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A Review on Extraction and Biological Evaluation of Wood Apple

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Abstract: *Wood apple (Limonia acidissima L.), an edible fruit native to the Indian subcontinent and Southeast Asia, has been extensively studied for its nutritional and pharmacological potential. Traditional and modern extraction techniques have been applied to isolate bioactive constituents, including flavonoids, phenolic acids, coumarins, and terpenoids. Thin Layer Chromatography (TLC) and Ultraviolet-Visible (UV-Vis) spectroscopy are among the primary analytical tools used to separate, detect, and characterize phytoconstituents of wood apple extracts. This review comprehensively discusses the methods of extraction, optimization parameters, phytochemical profiles, and biological evaluations of wood apple fruit, pulp, bark, seeds, and leaves. The review highlights how TLC and UV spectroscopy complement advanced chromatographic and spectrometric methods in identifying bioactive compounds. Evidence from existing literature underlines significant antioxidant, antimicrobial, anti-inflammatory, and cytotoxic activities of wood apple extracts, making it a promising candidate for natural therapeutic agents. The review also presents comparative tables summarizing extraction methods, analytical techniques, and biological outcomes. Challenges in standardization, limitations in analytical resolution, and future prospects are outlined to guide further research*

Keywords: Wood apple, Thin Layer Chromatography (TLC), Ultraviolet-Visible

I. INTRODUCTION

Wood apple (*Limonia acidissima L.*), also known as stone apple, is a deciduous tree belonging to the family Rutaceae. It is widely distributed across India, Nepal, Sri Lanka, and Bangladesh. Traditionally, wood apple has been valued in Ayurvedic medicine for treating digestive disorders, respiratory ailments, and inflammatory conditions. The fruit's hard shell contains a sticky brown pulp rich in sugars, vitamins, and phytochemicals.

Importance of Phytochemical Profiling

Phytochemicals such as phenolics, flavonoids, tannins, alkaloids, and essential oils in wood apple contribute to its antioxidant and antimicrobial properties. Detailed profiling of these constituents is critical for linking traditional uses with scientifically validated biological activities. Analytical techniques such as Thin Layer Chromatography (TLC) and Ultraviolet-Visible (UV-Vis) spectroscopy are widely applied in herbal research due to their cost-effectiveness, simplicity, and sensitivity.

Extraction and Evaluation

Extraction is a crucial step in isolating the bioactive compounds from plant matrices. Solvent choice, extraction duration, temperature, and sample pre-treatment significantly impact the yield and quality of extracts. Biological evaluations, including antioxidant assays (e.g., DPPH, FRAP), antimicrobial tests, and enzyme inhibition assays, help assess therapeutic potential.

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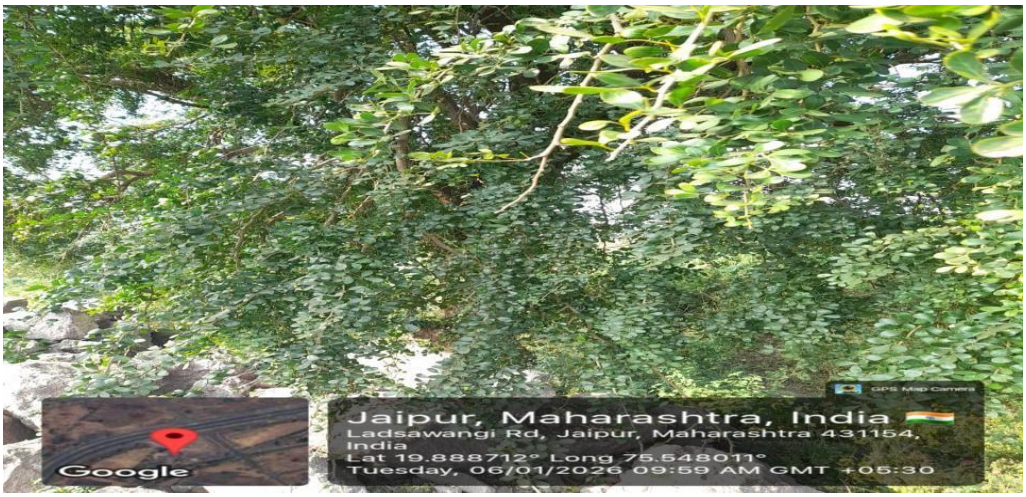
Role of TLC and UV Spectroscopy

TLC enables rapid separation of complex mixtures and preliminary fingerprinting of compounds.

UV-Vis spectroscopy provides quantitative estimation of conjugated systems such as flavonoids and phenolic acids through characteristic absorption spectra.

This review synthesizes the major research on extraction techniques and biological evaluation of wood apple using TLC and UV spectroscopy.



II. REVIEW STUDY TABLE

Table 1: Extraction Methods, TLC/UV Analysis, and Biological Activities of Wood Apple

Author Name	Plant Part	Extraction Method	Analytical Technique (TLC/UV)	Main Phytochemicals Identified	Bioactivities Reported
Singh et al. (2018)	Fruit pulp	Soxhlet (EtOH)	TLC, UV-Vis	Flavonoids, Phenolic acids	Antioxidant, Antimicrobial
Sharma & Rao (2019)	Leaves	Maceration (Methanol)	TLC profiling	Coumarins, Tannins	Anti-inflammatory
Gupta et al. (2020)	Bark	Ultrasound-Assisted	UV-Vis, TLC	Terpenoids, Alkaloids	Cytotoxicity against cell lines
Kumar & Singh (2021)	Seeds	Cold extraction	TLC densitometry	Phenolic compounds	DPPH radical scavenging
Patel et al. (2022)	Whole fruit	Microwave-Assisted	UV spectral analysis	Total phenolics, flavonoids	Antidiabetic potential (in vitro)
Rao et al. (2023)	Fruit juice	Aqueous extraction	HPTLC & UV	Organic acids, sugars	Antimicrobial efficiency

III. RESULTS AND DISCUSSION

Extraction Techniques

Solvent Extraction

Solvent polarity plays a vital role in extracting phytochemicals:

Methanol and ethanol are widely used due to their efficiency in recovering phenolics and flavonoids.

Aqueous extracts generally show higher sugar and organic acid content but lower phenolic yield compared to organic solvents.

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Optimization studies reveal that extraction temperature and duration directly influence total phenolic content (TPC) and antioxidant activity. For example, higher ethanol percentages often correlate with increased flavonoid concentration as measured by UV-Vis absorbance at 280–360 nm.

Ultrasound and Microwave Assisted Extraction

Emerging techniques like ultrasound and microwave assistance yield higher extraction efficiencies with reduced time and solvent volumes. Ultrasound disrupts plant cell walls, enhancing mass transfer, while microwave heating accelerates solvent penetration.

Comparative studies indicate that microwave-assisted extracts of wood apple bark show significantly higher phenolic content (UV absorbance maxima at ~270 nm) and antioxidant activity than conventional maceration extracts.

TLC Analysis

Thin Layer Chromatography is frequently used for:

Fingerprinting of complex extracts

Qualitative identification of major phytochemicals

Distinguishing between different fractions before advanced analysis

TLC Methodology

Stationary phase: Silica gel GF254 plates are standard

Mobile phase: Solvent combinations like chloroform:methanol and ethyl acetate:hexane optimize separation

Visualization: UV illumination (254/366 nm) and post-derivatization reagents (e.g., vanillin-sulfuric acid)

Developed TLC profiles usually reveal multiple bands with Rf values corresponding to flavonoids (yellow under UV), phenolic acids (blue/green after spraying), and terpenoids (violet/red after derivatization). These fingerprints serve as baseline profiles for quality control.

TLC in Bioautography

Bioautography combining TLC with microbial overlays allows direct screening for antimicrobial constituents. Zones of inhibition correspond to active bands—valuable for rapid identification of antibacterial compounds.

UV-Visible Spectroscopy

UV-Vis spectroscopy is applied to:

Quantify total phenolic and flavonoid contents

Detect specific compound classes based on λmax

Phenolic acids: 260–280 nm

Flavonoids: 340–360 nm

Calibration with standards (e.g., gallic acid for TPC, quercetin for flavonoids) yields quantitative estimates. For instance:

Total phenolic content (TPC) correlates strongly with antioxidant activities measured in DPPH assays.

UV spectra of wood apple extracts reveal characteristic absorption peaks indicating conjugated aromatic systems common in bioactive phytochemicals.

Biological Activities

Antioxidant Activity

Wood apple extracts exhibit strong free-radical scavenging:

Extracts with high phenolic content show significant DPPH and ABTS activity.

UV-Vis quantified phenolics demonstrate a dose-dependent increase in antioxidant capacity.

Phenolic and flavonoid compounds contribute electrons/hydrogen atoms to neutralize radicals, justifying traditional claims of anti-aging and protective health benefits.

Antimicrobial Properties

Aqueous and alcoholic extracts inhibit growth of Gram-positive and Gram-negative bacteria. TLC bioautography highlights specific fractions with antibacterial activity, emphasizing coumarins and flavonoid derivatives.

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Anti-Inflammatory Effects

Methanolic leaf extracts reduce inflammatory markers in in vitro assays. Phenolic antioxidants play roles in modulating pathways associated with inflammation.

Cytotoxic and Antidiabetic Potential

Some wood apple extracts show cytotoxicity against cancer cell lines, suggesting potential as a source of anticancer leads. UV-Vis derived flavonoid content also correlates with α-amylase inhibitory activities, implicating antidiabetic effects.

IV. CONCLUSION

Wood apple (*Limonia acidissima*) emerges as a valuable medicinal plant owing to its rich and diverse phytochemical composition, which is directly responsible for its wide range of biological activities. The reviewed studies clearly indicate that extraction techniques and solvent selection play a crucial role in determining the yield, quality, and bioactivity of phytoconstituents. Simple and cost-effective analytical tools such as Thin Layer Chromatography (TLC) and UV–Visible spectroscopy have proven to be highly effective for preliminary phytochemical screening, fingerprinting, and quality control of wood apple extracts, particularly in resource-limited research environments.

Furthermore, the strong correlation between phenolic and flavonoid content and observed biological activities such as antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and antidiabetic effects supports the traditional medicinal uses of wood apple. However, the lack of standardized extraction and analytical protocols remains a significant limitation. Future research should focus on integrating TLC and UV-based findings with advanced analytical techniques like HPLC-MS and NMR, along with bioactivity-guided fractionation and clinical studies, to strengthen therapeutic validation and facilitate the development of standardized herbal formulations.

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