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FTIR ANALYSIS OF ALKALOIDS IN RAUWOLFIA VOMITORIA (RV) LEAVES AND STEMS FOR POTENTIAL MEDICINAL APPLICATIONS

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ABSTRACT

Rauwolfia vomitoria (RV), a plant renowned for its medicinal properties, has garnered increasing attention for its potential therapeutic applications. The plant's leaves and stems are known to contain various bioactive compounds, including alkaloids and terpenoids, which contribute to its pharmacological significance. This research undertakes a thorough exploration of the alkaloid composition within the leaves and stems of *Rauwolfia vomitoria* (RV). In the case of RV, these alkaloids hold promise for applications in modern medicine, ranging from analgesics to anti-inflammatory agents. This study aims to conduct a comprehensive Fourier Transform Infrared Spectroscopy (FTIR) analysis of alkaloids present in RV leaves and stems. FTIR, a powerful analytical technique, enables the identification of functional groups within complex organic compounds. By focusing on alkaloids, this research seeks to elucidate the molecular composition of RV, laying the groundwork for understanding its medicinal properties. The investigation's significance lies in its potential to unveil specific alkaloid profiles, providing insights into the plant's therapeutic capabilities. This knowledge is vital for advancing drug discovery and development processes, ultimately contributing to the broader field of natural product-based pharmaceuticals.

INTRODUCTION

Rauwolfia vomitoria (RV), an indigenous tropical plant found in West Africa, has gained prominence in traditional medicine due to its diverse array of bioactive compounds, with alkaloids being a major focus of investigation (Ajazuddin *et al*., 2019; Hostettmann *et al*., 2006). Alkaloids, known for their pharmacological significance, contribute significantly to the therapeutic properties of medicinal plants (Li and Cui, 2014). The exploration of RV's alkaloid composition, particularly in different plant parts, holds promise for uncovering its medicinal potential.

Fourier Transform Infrared Spectroscopy (FTIR) has emerged as a valuable analytical technique for the rapid and non-destructive characterization of chemical functional groups in complex biological matrices (Stuart, 2004). In medicinal plant research,

FTIR has been successfully employed for the identification and quantification of bioactive compounds within plant extracts (Cicco *et al*., 2012). In the present study, we leverage FTIR to analyze the alkaloid content in distinct anatomical parts of RV, specifically the leaves and stems.

This study focuses on the application of FTIR spectroscopy to analyze the alkaloid content in different anatomical parts of RV, specifically the leaves and stems. Alkaloids, known for their diverse pharmacological activities, including anti-hypertensive, antiarrhythmic, and antipsychotic effects, make RV a promising candidate for further exploration in drug development (Izzo and Capasso, 2001; El-Readi *et al*., 2012). By employing FTIR as a sensitive and rapid analytical technique, we aim to provide a comprehensive characterization of alkaloids present in RV leaves and stems, laying the groundwork for understanding the plant's potential medicinal applications.

This investigation aligns with the global interest in natural products and traditional remedies as sources of novel therapeutic agents (Newman and Cragg, 2016). By contributing to the body of knowledge on the chemical composition of RV, this study seeks to bridge traditional medicinal knowledge with contemporary analytical methodologies. The resulting insights are expected to foster a more comprehensive understanding of RV's pharmacological potential and, consequently, contribute to the ongoing global efforts in drug discovery and development for improved healthcare.

MATERIALS AND METHODS

Sample Preparation:

The harvested leaves and stems of RV were air-dried indoors at room temperature. After desiccation, the leaves and stems underwent coarse grinding. The processed materials were then separated, with leaves and stems each placed in distinct amber bottles.

Maceration:

The plant matter was exposed to a freshly prepared 80% ethanol solution for 48 hours. After maceration, the resultant mixture underwent filtration using high-quality Whatman filter paper. The ethanol extracts were collected, stored in air-tight containers, and kept in the freezer for subsequent analysis. The residues obtained from the filtered ethanol extract underwent a secondary extraction using ammoniacal ethanol (2% ammonia). This process occurred over three days, with filtration at 24-hour intervals using Whatman filter paper. The resulting filtrate underwent concentration using a rotary evaporator at 40-45°C under pressure. The concentrated extracts were collected and stored in hermetically sealed containers in the freezer for further analysis.

Combining Extracts and Acidification:

The individual extracts were combined and mixed. The resulting solution underwent acidification using 0.1N hydrochloric acid to lower its pH. The acidified solution was then filtered to eliminate solid impurities or insoluble materials, ensuring the purity of the final product.

Extraction with Chloroform:

Weakly Basic Fraction: The acidified solution was subjected to extraction with three 500 mL portions of chloroform. The chloroform layers were collected, merged,

and then dried at 45°C, resulting in the isolation of a weakly basic fraction.

Intermediate Basic Fraction: The residual aqueous layer, containing compounds not extracted in the weakly basic fraction, was made alkaline (pH adjusted to 9 using ammonia). This alkaline solution underwent extraction with chloroform, and the collected layers were combined and dried at 45°C, isolating an intermediate basic fraction.

Strongly Basic Fraction: The remaining aqueous layer was further rendered strongly alkaline (pH raised to 11 using a combination of ammonia and sodium hydroxide solutions). The resulting strongly basic solution underwent another round of extraction with chloroform. The collected layers were combined and dried at 45°C, isolating the strongly basic fraction.

Thin-Layer Chromatography (TLC):

The isolated basic fractions were analyzed using thin-layer chromatography (TLC) on 20 x 20 cm silica-coated aluminum plates, cut into 10 cm x 5 cm sizes. Various solvent systems were employed for TLC optimization. Samples were applied to the TLC plates and run using different solvent systems. Plate visualization was performed using UV light at 254 nm and 365 nm. Additionally, Dragendorff's reagent was applied to determine the presence of alkaloids.

Fourier Transform Infrared Spectrometer (FT-IR) Analysis:
Fractions identified through spot testing with Dragendorff's reagent after being sprayed on the TLC plate were named A (for Leaves) and B (for Stem), and underwent further analysis. They were subjected to FT-IR (Fourier transform-infrared) spectroscopy to identify the alkaloids present in the fraction. This analytical technique provides insights into the molecular composition of the substance, aiding in the precise identification of the alkaloids within Fractions.

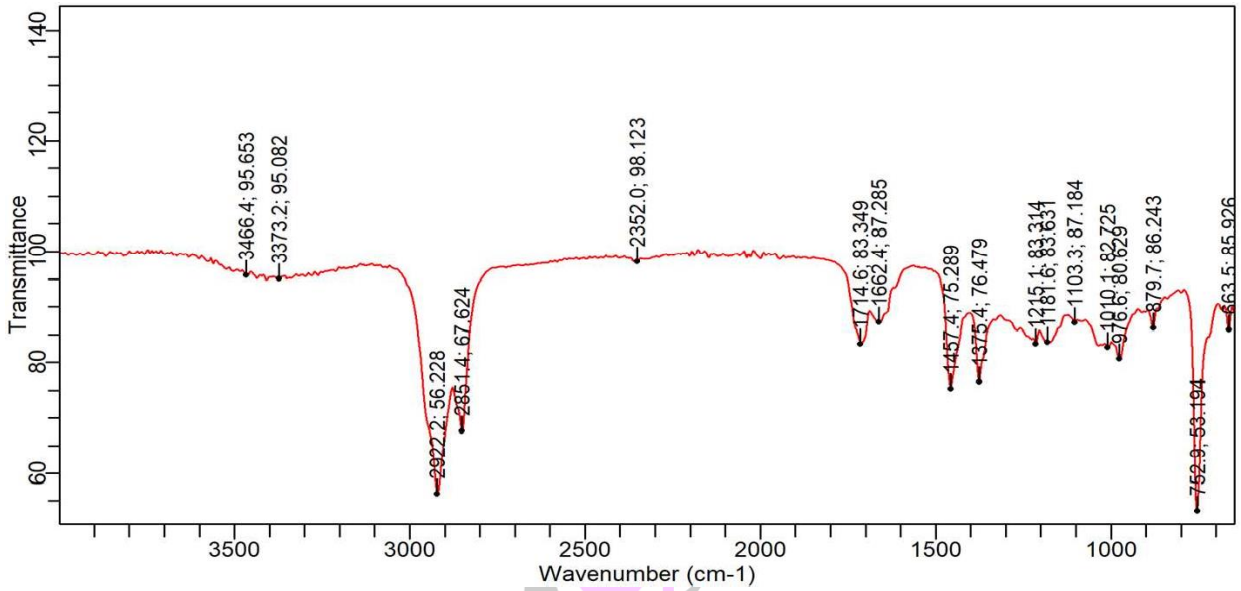
FOURIER TRANSFORM INFRARED SPECTROMETER (FT-IR) ANALYSIS

The Infrared spectra were recorded on an Agilent Cary 630 ATR-FT-IR analyzer in diamond crystals. The 0.01mg of the RV leaves and Stem 2% ethanol extracts were placed onto the sampling surface of the Agilent Cary 630 ATR-FT-IR analyzer. The samples were pressed against diamond crystal using the attached pressure clamp. A clutch on the clamp allows us not to overtight the samples against the sampling surface. Spectrum was acquired in between 30-45 seconds, using a wave number range from 4000 – 650 cm-1 using 32 scans.

RESULTS:

A. FTIR PROFILING OF ALKALOIDS IN *RAUWOLFIA VOMITORIA* (RV) LEAVES. REVEALING MOLECULAR SIGNATURES FOR MEDICINAL INSIGHT

Figure 1:



FTIR Spectral Peaks: Alkaloid Composition in *Rauwolfia vomitoria* (RV) Leaves)

Table 1: Functional groups of the FTIR spectral peaks of Alkaloid Composition in *Rauwolfia vomitoria* (RV) Leaves

Peak No	Wave No (Cm^{-3})	Intensity	Frequency Range (Cm^{-1})	Functional Groups
1	663.5	85.93	600-700	Alkyl halides, Halogen compounds, Alkane
2	752.9	53.19	750-850	Bending vibrations of C-H bonds are often found in alkyl halides, ethers,
3	879.7	86.24	880-950	Bending vibrations of C-H bonds (Alkyl or aryl groups)
4	976.6	80.63	980-1000	Ending vibrations of C-H bonds.
5	1010.1	82.73	1000-1050	C-N stretching vibrations are commonly found in Amines.
6	1103.3	87.18	1100-1150	C-O stretching vibrations in alcohols and ethers.
7	1181.6	83.63	1180-1250	C-N stretching vibrations in amines.
8	1215.1	83.31	1200-1350	C-O stretching in alcohols, C-C stretching in alkenes, and N-H bending in amines.
9	1375.4	76.29	1380-1400	C-H bending in methyl groups and C-H deformation in aromatic rings.
10	1457.4	75.29	1450-1600	C=O stretching in carbonyl compounds, C=C stretching in alkenes, and N-H stretching in amides and amines.

11	1662.4	87.29	1650-1700	The C=O stretching region typically corresponds to carbonyl compounds like ketones, aldehydes, and carboxylic acids
12	1714.6	83.35	1700-2300	C=O stretching in carbonyl compounds, C-N stretching in nitriles, and N-H stretching in amines.
13	2352.0	98.12	2350-2500	Isocyanates (N=C=O)
14	2851.4	67.62	2850-2900	C-H stretching vibrations in various organic compounds.
15	2922.2	56.23	2900-3200	CH stretching vibrations, often in alkanes and alkyl groups.
16	3373.2	95.08	3880-3950	Hydroxyl groups (alcohols and phenols).
17	3466.4	95.65	3980-3500	N-H stretching vibrations, particularly in amines.

B. FTIR PROFILING OF ALKALOIDS IN *RAUWOLFIA VOMITORIA* (RV) LEAVES. REVEALING MOLECULAR SIGNATURES FOR MEDICINAL INSIGHT

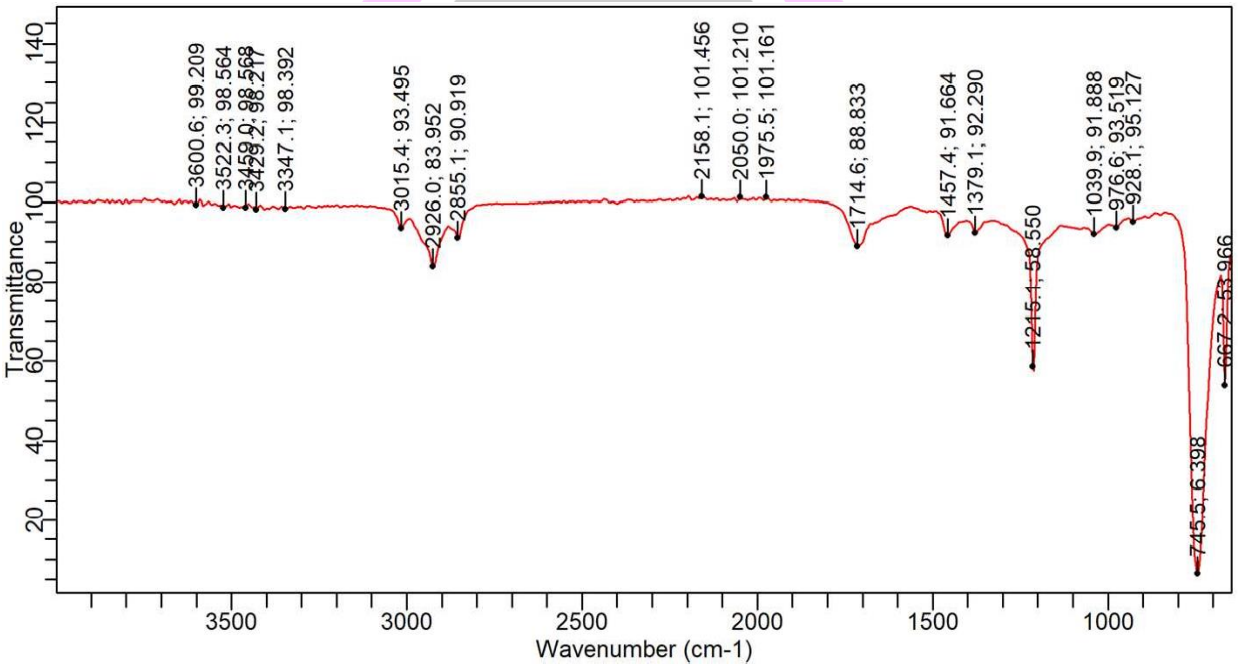


Figure 2: FTIR Spectral Peaks: Alkaloid Composition in *Rauwolfia vomitoria* (RV) Stems

Table 2: Functional groups of the FTIR spectral peaks of Alkaloid Composition in *Rauwolfia vomitoria* (RV) Stems

Peak No	Wave No (Cm^{-3})	Intensity	Frequency Range (Cm^{-1})	Functional Groups

1	667.2	53.97	600-650	**Alkynes (C≡C), A**romatic compounds
2	745.5	6.39	750-850	Alkyl halides and Ethers
3	928.1	95.13	910-950	Alkyl or aryl groups.
4	976.6	93.512	980-1000	Amines.
5	1039.9	91.89	1000-1100	Stretching vibrations of C-N bonds (Amines)
6	1215.1	58.55	1200-1350	C-O stretching vibrations in alcohols, C-C stretching vibrations in alkenes, and N-H bending vibrations in amines.
7	1379.1	92.29	1380-1400	C-H bending in methyl groups and C-H deformation in aromatic rings.
8	1457.4	91.66	1450-1650	Stretching vibrations of C=O bonds in carbonyl groups, C=C stretching vibrations in alkenes, and N-H stretching vibrations in amides and amines.
9	1714.6	88.83	1700- 1900	Ketones, aldehydes, and carboxylic acids.
10	1975;5	101.16	1980-2000	Triple bonds like C≡N or C≡C.
11	2050.0	101.21	2050-2100	Carbon-nitrogen triple bonds (C≡N).
12	2158.1	101.46	2150-2200	Alkynes.
13	2855.1	90.92	2850-2900	Stretching vibrations of C-H bonds
14	2926.0	83.95	2910-2950	Alkanes
15	3015.4	93.49	3000-3200	O-H bonds in alcohols and N-H bonds in amines.
16	3347.1	98.39	3350-3400	Alcohols and phenols.
17	3429.2	98.22	3420-3400	Primary and secondary amines.
18	3459.0	98.57	3450-3500	Amines
19	3522.3	98.56	3510-3550	Primary amines.
20	3600.6	99.21	3600-3800	Hydroxyl groups (OH), such as alcohols and phenols.

DISCUSSION:

FRACTION (RV LEAVES): C-N Stretching Vibrations (1000-1050 cm^{-1}): Amines containing C-N bonds can be found in various medications. Therapeutic applications include analgesics, antidepressants, and antihistamines (Brunton, et al., 2010). C-O Stretching Vibrations (1100-1150 cm^{-1}): Compounds with C-O bonds, such as alcohols and ethers, are used in anesthetics and cough suppressants (Katzung, et al., 2021). C-O Stretching Vibrations (1200-1350 cm^{-1}): These are common in alcohols, ethers, and alkenes, therapeutic applications include antiseptics, disinfectants, and muscle relaxants (Hardman et al., 2001). C=O

Stretching Vibrations (1450-1600 cm^{-1} and 1650-1700 cm^{-1}) are compounds with carbonyl groups, such as ketones and aldehydes, which are used in analgesics, antipyretics, and anti-inflammatory drugs (Katzung, et al., 2018). C-N Stretching Vibrations (1700-2300 cm^{-1}) include nitriles, amides, and various other compounds. Therapeutic applications are antifungals, antivirals, and chemotherapeutic agents. C≡N Functional Group (2350-2500 cm^{-1}): Isocyanates (N=C=O) are used in therapeutic applications including the production of polyurethanes (Tiedemann, et al., 2017). CH Stretching Vibrations (2850-2900 cm^{-1} and 2900-3200 cm^{-1}), C-H stretching vibrations are widespread in organic compounds. Therapeutic applications include anesthetics, analgesics, and various medications. OH Stretching (3880-3950 cm^{-1}) includes hydroxyl groups found in alcohols and phenols. Therapeutic applications include antipyretics, analgesics, and antiseptics. N-H Stretching (3980-3500 cm^{-1}) contains N-H stretching vibrations. Therapeutic applications include muscle relaxants and local anesthetics.

FRACTION B (RV STEM): Amines (1000-1100 cm^{-1}): Amines are organic compounds containing a nitrogen atom with a lone pair of electrons. They can be found in various drugs, including: Analgesics: Some pain relievers contain amine functional groups, such as morphine and codeine, Antidepressants: Many antidepressant medications are based on amine compounds, including selective serotonin reuptake inhibitors (SSRIs), Anti-histamines: These compounds are used to treat allergies and allergic reactions (Cipriani et al., 2018). Carbonyl Compounds (1450-1650 cm^{-1}), Carbonyl compounds contain the C=O functional group and are widely used in medicinal chemistry: Ketones and Aldehydes: These compounds are present in various drugs and play roles in metabolic pathways, Antibiotics: Some antibiotics, like penicillin and cephalosporins, contain carbonyl groups, Steroids: Steroids have carbonyl groups and are used as anti-inflammatory and immunosuppressant agents (Graham, et al., 2005). Hydroxyl Groups (3350-3400 cm^{-1}): The hydroxyl functional group (OH) is found in alcohols and phenols, and compounds containing OH groups have therapeutic uses: Antipyretics and Analgesics: Some pain relievers, such as acetaminophen, contain hydroxyl groups, Antiseptics, and Disinfectants: Phenol-based compounds are used as antiseptics and disinfectants (Li, et al., 2010). Amines (3420-3450 cm^{-1}): This region often corresponds to N-H stretching vibrations in amines: Muscle Relaxants: Some muscle relaxant drugs, like dantrolene, contain amine groups, Local Anesthetics: Local anesthetics, like lidocaine, may contain amine functional groups. Carboxylic acids (1700-1900 cm^{-1}): The C=O stretching region is often associated with ketones, aldehydes, and carboxylic acids: Anti-inflammatory drugs, Nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and naproxen, contain carbonyl groups, Antipyretics: Paracetamol (acetaminophen) contains a carbonyl group and is used as a fever reducer (Brune and Patrignani, 2015). Triple Bonds (1980-2100 cm^{-1}): Compounds with triple bonds (C≡C and C≡N) may have therapeutic applications:

Anti-Viral Drugs: Some antiviral medications contain triple bonds, such as nucleoside analogs like acyclovir, Chemotherapy Agents: Alkynes and nitriles with triple bonds are sometimes used in chemotherapy.

CONCLUSION:

The FTIR analysis of *Rauwolfia vomitoria* (RV) leaves and stems uncovered a diverse spectrum of functional groups indicative of potential therapeutic applications. Amines, carbonyl compounds, nitriles, and isocyanates present in both leaves and stems suggest a broad pharmacological profile encompassing analgesics, antidepressants, and anti-inflammatory drugs. The identified C-N stretching vibrations, C-O stretching vibrations, C=O stretching vibrations, and C≡N functional groups align with known compounds in these medicinal categories. These comprehensive insights into RV's molecular composition lay the groundwork for further exploration of its pharmaceutical potential, emphasizing its significance in drug discovery and development. The intricate chemical landscape revealed by FTIR analysis positions RV as a promising candidate for in-depth pharmacological investigations

RECOMMENDATION:

FTIR analysis of RV leaves and stems identified a diverse range of therapeutic functional groups. Recommendations include further compound identification, quantitative analysis, biological activity assessments, safety evaluations, and collaboration for a comprehensive understanding and potential pharmaceutical applications.

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