracting bioactive compounds from plant materials. Sheikh et al. (2013) also reported that methanol is one of the best solvents for the extraction of alkaloids and other phytochemicals from medicinal plants.

The hydro-alcohol extract also showed strong to moderate presence of alkaloids, especially in root extracts. This may be due to the combined effect of water and alcohol, which increases the permeability of plant cell walls and improves the extraction efficiency of alkaloids. Goswami et al. (2013) reported that hydro-alcoholic solvents are commonly used in phytochemical extraction because they can dissolve both polar and moderately polar compounds.

Chloroform extracts showed moderate presence of alkaloids in both leaf and root extracts. Chloroform is a semi-polar solvent and can extract certain types of alkaloids, particularly free base alkaloids. Similar results were reported by Sahu (1983), who observed that chloroform extracts contain moderate amounts of alkaloids in *Rauvolfia* species.

Ethyl acetate extracts showed weak to moderate presence of alkaloids. Ethyl acetate is a moderately polar solvent and can extract some alkaloids but not as effectively as methanol. The variation in alkaloid extraction among solvents may be due to differences in solvent polarity and solubility of alkaloids in different solvents (Spigno et al., 2007).

Hexane and petroleum ether extracts showed absence of alkaloids in both leaf and root extracts. These solvents are non-polar in nature and are mainly used for extraction of lipids, fats, and non-polar compounds. Alkaloids are generally polar compounds and therefore they are not soluble in non-polar solvents. Similar findings were reported by Sheikh et al. (2013), who observed that non-polar solvents are not suitable for alkaloid extraction.

The aqueous extract showed weak to moderate presence of alkaloids. Although alkaloids are soluble in acidic aqueous solutions, water alone may not be as effective as organic solvents in extracting alkaloids due to poor penetration into plant tissues and lower extraction efficiency.

The results of the present study are in agreement with previous studies on *Rauvolfia* species, which reported the presence of important indole alkaloids such as reserpine, ajmaline, serpentine, and yohimbine (Gao et al., 2012). These alkaloids are known for their pharmacological properties such as antihypertensive, antipsychotic, anticancer, anti-inflammatory, and antimicrobial activities (Weiss and Mann, 2000).

The study also showed that root extracts contained slightly higher alkaloid content compared to leaf extracts. This may be due to the accumulation of alkaloids in roots, which act as storage organs in many medicinal plants. Similar observations were reported in *Rauvolfia serpentina*, where roots contain higher alkaloid content compared to other plant parts (Sahu, 1983).

Overall, the study confirms that solvent polarity significantly influences alkaloid extraction and methanol was found to be the most effective solvent for extracting alkaloids from *Rauvolfia micrantha*. The presence of alkaloids in both leaf and root extracts indicates that the plant has significant medicinal value and can be used for further pharmacological and phytochemical studies.

5. Conclusion

The present study clearly demonstrated the presence of alkaloids in both leaf and root extracts of *Rauvolfia micrantha* through qualitative phytochemical analysis. The results revealed that the efficiency of alkaloid extraction largely depends on the type of solvent used. Among the various solvents employed in the study, methanol showed the highest efficiency for alkaloid extraction, indicating a strong presence of alkaloids in both leaf and root extracts.

Chloroform and ethyl acetate extracts showed moderate presence of alkaloids, whereas aqueous extracts showed mild presence. In contrast, non-polar solvents such as hexane and petroleum ether did not show the presence of alkaloids, confirming that alkaloids are mostly polar and semi-polar compounds and are best extracted using polar solvents.

The study also indicated that root extracts showed comparatively higher alkaloid presence than leaf extracts, suggesting that roots may serve as major storage sites for alkaloids in this plant. This supports earlier findings in other *Rauvolfia* species, where roots are known to contain important medicinal alkaloids such as reserpine, ajmaline, and serpentine.

Overall, the study concludes that *Rauvolfia micrantha* is a significant source of alkaloids and has considerable potential for medicinal and pharmaceutical applications. The findings of the present study provide a scientific basis for the use of this plant in traditional medicine and support further research on its bioactive compounds. Future research should focus on quantitative estimation, isolation, purification, and characterization of specific alkaloids, as well as evaluation of their pharmacological activities. Such studies may contribute to the development of plant-based drugs and promote the conservation and sustainable utilization of this important medicinal plant.

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