Research Paper

Preformulation Studies of Medicinal Plant Extract for Development of a Novel Drug Delivery System with Antibacterial Potential

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ABSTRACT
Preformulation studies are fundamental in the development of effective herbal drug delivery systems as they provide essential information regarding the physicochemical and phytochemical characteristics of plant extracts. The present study was undertaken to evaluate the preformulation parameters of Holoptelea integrifolia stem bark and Withania somnifera roots for the development of a novel antibacterial drug delivery system. Successive solvent extraction was carried out using petroleum ether, chloroform, methanol, and aqueous solvents. Among all extracts, methanolic extracts of both plants showed the highest extractive yields, with H. integrifolia and W. somnifera exhibiting yields of 8.42 ± 0.39% and 7.86 ± 0.34%, respectively. Preliminary phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, terpenoids, steroids, glycosides, and saponins, indicating significant therapeutic potential. TLC profiling established characteristic fingerprint patterns and confirmed the presence of major phytoconstituents. HPTLC analysis demonstrated reliable quantification of friedelin and withaferin A with excellent linearity, precision, and accuracy according to ICH guidelines. The quantified marker compounds supported the quality and consistency of the extracts. Overall, the findings suggest that the methanolic extracts possess promising phytochemical characteristics suitable for further formulation development and antibacterial investigations.

Keywords: Preformulation, Holoptelea integrifolia, Withania somnifera, Antibacterial, HPTLC, Phytochemical screening.
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INTRODUCTION:

Preformulation studies are essential for the effective development of pharmaceutical dosage forms. They offer important insights into the physicochemical and biopharmaceutical characteristics of drug substances, which aid in the selection of appropriate excipients and suitable formulation strategies. Antimicrobial resistance demands alternative therapeutic strategies. *Holoptelea integrifolia* and *Withania somnifera* are traditionally used medicinal plants, but their combined antibacterial potential remains unexplored. The convergence of rising AMR, the drying antibiotic pipeline, and the inherent limitations of conventional antibiotics has created an urgent need for alternative and novel therapeutic strategies.[2]

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The stem bark of *Holoptelea integrifolia* (Roxb.) Planch. was collected in March 2025 from the Medicinal Plant Garden of the Krishi College, Indore, India. The collection was made during the flowering season when the tree was in full bloom, as this period is considered optimal for maximum accumulation of bioactive constituents.

The roots of *Withania somnifera* (L.) Dunal was collected in the month of March 2025 from a certified Medicinal Plant Garden of the Krishi College, Indore, India. Plants of approximately 6-8 months age, at the flowering stage, were selected for root collection as this stage is reported to yield maximum withanolide content.

Authentication of Plant Materials

Both plant materials were authenticated by Dr. Prof. S. K. Sharma, Ret. Professor, Govt Mata Jija Bai Girls P G College, Moti Tabela, Indore, M.P., India. Voucher specimens were prepared for each plant and deposited in the institutional herbarium for future reference.

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade (AR) or high-performance liquid chromatography (HPLC) grade as appropriate.

Processing and Extraction of Plant Materials

For *Holoptelea integrifolia* bark: The fresh bark was washed thoroughly under running tap water to remove any adhering dust, dirt, and other contaminants. It was then rinsed with distilled water. The cleaned bark was cut into small pieces (approximately 2-3 cm in size) using a stainless-steel knife and spread in a thin layer on clean paper sheets. The bark pieces were shade-dried at room temperature (25±2°C) for 15 days with occasional turning to prevent fungal growth. Complete drying was confirmed by constant weight measurement.

For *Withania somnifera* roots: The washed roots were cut into small pieces (1-2 cm) and shade- dried at room temperature for 12 days. Drying was continued until constant weight was achieved.

Size Reduction

The dried plant materials were separately ground into coarse powder using a laboratory grinder. The powdered materials were passed through sieve No. 40 (420 µm) to obtain uniform particle size. The sieved powders were stored in

airtight amber-coloured glass containers, labelled appropriately, and kept in a cool, dry place until further use.

Extraction

Successive Solvent Extraction

The powdered plant materials were subjected to successive solvent extraction using solvents of increasing polarity in a Soxhlet apparatus. The extraction was carried out following the standard procedure described by Harborne (1998) with minor modifications.

Petroleum Ether Extraction:

Approximately 200 g of the powdered plant material was packed into a thimble and placed in the Soxhlet apparatus. Petroleum ether (60-80°C) was added to the round-bottom flask, and extraction was carried out at 65-70°C for 18-20 hours until the siphoning liquid became colorless. The extract was collected, filtered through Whatman No. 1 filter paper, and concentrated under reduced pressure using a rotary vacuum evaporator at 40°C. The concentrated extract was transferred to a pre-weighed glass vial and dried completely in a vacuum desiccator. The percentage yield was calculated, and the extract was stored at 4°C until further use.

Chloroform Extraction: The marc (residue) left after petroleum ether extraction was dried and then subjected to chloroform extraction in the Soxhlet apparatus. Extraction was carried out at 65°C for 18-20 hours. The extract was processed similarly, concentrated, dried, and stored.

Methanol Extraction: The chloroform-extracted marc was dried and subjected to methanol extraction in the Soxhlet apparatus at 70°C for 20-24 hours. The methanolic extract was concentrated, dried, and stored.

Aqueous Extraction: The methanol-extracted marc was dried and subjected to aqueous extraction by decoction method. The marc was boiled with distilled water (1:10 w/v) for 3 hours, filtered, and the filtrate was concentrated on a water bath. The concentrated extract was freeze-dried and stored.

Concentration and Drying of Extracts

All organic solvent extracts (petroleum ether, chloroform, methanol) were concentrated using a rotary vacuum evaporator (Buchi R-300) under reduced pressure. The conditions maintained were:

Water bath temperature: 40-45°C (depending on solvent boiling point)

Rotation speed: 80-100 rpm

Vacuum pressure: 175-350 mbar (adjusted according to solvent)

Condenser temperature: 5-10°C

The concentrated extracts were transferred to pre-weighed glass vials and dried completely in a vacuum desiccator over anhydrous calcium chloride until constant weight was achieved.

Calculation of Percentage Yield

The percentage yield of each extract was calculated using the following formula:

Percentage Yield (% w/w) = (Weight of dried extract / Weight of dried plant material taken for extraction) × 100

All extracts were stored in airtight amber-colored glass vials at 4°C in a refrigerator until further analysis. For long-term storage, extracts were kept at -20°C.

Preliminary Phytochemical Screening

The extracts obtained from both plants were subjected to qualitative phytochemical screening to identify the presence of various classes of phytoconstituents.

Preparation of Test Solutions

Each extract was dissolved in its respective solvent (petroleum ether, chloroform, methanol) or distilled water (for aqueous extract) to prepare a stock solution of 10 mg/mL concentration. These solutions were used for the phytochemical tests.

Thin Layer Chromatography (TLC)

TLC profiling was performed for all extracts to establish characteristic fingerprint patterns and to identify the presence of specific phytoconstituents.

Preparation of TLC Plates

Pre-coated aluminum TLC plates (Merck, Silica gel 60 F_{254}, 20 × 20 cm, 0.2 mm thickness) were used. The plates were activated by heating at 110°C for 30 minutes in a hot air oven before use.

Sample Application

Extracts were dissolved in their respective solvents (10 mg/mL concentration). Using a capillary tube, approximately 5-10 µL of each sample was spotted on the TLC plate, 1.5 cm above the bottom edge. The spots were dried using a hair dryer.

Selection of Mobile Phase

Different solvent systems were tried for each extract to achieve optimal separation. The solvent systems that gave the best resolution were selected. The details are shown in Table 1.

Table 1: Mobile Phases for TLC of Different Extracts

Extract	Mobile Phase Composition	Ratio (v/v)
Petroleum ether	Toluene: Ethyl acetate	9:1
Chloroform	Toluene: Ethyl acetate: Formic acid	7:3:0.5
Methanol	Ethyl acetate: Methanol: Water	10:1.5:1
Aqueous	n-Butanol: Acetic acid: Water	4:1:5 (upper layer)

Development of Chromatograms

The mobile phase was poured into a twin trough TLC chamber (CAMAG, 20×10 cm) and allowed to saturate for 30 minutes at room temperature. The spotted TLC plate was

placed in the chamber and developed ascendingly until the solvent front reached approximately 8 cm from the origin (about 20-25 minutes). The plate was removed and air-dried.

Calculation of Rf Values

The retention factor (Rf) for each spot was calculated using the formula:

Rf = Distance traveled by solute / Distance traveled by solvent front

High-Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis was performed for the quantitative estimation of marker compounds in the active extracts.

Preparation of Standard Solutions

Friedelin standard: Accurately weighed 5 mg of friedelin standard was dissolved in 5 mL of methanol in a volumetric flask to obtain a stock solution of 1000 µg/mL. From this stock, working standards of 10-100 µg/mL were prepared by appropriate dilution with methanol.

Withaferin A standard: Accurately weighed 5 mg of withaferin A standard was dissolved in 5 mL of methanol to obtain a stock solution of 1000 µg/mL. Working standards of 5-50 µg/mL were prepared by appropriate dilution.

Preparation of Sample Solutions

For *H. integrifolia* methanolic extract: Accurately weighed 100 mg of the extract was dissolved in 10 mL of methanol (10 mg/mL concentration). The solution was sonicated for 10 minutes and filtered through a 0.45 µm membrane filter.

For *W. somnifera* methanolic extract: Accurately weighed 100 mg of the extract was dissolved in 10 mL of methanol (10 mg/mL concentration). The solution was sonicated and filtered similarly.

Calibration Curve

Different volumes of standard solutions were applied to the HPTLC plate to obtain concentrations in the range of 100-1000 ng/band for friedelin and 50-500 ng/band for withaferin A. The plates were developed, scanned, and peak areas were recorded. Calibration curves were plotted as concentration vs. peak area.

Method Validation

The HPTLC method was validated for the following parameters as per ICH guidelines:

a. **Linearity:** Analyzed by preparing calibration curves with at least 5 concentrations in triplicate. The correlation coefficient (r^2) was calculated.

b. **Precision:**
   - **Intra-day precision:** Analyzed by applying the sample (3 concentrations) three times on the same day.
   - **Inter-day precision:** Analyzed by applying the sample (3 concentrations) on three different days.

c. **Accuracy (Recovery studies):** Performed by spiking known amounts of standard to pre- analyzed sample at three levels (80%, 100%, and 120% of the expected concentration). Recovery percentage was calculated.

d. **Limit of Detection (LOD) and Limit of Quantification (LOQ):** Calculated using the formulas:
   - **LOD = 3.3 × (SD / Slope)**
   - **LOQ = 10 × (SD / Slope)**

   Where SD is the standard deviation of the response and slope is the slope of the calibration curve.

e. **Specificity:** Assessed by comparing the Rf and spectra of standard and sample peaks.

f. **Robustness:** Evaluated by introducing small, deliberate changes in mobile phase composition and chamber saturation time.

Quantification of Marker Compounds in Extracts

The sample solutions (10 mg/mL) were applied to the HPTLC plate in triplicate (4 µL and 8 µL volumes). The plates were developed, scanned, and peak areas were recorded. The amount of marker compound present in the extract was calculated using the calibration curve.

Amount of marker (% w/w) = (Concentration from calibration curve × Dilution factor × 100) / (Weight of extract taken)

RESULT AND DISCUSSION

Extraction and Phytochemical Screening

Extractive Values

The powdered plant materials of *Holoptelea integrifolia* (stem bark) and *Withania somnifera* (roots) were subjected to successive solvent extraction using petroleum ether (60-80°C), chloroform, methanol, and water. The extractive values were calculated as percentage yield (% w/w) in terms of dried plant material. As shown in Table 2 & 3.

Table 2: Extractive Values of *Holoptelea integrifolia* in Different Solvents

Solvent	Color and Consistency	Weight of Extract (g)	Percentage Yield (% w/w)
Petroleum ether (60-80°C)	Pale yellow, semisolid	4.28 ± 0.32	2.14 ± 0.16
Chloroform	Greenish-brown, sticky	6.94 ± 0.45	3.47 ± 0.23
Methanol	Dark brown, viscous	16.84 ± 0.78	8.42 ± 0.39
Water	Brown, hygroscopic powder	12.36 ± 0.62	6.18 ± 0.31

*Values are expressed as mean ± SD (n=3) *

Table 3: Extractive Values of *Withania somnifera* in Different Solvents

Solvent	Color and Consistency	Weight of Extract (g)	Percentage Yield (% w/w)
Petroleum ether (60-80°C)	Light yellow, oily	3.62 ± 0.28	1.81 ± 0.14
Chloroform	Yellowish-brown, semisolid	5.18 ± 0.36	2.59 ± 0.18
Methanol	Dark brown, viscous	15.72 ± 0.68	7.86 ± 0.34
Water	Brownish-yellow, hygroscopic powder	10.84 ± 0.52	5.42 ± 0.26

Values are expressed as mean ± SD (n=3)

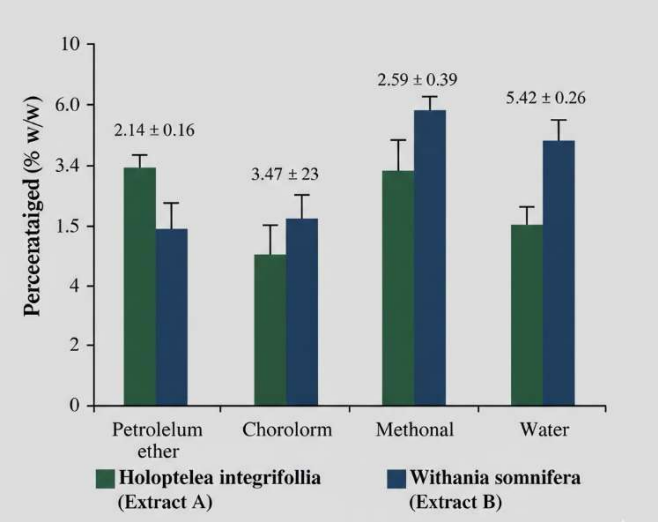


Figure 1: Extractive Yield Comparison of Different Solvents

The extractive values obtained in this study provide important information about the solubility profile of phytoconstituents present in both plants. For *Holoptelea integrifolia*, the highest yield was obtained with methanol (8.42 ± 0.39%), followed by aqueous (6.18 ± 0.31%), chloroform (3.47 ± 0.23%), and petroleum ether (2.14 ± 0.16%). Similarly, for *Withania somnifera*, the methanolic extract showed the maximum yield (7.86 ± 0.34%), followed by aqueous (5.42 ± 0.26%), chloroform (2.59 ± 0.18%), and petroleum ether (1.81 ± 0.14%).

Qualitative Phytochemical Screening

All extracts obtained from both plants were subjected to qualitative phytochemical screening to identify the presence of various classes of phytoconstituents.

Table 4: Qualitative Phytochemical Analysis of *H. integrifolia* Extracts

Phytoconstituent	Test Performed	Petroleum Ether	Chloroform	Methanol	Aqueous
Alkaloids	Dragendorff's test	-	+	++	-
	Mayer's test	-	+	++	-
	Wagner's test	-	+	++	-
	Hager's test	-	-	+	-
Flavonoids	Shinoda test	-	+	+++	+
	Alkaline reagent test	-	+	+++	+
	Lead acetate test	-	+	+++	+
Tannins	Ferric chloride test	-	+	+++	+++
	Gelatin test	-	-	++	++
Saponins	Foam test	-	-	+	++
Steroids	Salkowski test	++	++	+	-
	Liebermann-Burchard test	++	++	+	-
Terpenoids	Salkowski test	++	+++	+++	-
Glycosides	Keller-Kiliani test	-	-	+	+
	Borntrager's test	-	-	-	-

Carbohydrates	Molisch's test	-	-	+	++
	Fehling's test	-	-	-	+
Proteins/Amino Acids	Biuret test	-	-	-	-
	Ninhydrin test	-	-	-	-
Fixed Oils/Fats	Spot test	++	-	-	-

(+++): Highly present; (++): Moderately present; (+): Present; (-): Absent

Table 5: Qualitative Phytochemical Analysis of *W. somnifera* Extracts

Phytoconstituent	**Test Performed**	**Petroleum Ether**	**Chloroform**	**Methanol**	**Aqueous**
Alkaloids	Dragendorff's test	-	++	+++	+
	Mayer's test	-	++	+++	+
	Wagner's test	-	++	+++	+
	Hager's test	-	+	++	-
Flavonoids	Shinoda test	-	+	+++	++
	Alkaline reagent test	-	+	+++	++
	Lead acetate test	-	+	+++	++
Tannins	Ferric chloride test	-	-	++	++
	Gelatin test	-	-	+	+

Saponins	Foam test	-	-	++	+++
Steroids	Salkowski test	++	+++	++	-
	Liebermann-Burchard test	++	+++	++	-
Terpenoids	Salkowski test	+	+++	+++	-
Glycosides	Keller-Kiliani test	-	-	+	+
	Borntrager's test	-	-	-	-
Carbohydrates	Molisch's test	-	-	++	++
	Fehling's test	-	-	+	+
Proteins/Amino Acids	Biuret test	-	-	-	-
	Ninhydrin test	-	-	-	-
Fixed Oils/Fats	Spot test	+++	-	-	-

(+++): Highly present; (++): Moderately present; (+): Present; (-): Absent

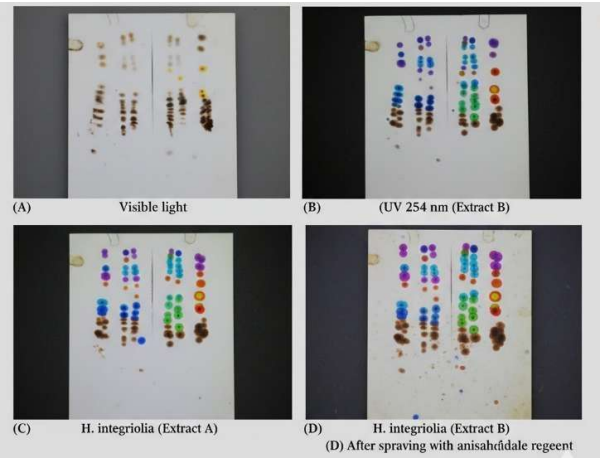
Thin Layer Chromatography (TLC) Profiling

The extracts were subjected to TLC profiling to establish characteristic fingerprint patterns and to confirm the presence of specific phytoconstituents. The results are presented in Tables 6 & 7 and Figure 2.

Table 6: TLC Profile of *H. integrifolia* Methanolic Extract

Spot No.	Rf Value	Visible Light	UV 254 nm	UV 366 nm	After Spraying (Anisaldehyde)	Probable Compound Class
1	0.12	Colorless	Quenching	-	Violet	Phenolics
2	0.24	Pale yellow	Quenching	Blue fluorescence	Green	Flavonoids
3	0.31	Colorless	Quenching	-	Pink	Terpenoids
4	0.45	Yellow	Quenching	Yellow fluorescence	Yellow	Flavonoids
5	0.53	Colorless	Quenching	-	Violet-blue	Triterpenes
6	0.62	Brown	Quenching	-	Brown	Tannins
7	0.78	Colorless	-	-	Purple	Steroids

Table 7: TLC Profile of *W. somnifera* Methanolic Extract



Spot No.	Rf Value	Visible Light	UV 254 nm	UV 366 nm	After Spraying (Anisaldehyde)	Probable Compound Class
1	0.08	Colorless	Quenching	-	Violet	Phenolics
2	0.16	Pale yellow	Quenching	Blue fluorescence	Green	Flavonoids
3	0.27	Colorless	Quenching	-	Pink	Withanolides
4	0.35	Yellow	Quenching	Yellow fluorescence	Yellow	Flavonoids
5	0.44	Colorless	Quenching	-	Blue-violet	Withanolides
6	0.56	Colorless	Quenching	-	Violet	Triterpenes
7	0.67	Orange	-	Orange fluorescence	Orange	Alkaloids
8	0.82	Colorless	-	-	Purple	Steroids

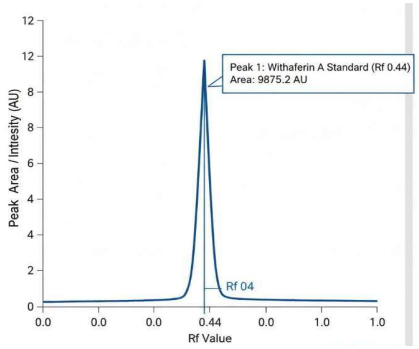
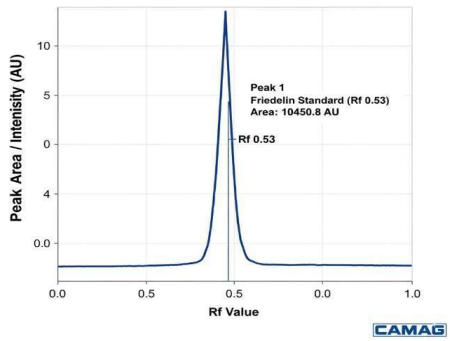


Figure 2: TLC Plates of *H. integrifolia* and *W. somnifera* Extracts.

Photographs showing TLC plates under different



visualization conditions: (A) Visible light, (B) UV 254 nm, (C) UV 366 nm, (D) After spraying with anisaldehyde reagent.

The TLC profiles established in this study serve as reference fingerprints for the methanolic extracts and confirm the presence of the major classes of bioactive compounds that are likely responsible for the antibacterial activity.

High-Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC analysis was performed for the quantitative estimation of marker compounds in the methanolic extracts. Friedelin was selected as the marker for *H. integrifolia*, and withaferin A was selected as the marker for *W. somnifera* based on literature reports indicating their abundance and biological activities [19, 21].

Method Validation

The HPTLC method was validated for linearity, precision, accuracy, LOD, and LOQ as per ICH guidelines. The results are shown in Table 8 & 9

Table 8: HPTLC Method Validation Parameters for Friedelin

Parameter	Result
Linearity range	100-1000 ng/band
Regression equation	y = 8.642x + 124.36
Correlation coefficient (r^2)	0.9984
Slope	8.642
Intercept	124.36
LOD	28.42 ng/band
Intra-day precision (% RSD)	0.84-1.26%
Inter-day precision (% RSD)	1.12-1.68%
Accuracy (% recovery)	98.24-101.36%

Table 9: HPTLC Method Validation Parameters for Withaferin A

Parameter	Result
Linearity range	50-500 ng/band
Regression equation	y = 12.384x + 86.42
Correlation coefficient (r^2)	0.9992
Slope	12.384
Intercept	86.42
LOD	12.36 ng/band
LOQ	37.45 ng/band
Intra-day precision (% RSD)	0.62-0.98%
Inter-day precision (% RSD)	0.94-1.42%
Accuracy (% recovery)	98.86-102.12%

Figure 3: HPTLC Chromatogram of Friedelin Standard

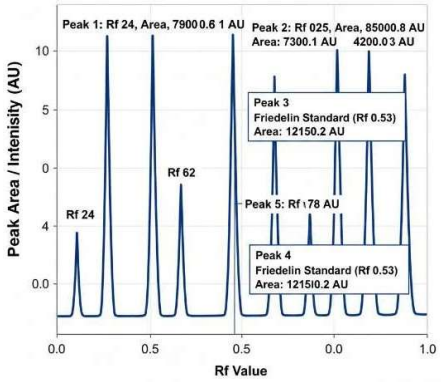


Figure 4: HPTLC Chromatogram of *H. integrifolia* Methanolic Extract

Figure 5: HPTLC Chromatogram of Withaferin A Standard

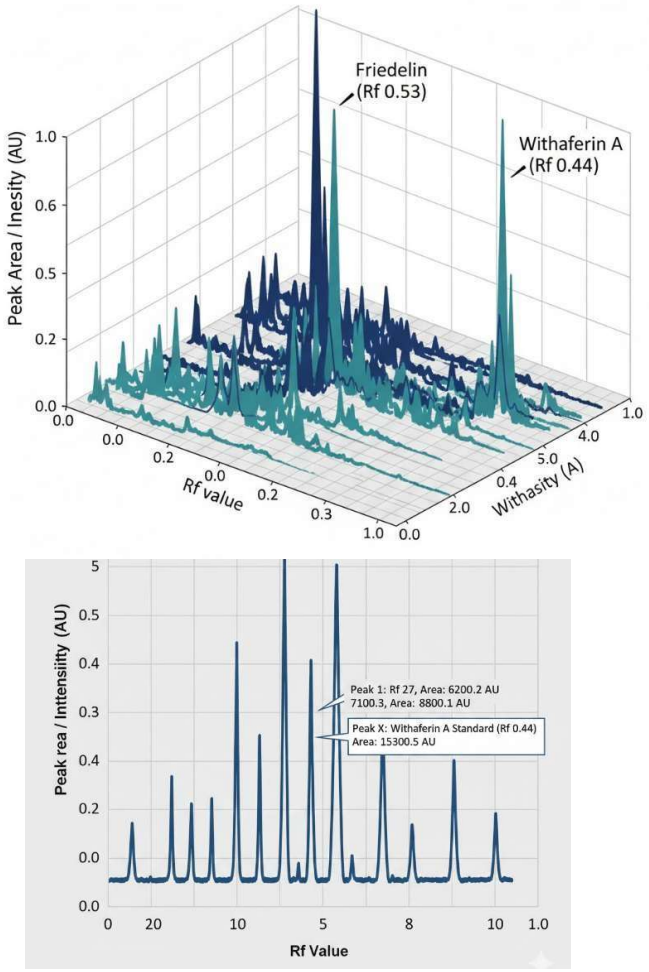


Figure 6: HPTLC Chromatogram of *W. somnifera* Methanolic Extract

Quantification of Marker Compounds

The amount of marker compounds present in the methanolic extracts was calculated using the calibration curves. The results are shown in Table 10 and Figure 7.

Table 10: Quantification of Marker Compounds in Extracts

Marker Compound	Plant	Amount in Extract (% w/w)
Friedelin	*H. integrifolia*	0.142 ± 0.008
Withaferin A	*W. somnifera*	0.218 ± 0.012

Values are expressed as mean ± SD (n=3)

Figure 7: 3D HPTLC Densitogram of Marker Compounds

The successful quantification of these markers establishes a quality control parameter for the extracts and ensures consistency for formulation development. The presence of these bioactive compounds supports the selection of these plants for antibacterial studies and provides a link between traditional use and modern phytochemical analysis.

Discussion:

The validated HPTLC method showed good linearity for both markers with correlation coefficients (r^2) greater than 0.998, indicating excellent correlation between concentration and peak area. The low % RSD values for intra-day and inter-day precision (<2%) indicate that the method is precise and reproducible. Recovery values within the range of 98-102% confirm the accuracy of the method.

The quantification revealed that the methanolic extract of *H. integrifolia* contained 0.142 ± 0.008% w/w of friedelin. This value is within the range reported by Chaturvedi et al. (2024), who found 0.12-0.16% friedelin in *H. integrifolia* bark extracts [24]. Friedelin is a pentacyclic triterpene with documented antibacterial, anti-inflammatory, and wound-healing activities [25]. Its presence in the extract contributes to the overall biological activity of the plant.

The methanolic extract of *W. somnifera* contained 0.218 ± 0.012% w/w of withaferin A. This is consistent with the range (0.15-0.30%) reported in the literature for root extracts of *W. somnifera*. Withaferin A is one of the most important bioactive withanolides, known for its antibacterial, anti-inflammatory, and anticancer properties. The presence of withaferin A confirms the quality of the plant material and provides a basis for the antibacterial activity observed.

Conclusion:

The present investigation successfully established the preformulation profile of *Holoptelea integrifolia* and *Withania somnifera* extracts intended for the development of a novel herbal drug delivery system with potential antibacterial applications. The extraction studies demonstrated that methanol was the most suitable solvent for obtaining higher extractive yields from both plant materials, indicating the abundance of polar bioactive constituents. Qualitative phytochemical screening confirmed the presence of several therapeutically important phytoconstituents such as flavonoids, alkaloids, tannins, terpenoids, steroids, glycosides, and saponins, which are known to contribute to antimicrobial and synergistic biological activities.

The TLC fingerprint profiles generated for the methanolic extracts provided characteristic identification patterns and confirmed the presence of diverse phytochemical groups. Furthermore, the HPTLC method developed for the quantification of friedelin and withaferin A was found to be accurate, precise, reproducible, and compliant with ICH validation

guidelines. The presence and quantification of these marker compounds established an important quality control parameter for standardization of the extracts.

The overall findings indicate that both plant extracts possess suitable physicochemical and phytochemical properties for further pharmaceutical development. These preformulation studies provide a scientific basis for the selection and standardization of extracts for future formulation studies and antibacterial evaluations. The results also support the traditional medicinal importance of both plants and highlight their potential as promising candidates for the development of effective herbal therapeutic systems against antimicrobial resistance.

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