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Alkaloid Distribution in the Roots of Papaver Somniferum (Opium Poppy)

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Research Article

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Abstract

Papaver somniferum (opium poppy) is a medicinally and forensically significant plant known for producing benzylisoquinoline alkaloids such as morphine, codeine, thebaine, papaverine, and noscapine, predominantly within the latex of its capsules. While extensive research has characterized alkaloid content in capsules and aerial plant parts, the root system remains comparatively understudied despite its forensic relevance in cases involving uprooted or fragmented plant evidence. This review and experimental study examine the qualitative alkaloid distribution in Papaver somniferum roots, with the objective of identifying opium plant roots through presumptive and colorimetric testing methods.

Root extracts were prepared by acid digestion of dried root powder and subjected to a tiered testing protocol: general alkaloid screening (Mayer's, Dragendorff's, Wagner's, and Hager's tests), specific opium alkaloid color tests (Marquis, Mecke's, Froehde's, and Mandelin reagents), and the meconic acid test using ferric chloride, regarded as the most specific confirmatory test for opium. All tests produced characteristic positive reactions — including cream, orange-red, reddish-brown, and yellow precipitates in the screening tests, purple-to-violet and blue-to-green color changes in the specific tests, and a blood-red coloration confirming meconic acid presence.

Literature review further revealed that sanguinarine and its hydroxylated/reduced derivatives dominate root alkaloid composition, while NDPS-scheduled alkaloids (morphine, codeine, thebaine) occur only in trace-to-low concentrations, localized to vascular and phloem tissue. These findings establish that opium poppy roots, despite low overall alkaloid yield, can be reliably identified through simple presumptive colorimetric tests, offering forensic laboratories a practical, low-cost supplementary method for plant identification in illicit cultivation investigations.

Keywords: Papaver Somniferum; Opium; Alkaloids; Morphine; Codeine; Forensic Toxicology

Abbbreviations

BIA: Benzylisoquinoline Alkaloid; BBE: Berberine Bridge Enzyme; COR: Codeinone Reductase.

Introduction

The opium poppy, Papaver somniferum, is one of the most significant medicinal and narcotic plants due to its production of pharmacologically active benzylisoquinoline alkaloids, including morphine, codeine, thebaine, papaverine, and noscapine. These alkaloids are synthesized through complex metabolic pathways and are predominantly accumulated in the latex of immature capsules, making the capsule the principal commercial and forensic source of opium alkaloids. While extensive research has focused on the latex, capsules, and aerial parts of the plant, comparatively little attention has been given to the roots, resulting in a significant gap in

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the understanding of root-specific alkaloid distribution [1].

From a forensic perspective, the identification and characterization of alkaloids in different plant tissues have considerable evidentiary value. During investigations into suspected illicit cultivation, law enforcement agencies may recover uprooted or partially destroyed Papaver somniferum plants. In such situations, root material may be among the available botanical evidence alongside stems and residual plant fragments. The chemical examination of roots could therefore contribute to plant identification and complement other botanical and analytical findings, although current evidence indicates that root alkaloid concentrations are generally much lower than those in the latex and capsules.

The biosynthesis of opium alkaloids begins in root tissues before intermediates and end products are transported and accumulated in other plant organs. This biological role suggests that roots may contain detectable amounts of certain alkaloids or biosynthetic intermediates, making them scientifically relevant despite their comparatively low alkaloid content. However, published studies describing the qualitative and quantitative distribution of alkaloids in Papaver somniferum roots remain limited, and reported findings vary depending on plant genotype, developmental stage, environmental conditions, and analytical methodology [2].

In India, unauthorized cultivation of opium poppy occasionally results in the seizure of whole plants by enforcement agencies. In such cases, comprehensive chemical characterization of all available plant parts, including roots, may support forensic examination when interpreted alongside botanical, morphological, and other analytical evidence. Nevertheless, root alkaloid analysis should not be regarded as a standalone method for establishing the legality or origin of cultivation.

This review critically examines the current scientific literature on alkaloid distribution in the roots of Papaver somniferum, discusses the biosynthesis and transport of major opium alkaloids, evaluates analytical techniques used for their detection, and highlights the forensic significance and existing research gaps. By consolidating the available evidence, this review aims to provide a foundation for future studies on root alkaloids and their potential applications in forensic science and plant-based drug investigations (Table 1).

S. NO	Alkaloid	Molec Ular Formu La	Chemic Al Class	Concentr Ation In Root	Location In Root	Forensi C Signific Ance
1	sanguinarine	$C_{20}H_{14}NO_4^+$ (cation); $C_{20}H_{13}NO_4$ (neutral form)	Benzo(c) phenanthr idine (Quaterna ry Ammonium Salt)	HIGH – Dominant root alkaloid	Root parenchyma cells	PRIMARY root – specific marker for opium poppy plant
2	Dihydrosanguinari ne	$C_{20}H_{15}NO_4$	Benzo(c) Phenanth ridine (reduced form)	HIGH – Co-dominant root alkaloid	Root cells; biosynthetic product from protopine via P6H enzyme	Co-dominant root marker
3	10-hydroxysanguinari ne	$C_{20}H_{14}NO_5^+$	Benzo(c) Phenanth ridine (hydroxyl ated derivative)	MODERATE	Root cells; hydroxylated form of sanguinarine	Root – specific derivative; Benzo(c) Phenanthri dine fingerprint
4	10 -Hydroxydihydrosa nguinarine	$C_{20}H_15NO_5$	Benzo(c) Phenanth ridine (hydroxyl ated reduced form)	MODERATE	Root cells; co – occurs with 10-hydroxysang uinarine	Root – specific derivative; part of species identificati on profile
5	Morphine (NDPS Scheduled)	$C_{17}H_{19}NO_3$	Morphina n (Phenant hrene-type)	LOW – 0.001 TO 0.24% (DRY WEIGHT)	Mature root tissue; vascular bundle; pericycle of stele	CRITICA L under NDPS Act 1985
6	Codeine (NDPS Scheduled)	$C_{18}H_{21}NO_3$	Morphina n	TRACE	Mature root tissue; secondary phloem	NDPS Scheduled substance
7	Thebaine (NDPS Scheduled)	$C_{19}H_{21}NO_3$	Morphina n (methylated)	TRACE	Mature root tissue; secondary phloem	NDPS Scheduled substances

Table 1: Alkaloid Distribution.

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Objective of this Research Paper

- To understand the Alkaloid Distribution in Roots of Opium Plant
- The Objective of this Research Paper is to Identify Roots of Opium Plants with Help of Presumptive Tests
- To Identify Roots of Opium Plants by its Colorimetric Reaction

Materials and Methods

Materials Used

Sample Preparation

- Dilute HCL
- Filter paper
- Dried opium roots powder

Reagents Used

- Mayer's reagent – 1.36 g mercuric chloride + 5.0 g potassium iodide (KI), dissolved in distilled water and made up to 100 ml
- Dragendorff's reagent – Potassium bismuth iodide solution
- Wagner's reagent – Iodine in potassium iodide solution
- Hager's reagent – Saturated aqueous solution of picric acid
- Marquis reagent – Concentrated sulfuric acid (100 ml) + 40% formaldehyde (5 ml)
- Mecke's reagent – Selenious acid (0.5 g) + concentrated sulfuric acid (100 ml)
- Froehde's reagent – 0.5 g sodium molybdate in 100 ml concentrated sulfuric acid (H_2SO_4)
- Mandelin reagent – Concentrated sulfuric acid (100 ml) + ammonium metavanadate (0.5–1 g)
- Ferric chloride reagent (Meconic acid test) – 5% w/v aqueous ferric chloride solution; dissolve 5 g of ferric chloride in distilled water and make up to 100 ml

Glassware and Consumables

- Beakers
- Dropper
- Porcelain dish
- Spot plate
- Measuring cylinders
- Digital camera for recording color changes and results

Safety Equipment

- Laboratory coat
- Nitrile gloves
- Protective goggles
- Fume hood for handling chemicals and reference standards

Method Used

Sample Preparation

A small quantity of dried root material is ground into powder. The extract is prepared by dissolving ~100 mg of root powder in 5–10 mL of dilute hydrochloric acid (2N HCl) or acidified water/ethanol, then filtering. This filtrate is used for all tests below

Testing of Sample: Perform presumptive test on sample and note down the color change observation between reagents and prepared sample of roots of opium

Result of Presumptive Tests for Opium Plant Roots Extracts

S.No.	Test Name	Reagent Composition	Procedure	Positive Result	Specificity
1	Mayer's test	1.36 g mercuric chloride and 5.0 g potassium iodide (KI) dissolved in distilled water and made up to 100 ml	Add 2-3 drops of Mayer's reagent to 2ml of acidified root extract	Cream – colored precipitate	General alkaloid detection
2	Dragendorff's test	Potassium bismuth iodide solution	Add 2-3 drops of reagent to 2ml of acidified root extract	Orange – red precipitate	General alkaloid detection
3	Wagner's test	Iodine in potassium iodide solution	Add 2-3 drops of reagent to 2ml of acidified root extract	Reddish – brown precipitate	General alkaloid detection
4	Hager's test	Saturated aqueous solution of picric acid	Add 2-3 drops of reagent to 2ml of acidified root extract	Yellow – precipitate	General alkaloid detection

Table 2: General Alkaloid Screening Tests.

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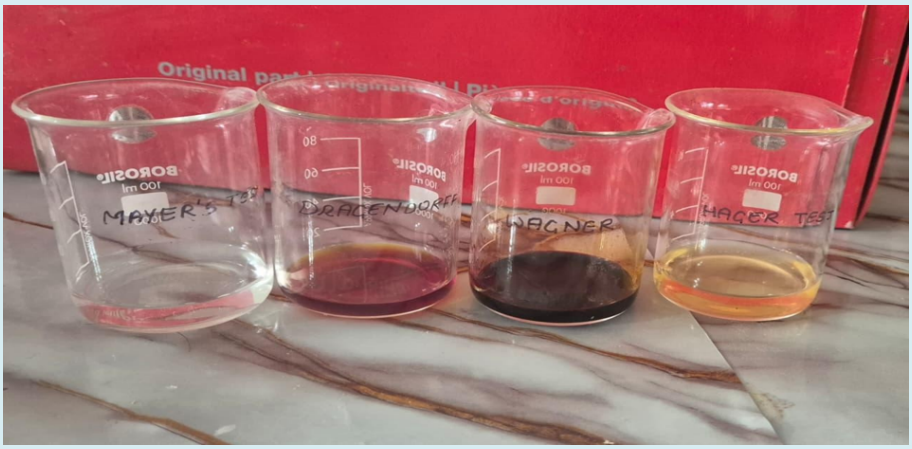


Figure 1: Result for Mayers, Dragendorff's, Wagner's and Hager's Test.



Figure 2: Precipitate for Mayers, Dragendorff's, Wagner's and Hager's Test.

S.No.	Test Name	Reagent Composition	Procedure	Positive Result	Specificity
1	Marquis test	Conc. Sulfuric Acid (100ML) + 40% FORMALDEHYD E (5ML)	Add 1-2 drops to dried root extract on white porcelain tile	Purple to violet	Opium alkaloid detection
2	Mecke 's test	Selenious Acid (0.5g) + Conc. Sulfuric Acid (100ml)	Add 1-2 drops to dried root extract on white porcelain tile	Blue to dark bluish - green	Opium alkaloid detection
3	Froehde's test	0.5 g sodium molybdate in 100 mL concentrated sulfuric acid (H_2SO_4).	Add 1-2 drops to dried root extract on white porcelain tile	Violet àGreen àBlue	Opium alkaloid detection UNODC Recommended test
4	Mandelin test	Conc. Sulfuric Acid (100ML) + AMMONIUM METAVANADATE (0.5 – 1g)	Add 1-2 drops to dried root extract on white porcelain tile	Dark olive color	Opium alkaloid detection

Table 3: Specific Opium Alkaloid Color Test.

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Figure 3: Mecke, Marquis, Mandelin And Froehde Reagent.

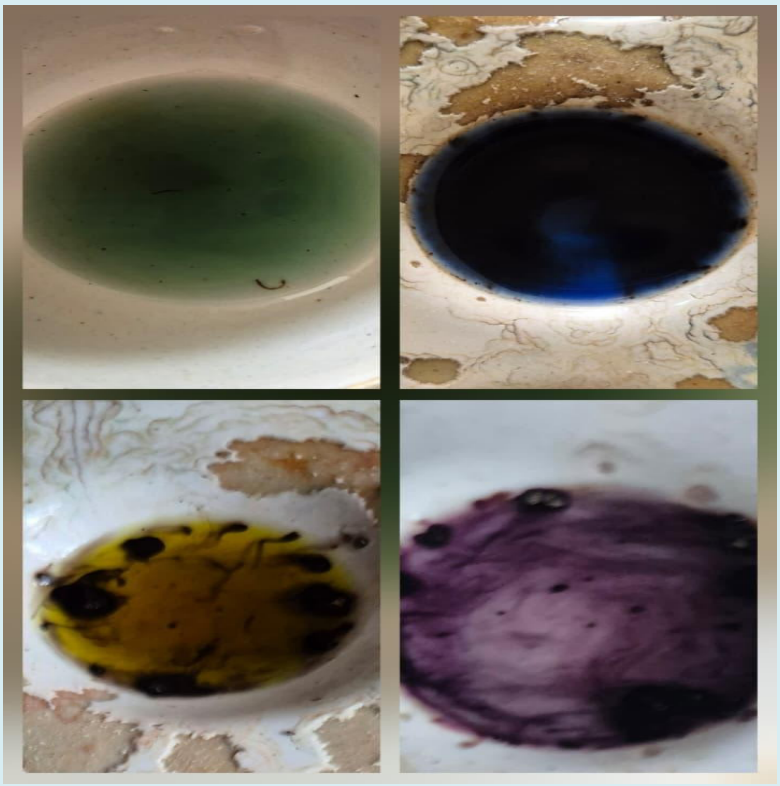


Figure 4: Results for Mecke , Frohede, Mandelin and Marquis Test.

S.No.	Test Name	Reagent Composition	Procedure	Positive Result	Specificity
1	Meconic acid test - most specific test for opium	5% w/v aqueous ferric chloride (solution — dissolve 5 g of ferric chloride in distilled water and make up to 100 ml	Add solution drops to concentrated aqueous root extract	Blood red / Dark blood red color	Highly specific test for opium – detects meconic acid in opium

Table 4: Meconic Acid Test - Most Specific Test for Opium.

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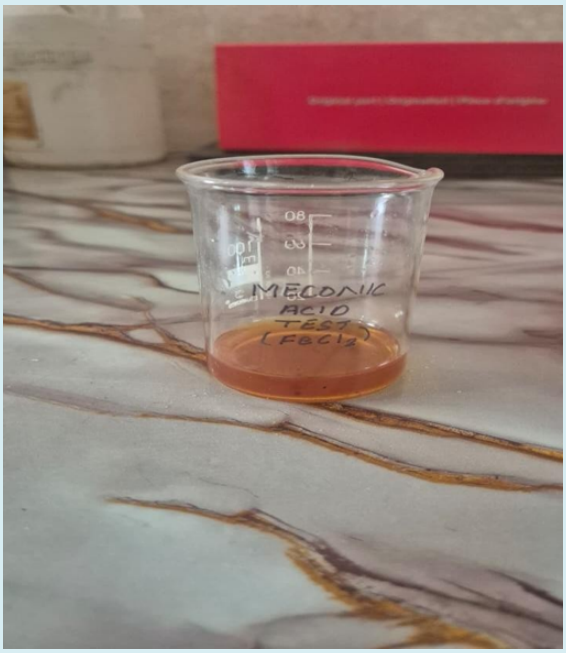


Figure 5: Meconic Acid Reagent.



Figure 6: Result for Meconic Test.

Discussion

Botanical Background

In a forensic lab (like FSL, UP), roots received as dried seized exhibits typically show:

- Shrunken, wrinkled, brownish external surface
- Brittle texture upon bending or snapping
- Yellowish-brown resinous streaks at cut ends (dried latex)
- Root hairs absent (lost during drying)
- Anatomical features still visible under microscopy after rehydration with lacto-phenol or FAA solution

Opium poppy plant's roots have fairly ordinary botanical features compared to the rest of the plants:

- Root type – Taproot system root
- Structure – slender, fibrous and relatively shallow compared to taproot's length; not fleshy like carrots or beets
- Function – primarily anchorage and water / nutrient uptake
- Growth habit - As an annual, the root system develops relatively quickly within a single growing season and doesn't persist or thicken like a perennial would.

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Figure 7: Alkaloid Biosynthesis.

Benzylisoquinoline alkaloid (BIA) biosynthesis in Papaver somniferum is compartmentalized across three specialized, phloem-associated cell types: companion cells, sieve elements, and laticifers which together form a system in which sieve elements, though enucleate and incapable of independent RNA and protein synthesis, are sustained through plasmodesmatal connections with adjacent companion cells Critically, this cell-specific architecture is not restricted to aerial tissue; immunofluorescence labeling has localized seven BIA biosynthetic enzymes — including (S)-N-methylcoclaurine 3′-hydroxylase (CYP80B1), berberine bridge enzyme (BBE), codeinone reductase (COR), 6OMT, CNMT, 4′OMT, and SAT — specifically to the sieve elements of roots and hypocotyls in opium poppy seedlings Root-localized enzyme abundance was not static during early development: these enzymes increased rapidly in abundance between one and three days after seed germination , indicating that root tissue is biosynthetically active from the earliest stages of plant growth [3].

Presumptive Test Observation with Opium Root Extract

The presumptive tests performed on the opium plant root extract collectively indicate the presence of alkaloids characteristic of opium. The general alkaloid screening tests (Mayer's, Dragendorff's, Wagner's, and Hager's tests) produced their respective characteristic precipitates (cream, orange-red, reddish-brown, and yellow), confirming the presence of alkaloids in the sample. Further, the specific color tests (Marquis, Mecke's, Froehde's, and Mandelin tests) gave positive color reactions (purple-violet, blue-green, violet-to-blue, and dark olive respectively), narrowing identification toward opium-derived alkaloids specifically. Finally, the meconic acid test, regarded as the most specific and confirmatory test for opium, produced a blood-red/dark blood-red coloration with ferric chloride, providing strong confirmatory evidence for the presence of meconic acid — a marker compound unique to opium [4-7].

Taken together, the consistent positive results across all three tiers of testing (general screening, specific color tests, and the confirmatory meconic acid test) provide reliable evidence that the analyzed root extract contains opium alkaloids

Conclusion

This study consolidates the limited but growing evidence on alkaloid distribution in the roots of Papaver somniferum, highlighting that root tissue is not the primary site of alkaloid accumulation, is far from chemically inert. Sanguinarine and its derivatives (dihydrosanguinarine, 10-hydroxysanguinarine, and 10-hydroxydihydrosanguinarine) emerge as the dominant root-specific alkaloids, while morphine, codeine, and thebaine occur only in low to trace concentrations, primarily localized in mature vascular and phloem tissue. The presence of BIA biosynthetic enzymes in root sieve elements from early seedling stages confirms that roots are biosynthetically active tissue, not merely passive conduits for alkaloids produced elsewhere in the plant.

From an applied forensic standpoint, the presumptive testing protocol employed in this study — general alkaloid screening (Mayer's, Dragendorff's, Wagner's, Hager's), specific opium color tests (Marquis, Mecke's, Froehde's,

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Mandelin), and the highly specific meconic acid test-demonstrated consistent positive reactions on root extracts. The convergence of results across all three tiers of testing supports the conclusion that opium poppy roots retain detectable, testable levels of characteristic alkaloids and marker compounds, making root material a potentially useful supplementary exhibit in cases where seized plants are uprooted or fragmented prior to recovery.

However, root alkaloid analysis should be interpreted as a corroborative tool rather than a standalone method of identification. Given the low concentration of NDPS-scheduled alkaloids in roots relative to capsule latex, findings from root testing must be considered alongside botanical, morphological, and microscopic evidence to build a complete forensic picture. This study underscores the need for further quantitative and species-comparative research to establish standardized reference values for root alkaloid content, which would strengthen the evidentiary reliability of root-based analysis in forensic investigations of illicit opium poppy cultivation.

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