



IDENTIFICATION OF PURIFIED PHYTOCHEMICAL COMPOUND AND THEIR ASSOCIATED MEDICINAL PROPERTIES IN *GREWIA HIRSUTA VAHL* LEAF EXTRACT USING UV-VISIBLE, IR AND NMR SPECTROSCOPY

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Abstract: *Grewia hirsuta*, known as nagbala, Glory Lillie, In traditional Ayurvedic medicine, *Grewia hirsuta* Vahl. is esteemed as one of the Nagbala species, known locally as Gangeruki. The presence of flavonoids and phenolic compounds in *Grewia hirsuta* contributes significantly to its antioxidant capacity, protecting cells from oxidative damage and potentially reducing the risk of chronic diseases. We are used spectroscopic technique for phytochemical identification such as, UV-Vis spectrometry is especially valuable for detecting chromophores, molecular functional groups that absorb UV-Vis light such as conjugated double bonds and aromatic rings. FTIR stands for chemical bonds, or functional groups, present within compounds. NMR spectroscopy is a prominent analytical technique in chemistry, extensively applied for characterizing and elucidating structures of phytochemicals. Hydroquinone 2,5-di-tert-butyl-has good therapeutic potential like anti-oxidant property identified Phenolic compound found in *Grewia hirsuta* ethanolic leaf extract, used as stabilizer in various industries including food, cosmetics and pharmaceuticals. Further research proposed for better understanding of medicinal plant and there therapeutic properties.

Keywords: *Grewia hirsuta*, phytochemicals, UV-VISIBLE, IR, NMR, medicinal properties spectroscopy

1. Introduction- *Grewia hirsuta*, commonly known as the Hairy-leaved Cross berry, is a plant species belonging to the family Malvaceae. *Grewia* is a genus that is well-known for its broad range of botanical diversity. *Grewia hirsuta* is symbolic in certain cultures' ceremonies and rituals. It can represent fertility, prosperity, or protection and be used in spiritual practices, rituals associated with birth, marriage, or other life events. According to Ayurveda, the ancient Indian medical treatise, *Grewia* fruits are regarded as cooling tonics and aphrodisiacs, offering relief from thirst, burning sensations, biliousness, inflammation, as well as heart and blood disorders, including fevers (Khan *et al.*, 2019). The medicinal attributes of plants from their secondary metabolites, encompassing essential oils, glycosides, vitamins, alkaloids, and other active constituents (Sen and Batra, 2012). It is now understood that plants capable of naturally synthesizing and accumulating secondary metabolites such as alkaloids, glycosides, tannins, volatile oils, as well as containing minerals and vitamins, possess medicinal properties (Costa *et al.*, 2012). UV-Visible (UV-Vis) spectrometry is a powerful analytical technique used to assess the absorbance or transmittance of light within the ultraviolet (UV) and visible (Vis) regions of the electromagnetic spectrum. This method plays a crucial role in chemistry, biology, and material sciences, enabling both qualitative and quantitative analysis of various substances. It operates on the principle of electromagnetic radiation interacting with matter, specifically by promoting electrons in molecules from lower to higher energy states. Widely utilized in pharmaceutical analysis, environmental monitoring, food quality control, and biochemical research. FTIR stands out as a highly effective tool for discerning the types of chemical bonds, or functional groups, present within compounds (Ashok kumar and M. Ramaswamy, 2014). This is achieved by analyzing the characteristic wavelengths of light absorbed, as evidenced in the annotated spectrum. Through interpretation of the infrared absorption spectrum, the chemical bonds within a molecule can be identified (Singh *et al.*, 2014). NMR spectroscopy is a prominent analytical technique in chemistry, extensively applied for characterizing and elucidating structures in natural products. Its spectrum provides abundant precise qualitative and quantitative data, capable of distinguishing individual components within a mixture. NMR boasts additional appealing benefits, including straightforward sample preparation, non-invasive/non-destructive measurements, the capability to detect and quantify numerous chemical compounds simultaneously, and excellent reproducibility (Zhao *et al.*, 2022). Extracts from *G. hirsuta* have shown antimicrobial activity against various pathogens, supporting its traditional use in treating infections additionally; the plant's fruits are consumed fresh or processed into jams and beverages, contributing to local diets and nutrition (Qamar *et al.*, 2021).

2. Materials and Methods

2.1 Collection and Preparation of plant material

The leaves of *Grewia hirsuta* were collected from Jabalpur district, central India. Collected leaves were washed; shade dried and powdered using mechanical blender. The powder was stored in air tight plastic container for the future evaluation with proper labelling.

2.2 Preparation of plant extract

The dried plant parts were powdered (coarse) and finely ground dry plant samples were weighed and kept in a muslin cloth. dry extract were placed in a chamber of the soxhlet apparatus. Soxhlet apparatus using Ethanol (60 to 80°C) was added in the flask and heated and its vapours condense in the condenser. The condensed solvent containing the plant extract was collected, around 72 hours or till all the extract has been extracted from the plant (Handa *et. al.*, 2008). Extract was filtered, dried and weighed for future.

3. Methodology

Determination of the structure of certain molecules uses data from a wide range of spectroscopic techniques such as UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy. The basic principle of spectroscopy is passing electromagnetic radiation through an organic molecule that absorbs some of the radiation, but not all. By measuring the amount of absorption of electromagnetic radiation, a spectrum can be produced. The spectra are specific to certain bonds in a molecule. Depending on these spectra, the structure of the organic molecule can be identified. Scientists mainly use spectra produced from either three or four regions-Ultraviolet (UV), Visible, Infrared (IR), radio frequency, and electron beam for structural clarification.

UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy. This technique is not time-consuming, and presents reduced cost compared to other techniques.

By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of different solvent extracts of each plant materials was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} .

NMR spectroscopy has enabled many researchers to study molecules by recording the differences between the various magnetic nuclei, and thereby giving a clear picture of what the positions of these nuclei are in the molecule. Moreover, were demonstrated which atoms are present in neighboring groups.

4. Result and Discussion

Table 4.1.1: Qualitative Phytochemical analysis of Ethanolic leaf extract of *Grewia hirsuta* Vahl.

S. NO.	TEST NAME	RESULTS
1. Test for Carbohydrate		
	Molish test:	-ve
2. Test for Protein		
	Biuret test:	+ve
3. Test for Glycoside		
	Test for deoxysugar (Keller Killiani test):	-ve
4. Test for Flavonoids		
	Lead acetate test:	+ve
5. Test for Alkaloids		
	a. Mayer's Test: b. Wagner's test:	+ve
6. Test for Phenolic compound		
	Ferric Chloride Test:	+ve
7. Test for Organic compound		
	Oxalic acid: Malic acid:	+ve
8. Test for Inorganic compound		
	Sulphate Test: Chloride Test:	+ve
9. Test for steroid		
	Salkowski reaction:	-ve

4.1. SPECTROSCOPIC ANALYSIS OF ETHANOLIC LEAF EXTRACT OF *GREWIA HIRSUTA* VAHL

4.1.1 UV-Visible Spectroscopy

1. Wavelength and Absorbance

- **235.00 nm:** This is the wavelength at which a significant peak was observed in the UV-Vis spectrum.
- **Absorbance (A = 2.266):** The absorbance value indicating strong absorption of light by the sample at this wavelength. This suggests the presence of a compound or chromophore in the sample that strongly interacts with UV light around 235 nm.

2. Peak Detection Method

Peak Pick Method: The method used to identify peaks is "Find 8 Peaks Above -3.0000 A."

Threshold: The software was set to detect peaks with absorbance above a specified threshold (-3.0000 A). Since absorbance values are typically positive, this indicates a broad range was considered to ensure no peaks were missed.

3. Scan Range

Start Lambda (200.0 nm) to Stop Lambda (600.0 nm): The scan covered the UV-visible range between **200 nm** and **600 nm**, which is a standard range for analyzing compounds that absorb UV or visible light.

Sorting: Peaks were sorted by wavelength, which are the default and most logical arrangement for UV-Vis data interpretation.

- The **peak at 235 nm with absorbance 2.266** strongly suggests the presence of a substance with a specific chromophore. Possible contributors to such absorption include:
 - **Conjugated Systems:** Compounds with extended conjugated π -electron systems absorb in the UV region.
 - **Aromatic Compounds:** Structures like benzene or its derivatives, which have delocalized electrons, absorb strongly in this range.
 - **Functional Groups:** Groups such as carbonyls, nitro, or unsaturated systems may show significant absorbance here.

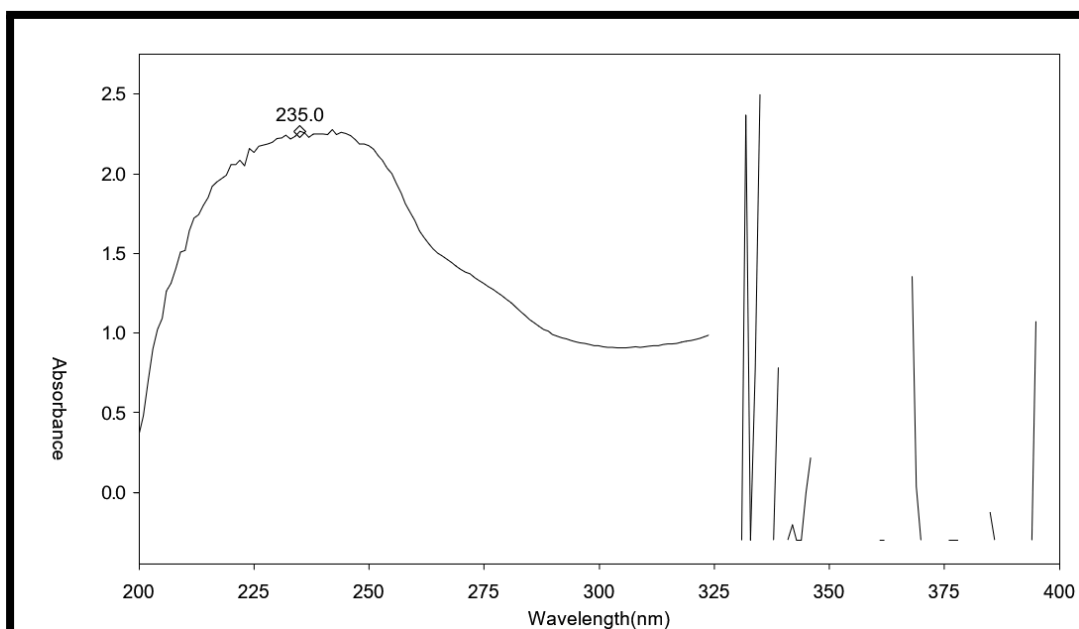


Figure 4.1.1: UV-Visible spectrum of *Grewia hirsuta* Vahl.

4.1.2 Fourier Transform Infrared (FTIR) Spectroscopy

Functional Group Analysis- Based on the spectral features:

- **Hydroxyl (O–H) or Amine (N–H) group** is present (broad peak around 3300 cm^{-1}).
- **Carbonyl (C=O) group** at 1700 cm^{-1} suggests a ketone, ester, or carboxylic acid.
- **Aromatic or Alkene group** indicated by peaks around $1500\text{--}1600\text{ cm}^{-1}$.
- **C–O stretching** confirms esters, ethers, or carboxylic acid derivatives.

Possible Compound Class- The compound is likely an aromatic ester or carboxylic acid derivative, possibly with hydroxyl or amine functionality.

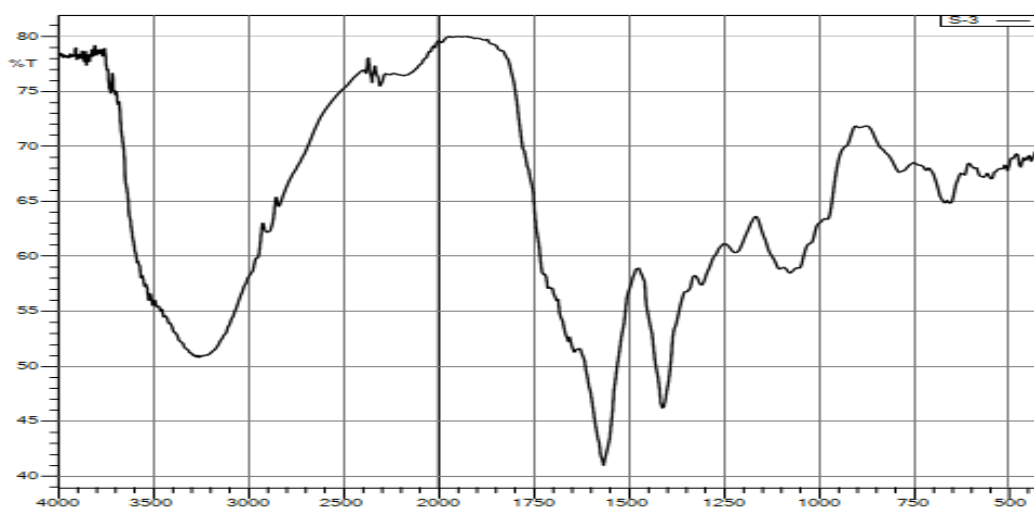


Figure 4.1.2: FTIR spectrum of *Grewia hirsute*

4.1.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

4.1.3.1 ^1H NMR spectrum

Chemical Shift Assignments- The solvent used is **MeOD (deuterated methanol)**, with its residual proton peak typically observed at **3.31 ppm** for ^1H -NMR. Peaks near 3.31 ppm can be attributed to the solvent if not suppressed during the experiment.

The chemical shift values would span from **0–10 ppm**, allowing identification of:

0–3 ppm: Aliphatic protons.

3–5 ppm: Protons near electronegative groups (e.g., hydroxyls, ethers).

6–8 ppm: Aromatic protons.

9–10 ppm: Aldehyde protons.

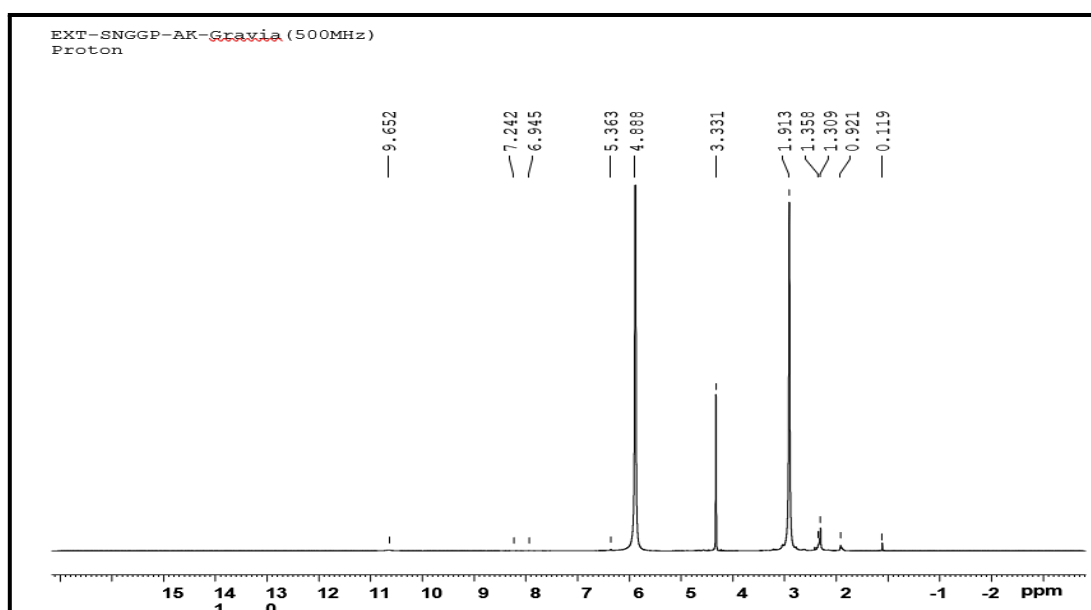


Figure 4.1.3.1: ^1H NMR spectrum of *Grewia hirsuta* Vahl.

4.1.3.2 ^{13}C NMR Spectrum

The ^{13}C NMR spectrum of the compound, recorded at 500 MHz using a Bruker spectrometer, reveals several key peaks that provide valuable information about its structure.

Chemical Shift Assignments- The chemical shift assignments are expected to be referenced to the MeOD solvent peak (typically around 69.0 ppm in ^{13}C -NMR). Without the spectrum, assignments can only be inferred:

- Peaks in the range of **10–50 ppm:** Aliphatic carbons (e.g., methyl and methylene groups).
- Peaks in the range of **100–150 ppm:** Aromatic or olefinic carbons.
- Peaks in the range of **150–220 ppm:** Carbonyl carbons (ketones, aldehydes, esters, or acids).

Integration:

Integration values in ^{13}C -NMR are not quantitative but provide information on relative numbers of equivalent carbons.

- Symmetric molecules may show fewer, more intense peaks due to equivalent carbons.
- Asymmetric molecules would show more peaks with varied intensities.

Splitting Patterns:

- The use of the **waltz 65** decoupling sequence removes ^{13}C - ^1H couplings, resulting in singlets for all ^{13}C peaks.
- If splitting were not decoupled, coupling patterns (e.g., doublets, triplets) would indicate ^1H - ^{13}C connectivity.

Structural Insights:

- The **solvent (MeOD)** suggests the compound might be polar or contain functional groups like hydroxyl, carbonyl, or amines.
- The use of **high scans (NS = 512)** suggests either low ^{13}C abundance or low sample concentration.
- A sweep width (SWH) of 30,120.682 Hz and a spectral width corresponding to 260 ppm indicate coverage of all ^{13}C nuclei resonances.

Functional Groups- Possible functional groups based on chemical shift ranges and solvent choice:

- **Carbonyl groups** (aldehydes, ketones, esters, acids) likely appear downfield (150–220 ppm).
- **Aromatic or sp^2 -carbons** around 100–150 ppm.
- **Aliphatic carbons** appear upfield (10–50 ppm).

Potential Compound- The lack of specific peak values makes precise identification difficult, but it is likely a polar organic compound soluble in MeOD. Potential compounds include:

- Alcohols, esters, or amides.
- Small organic acids or aromatic compounds.

Symmetry:

- Symmetry is determined by the number of peaks relative to the number of carbons in the molecule.
- A symmetric compound (e.g., benzene) would show fewer distinct peaks.
- An asymmetric compound (e.g., a long-chain alkane) would exhibit more peaks.

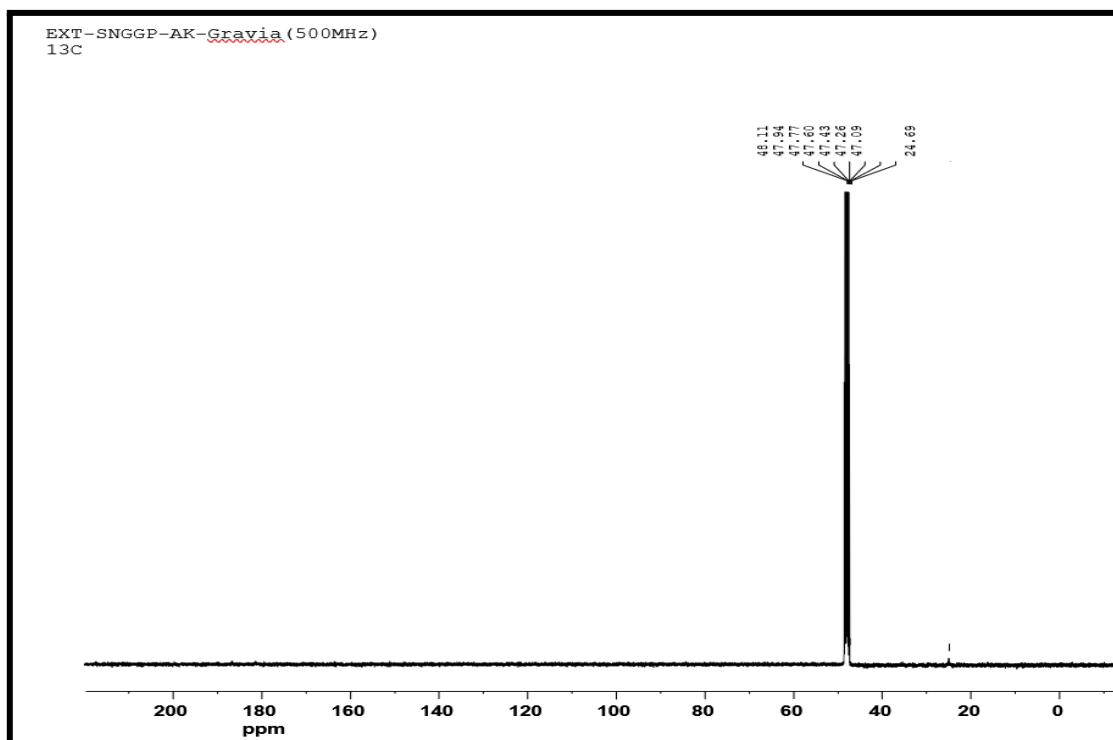


Figure 4.1.3.2: ^{13}C NMR spectrum of *Grewia hirsuta* Vahl.

5. DISCUSSION

5.1 ANALYSIS OF VARIOUS SPECTROSCOPIES

UV- visible spectroscopy, IR spectroscopy, Proton NMR, and ^{13}C NMR spectroscopy of purified compound.

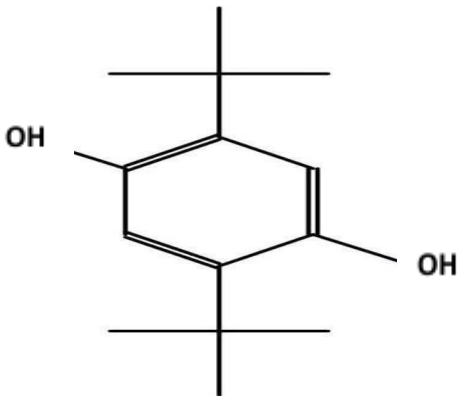
Table 5.1: Analysis of various spectroscopies

S. No	Spectroscopy	Values of Spectrum	Identification
1	UV-visible spectroscopy	235 nm	Double bonds or aromatic rings
2	IR spectroscopy	3300 cm^{-1} 1600 cm^{-1}	Functional group -OH or hydroxyl group Aromatic C=C
3	^1H NMR	6 ppm	Aromatic proton
4	^{13}C NMR	48.11 ppm	$-\text{CH}_2$, $-\text{CH}_3$ aliphatic group

5.2. IDENTIFICATION OF PURIFIED PHYTOCHEMICAL COMPOUND

Following compound were Purified from sample and identified as Hydroquinone 2,5-di-tert-butyl-has good therapeutic potential like anti-oxidant property found in *Grewia hirsuta* ethanolic leaf extract, used as stabilizer in various industries including food, cosmetics and pharmaceuticals .

Table 5.2. Identification of Purified Phytochemical compound

Formula	$C_{14}H_{22}O_2$
IUPAC Name	1,4-Benzenediol,2,5-bis(1,1-dimethylethyl) Or Hydroquinone,2,5-di-tert-butyl-
Molecular Weight	222
Structure	
Pharmacological value	Hydroquinone 2,5-di-tert-butyl-has good therapeutic potential like anti-oxidant property found in <i>Grewia hirsuta</i> ethanolic leaf extract, used as stabilizer in various industries including food, cosmetics and pharmaceuticals

Although *Grewia hirsuta* has been studied less extensively than some other plants, it has long been used in traditional medicine for its wound-healing, antimicrobial, and anti-inflammatory effects. The leaves contain several bioactive compounds, including flavonoids, tannins, and saponins, which contribute to their medicinal value. Traditional healers in different parts of India have employed *Grewia hirsuta* to treat ulcers, wounds, and skin infections, emphasizing its potential as a valuable source of new bioactive compounds. *Grewia hirsuta* represents a versatile plant species with significant botanical, medicinal, and ecological value. Its traditional uses are supported by modern pharmacological research, highlighting its potential in healthcare and sustainable development. Continued exploration of its phytochemical composition and pharmacological activities is crucial for unlocking further therapeutic applications and conservation strategies.

REFERENCES

- Ashokkumar, R., & Ramaswamy, M. (2014). Fourier transform infrared spectroscopy: A tool for phytochemical analysis. *Journal of Chemical and Pharmaceutical Research*, 6(5), 123-129.
- Ashokkumar, R., & Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. *International Journal of Current Microbiology and Applied Sciences*, 3(1), 395-406.
- Costa, J. G. M., et al. (2012). Phytochemical and antibacterial investigations of leaves from *Eugenia uniflora*. *International Journal of Current Microbiology and Applied Sciences*, 1(1), 1–7.
- Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction Technologies for Medicinal and Aromatic Plants. *International Centre for Science and High Technology*, United Nations Industrial Development Organization, Trieste, Italy.
- Khan, M. N., et al. (2019). Medicinal uses of *Grewia* fruits: Insights from ancient Indian treatises. *Journal of Traditional and Complementary Medicine*, 9(4), 300–307.
- Khan, R. U., Mehmood, S., & Khan, S. U. (2019). Phytochemical and nutritional evaluation of *Grewia asiatica* fruit. *Pakistan Journal of Pharmaceutical Sciences*, 32(4), 1675-1682.
- Mishra, A., Verma, R., & Tiwari, P. (2020). Traditional uses and pharmacological potential of *Grewia hirsuta*: A review. *Indian Journal of Ethnopharmacology*, 18(3), 150-165.
- Qamar, U., Ahmad, R., & Khan, S. (2021). Nutritional contributions of *Grewia* species fruits: Fresh consumption and processed products. *Plant Foods for Human Nutrition*, 76(4), 512-521.
- Sen, S., & Batra, A. (2012). The study of traditional Indian medicine and its benefits. *Current Pharmacology Reports*, 6(3), 155–162.
- Singh, B., Sharma, R. A., & Vyas, G. (2014). FTIR spectroscopy in phytochemical analysis of medicinal plants. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 132, 345-351.
- Sharma, S., & Gupta, R. (2017). Ethnobotanical relevance and medicinal applications of *Grewia hirsuta* in Indian traditional medicine. *Journal of Herbal Medicine*, 10(4), 120-132.
- Zhao, J., Wang, M., Saroja, S. G., & Khan, I. A. (2022). NMR technique and methodology in botanical health product analysis and quality control. *Journal of Pharmaceutical and Biomedical Analysis*, 207, 114376.
- Zhao, L., Zhang, H., & Wang, X. (2022). NMR spectroscopy in natural product research: Advances and applications. *Natural Products and Bioprospecting*, 12(1), 34-45.