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BIOACTIVE CHARACTERIZATION OF JUGLANS REGIA L. BARK: A COMBINED UV-VIS AND FTIR SPECTROSCOPIC APPROACH

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Abstract
Background: English walnut The bark of many plants is phenolic-rich botanical material that has ethnomedicinal significance and is gaining interest as a source of natural antioxidants and antimicrobial phytochemicals. The current study gives a qualitative spectroscopic and phytochemical analysis of J. The uploaded UV-Vis and FTIR spectral files provide the primary data source for the regia bark extract. The objective was to interpret the UV-Vis and FTIR fingerprints using probable bioactive constituents without making use of any quantitative or chromatographic data. The UV-Vis interpretation file, as discussed before, had an absorption band centred at 420 nm. The FTIR data had 3295.6, 2921.5, 1618.0, 1317.1, 1018.0 and 778.7 cm^-1 diagnostic bands. Standard principles of spectroscopic interpretation were used to assign the peaks which were then compared with published phytochemical data on phytoconstituents isolated from Juglans regia and plant phenolic. Results: The 420 nm UV-Vis band aligning with electronic transitions in conjugated chromophoric systems was interpreted as supportive evidence for phenolic and related aromatic constituents that included possible presence of ferulic acid, vanillic acid, coumaric acid, syringic acid and other conjugated metabolites. The bands observed in FTIR are due to the vibrations of bonds such as hydroxyl, aliphatic C-H, aromatic C=C/conjugated carbonyl, phenolic C-O, glycosidic/polysaccharide C-O-C, substituted aromatic ring vibrations (Cyclic) etc. Fingerprinting through combined UV-Vis and FTIR. There exist significant phenolic, flavonoid/tannin like, aromatic and glycosidic constituents in J. Extract of regia bark. Because compound identity requires confirmatory HPLC, LC-MS/MS, GC-MS analyses and, where appropriate, quantitative phytochemical assays these findings are preliminary.

Keywords Juglans regia L.; walnut bark; UV-Vis spectroscopy; FTIR fingerprinting; phenolic compounds; phytochemical evaluation.

INTRODUCTION

Complex mixtures of phenolic acids, flavonoids, tannins, quinones, glycosides, terpenoids and other secondary metabolites are present in medicinal plants in varying proportions. Conducting spectroscopic fingerprinting for preliminary characterization is useful as it immediately, and inexpensively, reveals chromophores and functional groups before any costly targeted chromatographic analyses are done. Both UV-Vis spectroscopy is capable of providing electronic transition information of conjugated systems. At the same time, FTIR spectroscopy deals with vibrational information according to the functional groups and molecular bonding patterns (Aleixandre-Tudo and du Toit, 2018, Coates, 2000, Pavia et al., 2015, Socrates, 2001).

Walnut that is Juglans regia L. is a member of the Juglandaceae family. The walnut is grown for a long time, with edible kernels which are also packed with a number of non-edible plant parts like bark, leaves, green husk, roots, buds and septa. Research on phytochemicals regularly refers to walnut tissue as the source of phenolics,

flavonoids, tannins, naphthoquinones, hydroxybenzoic and hydroxycinnamic acids and related antioxidant compounds (Bhat et al., 2023; Delaviz et al., 2017; Jahanban-Esfahlan et al., 2019; Medic, Jakopic et al., 2021). Through HPLC-MS/MS profiling of walnut's tissues, bark is one of the organs that is said to contain important phenolic and naphthoquinone groups which will support its value for phytochemical analysis.

The Bark of J The significance of regia in ethnopharmacology is high since a number of studies have been done on bark and root bark preparations for their antimicrobial, oral health, anti-inflammatory, and antioxidant-related properties (Wani & Kumar, 2020; Zakavi et al., 2013). Recent analytical studies have intensified this interest with the demonstration of the presence of phenolic constituents in walnut bark including gallic acid, vanillic acid, quercetin, catechin, epicatechin, syringic acid, and juglone in regional materials (Boukettaya et al., 2025; Bourais et al., 2022). These reports support the use of preliminary spectral fingerprints to screen bark extracts before marker-based quantification.

Phenolic compounds are particularly amenable to UV-Vis analysis owing to the electronic transitions of aromatic rings and conjugated pi-electron systems which could be observed in the ultraviolet and visible region. Phenolic acids and flavonoids typically possess absorptions owing to pi to pi* transitions. In addition, in the presence of a carbonyl function or other extensive conjugation, n to pi* transitions can be seen (Aleixandre-Tudo& du Toit, 2018; Pavia et al., 2015). A band in the visible region centered around 420 nm can indicate the presence of colored or highly conjugated phytochemicals. However, such a band by itself without UV-Vis cannot identify whether or not a particular phytochemical is present.

FTIR fingerprinting is complementary as it indicates the presence of hydroxyl, aliphatic, aromatic, carbonyl, C-O, C-O-C and substituted ring vibrations describing major phytochemical classes. The broad bands observed in the range of 3200 to 3400 cm^-1 correspond to hydrogen-bonded hydroxyls of phenolics and alcohols in plant extracts. The bands appearing in the 2850-2950 cm^-1 region arise from the stretching of aliphatic C-H. The bands appearing near 1600 cm^-1 are often due to the stretching of aromatic C=C or conjugated carbonyl systems. The presence of bands in the range of 1300-1000 cm^-1 often indicates phenolic C-O and glycosidic C-O-C stretching (Coates, 2000; Socrates, 2001).

The present manuscript interprets the ways UV-Vis and FTIR data has uploaded for J. Regia bark extract based on credible evidence. The aim is not to assert the conclusive identification of the compounds but to link spectral features to likely classes of bioactive phytochemicals and arrive at a rational analytical pathway for chromatographic confirmation.

MATERIALS AND METHODOLOGY

Study Design

This study was created as experimental qualitative spectroscopic phytochemical evaluation on interpretation of experimental spectral data. The study examined two complementary fingerprints, namely, the UV-Vis absorption behaviour and FTIR vibrational bands..

Source of Plant Material and Extract

The material for the study was J. Regia L. Bark extract. The dried bark of Juglans Regia L was procured from the open market and same was authenticated by NISCAIR Delhi .

Dried J. regia plant material was and powdered using a sterile grinder. A measured quantity of powder was placed in a thimble and extracted with Ethanol as solvent in a Soxhlet apparatus for repeated solvent cycling. The extract was concentrated under reduced pressure using a rotary evaporator and stored in amber containers at 4°C until testing.

UV-Vis

The UV–Visible spectral analysis of the ethanolic bark extract of Juglans regia was carried out using a UV–Visible spectrophotometer. A stock solution of the dried extract was prepared filtered through a 0.45 µm membrane filter before analysis. The absorbance spectrum was recorded in the wavelength range of 200–800 nm using ethanol as the blank

UV-Vis Spectral Analysis

The assessment for UV-Vis was based on the uploaded interpretation file and the graph. Spectral finding documented an absorption band at 420 nm. This band was interpreted in relation of electronic transition in conjugated organic constituents particularly pi to pi* transitions in aromatic phenolic system. The UV-Vis graph could have been used as a visual spectral record; however, the peak area, concentration, molar absorptivity and calibration calculations were not attempted. This is because no absorbance values in numbers and details regarding dilution of samples were provided.

FTIR

FTIR Analysis

Fourier Transform Infrared (FTIR) Spectroscopy
The functional groups present in the ethanolic bark extract of Juglans regia were identified using Fourier Transform Infrared (FTIR) spectroscopy. The dried extract was finely powdered and analyzed using an FTIR spectrophotometer at room temperature. The FTIR spectrum was recorded, and characteristic absorption bands were interpreted to identify the major phytochemical functional groups present in the extract.

FTIR Spectral Analysis
FTIR analysis was conducted using the FTIR graph and raw FTIR result file uploaded. The graph displays six principal bands at the frequencies ranging from 778.7 to 3295.6 cm^-1. The file of raw FTIR result covers mid infrared which is from about 4000 to 650 cm^-1 and it is a transmittance value. The peak assignment was done by looking at the wave numbers used and comparing it with standard group-frequency ranges for plant phenolics, aromatic compounds, aliphatic residues, glycosides, and polysaccharide-like constituents (Coates, 2000; Socrates, 2001).

Data Interpretation Strategy
The interpretation was based on three principles: i) as observed peaks are reported, ii) Assignments are reported as likely functional groups or phytochemical classes (not necessarily compounds), and iii) articles must be genuine published papers on J and not non-journal citations. The phytochemistry, plant phenolics, UV-Vis spectroscopy, and FTIR group-frequency interpretation of Regia. The strategy intentionally adopts a cautious stance since UV-Vis and FTIR are used for fingerprinting surveillance: any compound(s) needs to be confirmed at the appropriate level by HPLC, LC-MS/MS, GC-MS and/or NMR.

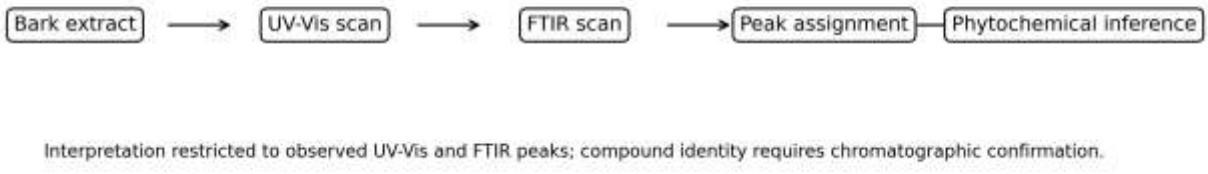

Figure 1. Analytical workflow used for conservative interpretation of the uploaded UV-Vis and FTIR spectral data.

RESULTS

UV-Vis Spectral Fingerprint
The UV-Vis interpretation file reported an absorption band centered around 420 nm. This band was assigned to an electronic transition linked with conjugated pi-electron density, particularly a pi to pi* transition. In the context of J. regia bark, this observation is compatible with phenolic and aromatic constituents because phenolic acids, flavonoid-related structures, tannins, and quinone-type compounds contain conjugated systems that can absorb in UV and visible regions (Aleixandre-Tudo& du Toit, 2018; Pavia et al., 2015).

The uploaded graph also showed intense low-wavelength absorbance features and an extended spectral trace across the visible range. These regions were not assigned quantitatively because the graph alone does not provide baseline correction, dilution status, or validated raw absorbance values. Therefore, the 420 nm band is treated as the principal reported UV-Vis finding, and all phytochemical inference is qualitative.

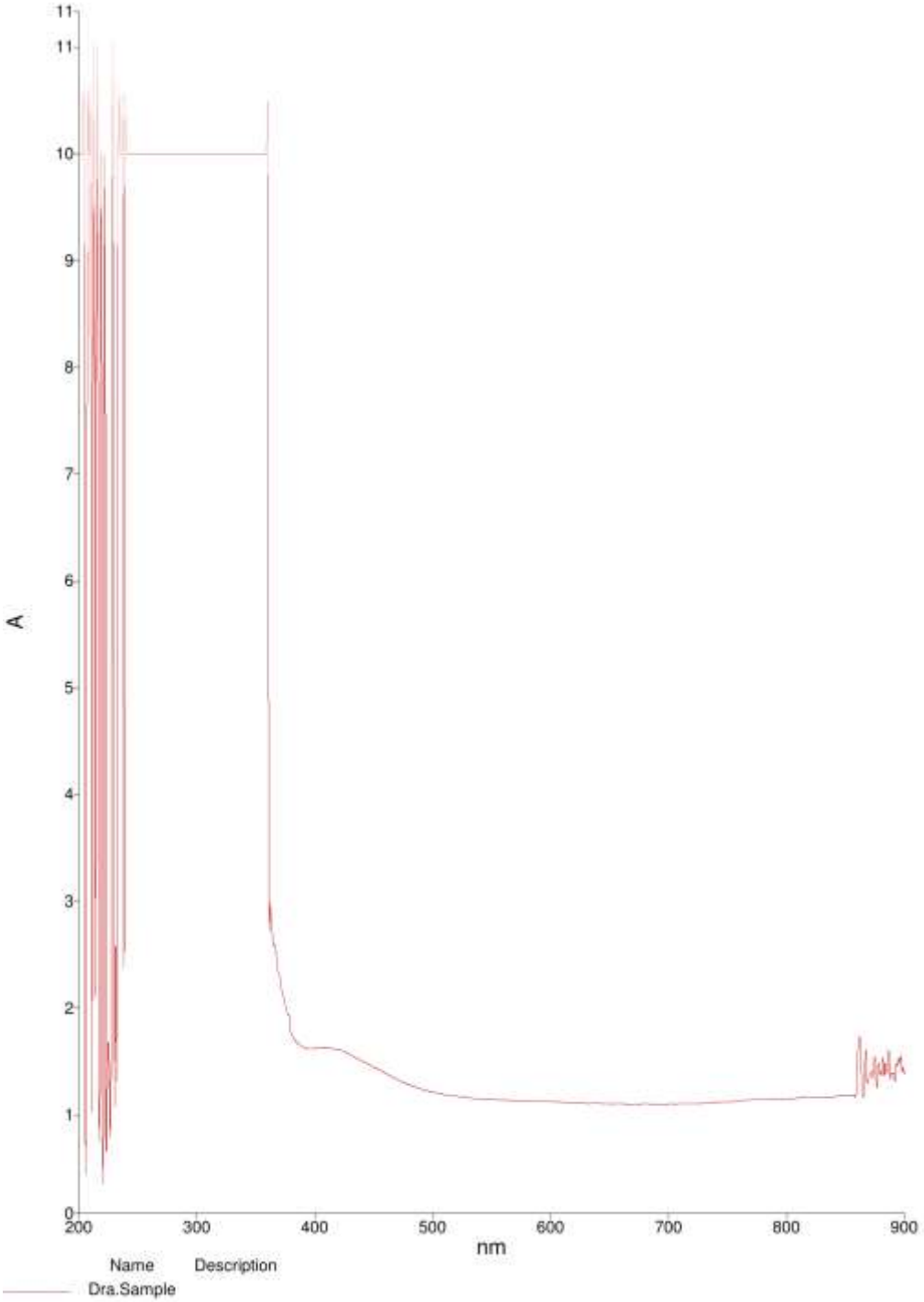

Figure 2. Uploaded UV-Vis absorbance spectrum of the bark extract. The corresponding interpretation file reported a band centered around 420 nm.

Observed UV-Vis feature	Probable electronic transition	Probable phytochemical implication	Interpretive strength
Band centered around 420 nm	pi to pi* transition in conjugated aromatic/chromophoric systems	Consistent with phenolic and conjugated constituents; possible ferulic acid, vanillic acid,	Preliminary qualitative evidence; individual compounds require HPLC/LC-MS

		coumaric acid, syringic acid, flavonoid/tannin-like compounds, or quinone-related chromophores	confirmation
High absorbance/noisy low-UV region in graph	Not assigned as a validated discrete band	May reflect concentrated extract, baseline effects, solvent/background contribution, or multiple overlapping chromophores	Not used for compound inference due to lack of raw absorbance and dilution data

Table 1. UV-Vis spectral feature and phytochemical interpretation of J. regia L. bark extract.

FTIR Spectral Fingerprint

FTIR fingerprinting revealed six clearly labelled bands at 3295.6, 2921.5, 1618.0, 1317.1, 1018.0, and 778.7 cm^-1. The strongest interpretive evidence was the combination of a hydroxyl-associated band around 3295.6 cm^-1, an aromatic/conjugated band around 1618.0 cm^-1, and pronounced C-O/C-O-C region features around 1317.1 and 1018.0 cm^-1. This pattern supports the presence of phenolic, aromatic, glycosidic, and polysaccharide-associated plant constituents.

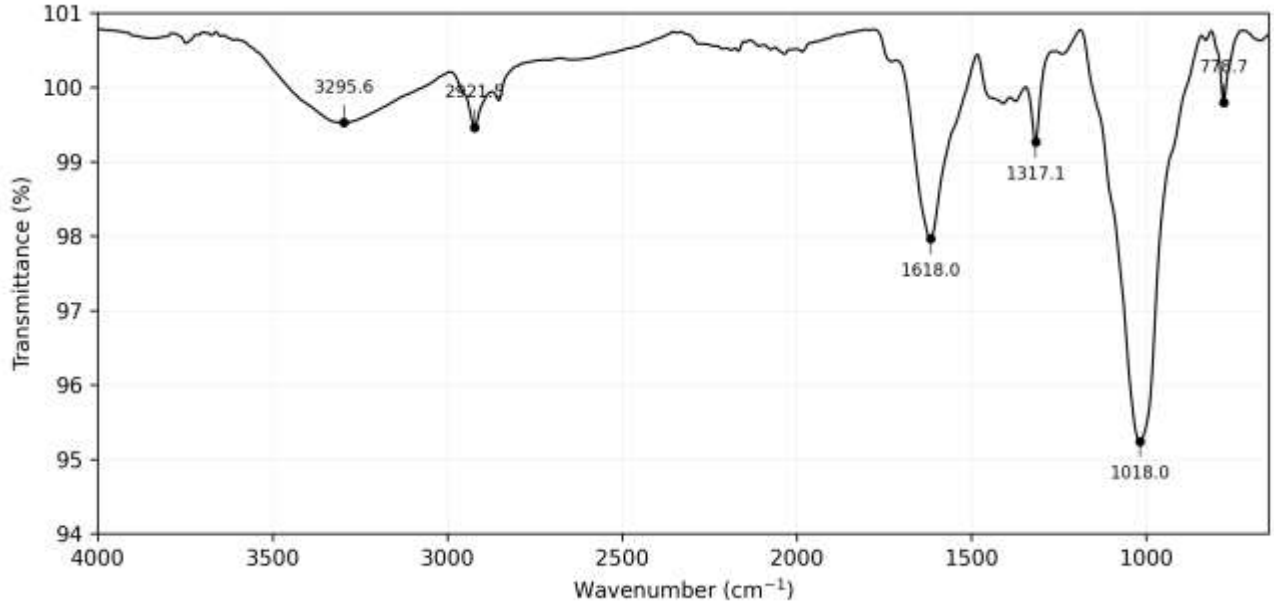

Figure 3. Reconstructed FTIR transmittance fingerprint from uploaded raw FTIR data, showing the principal labelled bands used for functional-group interpretation.

Peak position (cm^-1)	Probable functional group	Likely vibration	Phytochemical significance
3295.6	Hydroxyl groups of phenols/alcohols; hydrogen-bonded O-H	O-H stretching	Supports phenolic compounds, flavonoid/tannin-like hydroxylated metabolites, and carbohydrate-associated hydroxyl groups
2921.5	Aliphatic C-H groups	C-H stretching of CH2/CH3 groups	Indicates aliphatic side chains, lipid/wax residues, or methylene groups in complex plant extract

			matrices
1618.0	Aromatic ring C=C and/or conjugated C=O/C=C systems	Aromatic skeletal stretching; conjugated carbonyl/ring vibration	Supports aromatic phenolics, flavonoid/tannin-like structures, lignin-derived aromatic units, or quinone-related chromophores
1317.1	Phenolic C-O and O-H deformation region	C-O stretching and/or O-H bending	Suggests phenolic acids, tannins, flavonoids, or other oxygenated aromatic compounds
1018.0	C-O, C-O-C, and glycosidic groups	Alcohol/ether/glycosidic stretching	Consistent with glycosides, polysaccharides, carbohydrate residues, and phenolic glycoside-related structures
778.7	Substituted aromatic ring C-H	Aromatic C-H out-of-plane bending	Supports substituted benzene/aromatic ring systems common in phenolic acids and related compounds

Table 2. FTIR peak assignments and phytochemical significance of J. regia L. bark extract.

Consolidated Phytochemical Inference

The combined UV-Vis and FTIR fingerprints indicate a phytochemical profile dominated by aromatic, hydroxylated, oxygenated, and conjugated constituents. UV-Vis evidence supports a conjugated chromophoric system, whereas FTIR evidence supports hydroxyl-rich phenolics, aliphatic components, aromatic ring structures, and glycosidic/polysaccharide-associated C-O vibrations. The spectral pattern is consistent with the broader literature reporting phenolic acids, flavonoids, tannins, and naphthoquinones in J. regia tissues, including bark (Boukettaya et al., 2025; Bourais et al., 2022; Medic, Zamljen, et al., 2021).

Evidence source	Key observation	Supported phytochemical class	Caution
UV-Vis	420 nm band	Conjugated phenolics, flavonoid/tannin-like chromophores, quinone-type constituents	Does not identify individual molecules
FTIR	3295.6 cm^-1 O-H band	Phenols, alcohols, tannins, flavonoids, carbohydrate hydroxyls	O-H bands are broad and non-specific
FTIR	1618.0 cm^-1 aromatic/conjugated band	Aromatic phenolics, lignin-derived units, conjugated carbonyl/quinone systems	Overlapping bands possible
FTIR	1317.1 and 1018.0 cm^-1 C-O-rich region	Phenolic C-O, glycosides, polysaccharides, oxygenated metabolites	Needs chromatographic confirmation
FTIR	778.7 cm^-1 aromatic C-H out-of-plane band	Substituted aromatic rings	Substitution pattern cannot be resolved by this single band

Table 3. Integrated interpretation matrix from UV-Vis and FTIR data.

DISCUSSION

Interpretation of the 420 nm UV-Vis Band

A key UV-Vis finding of this study is the 420 nm band. Phytochemicals can be pigments or colored secondary metabolites, exhibiting visible-region absorption due to conjugation. The presence of hydroxyl, methoxyl, carbonyl substituents, and extension of conjugation leads to UV or visible absorption of many diametric derivatives phenols. However, these compounds contain an aromatic ring with delocalized pi-electron systems (Aleixandre-Tudo& du Toit, 2018; Pavia et al., 2015). Thus, the 420 nm band can be interpreted as an evidence of a conjugated phytochemical fraction rather than a marker of a single compound.

The interpretation included in the upload linked 420 nm band with pi to pi* transition which leads to the identification of phenolic compounds such as ferulic acid, vanillic acid, coumaric acid and syringic acid. A class-level interpretation of this assignment is feasible as these phenolic acids have aromatic systems and oxygenated substituents. On the other hand, UV-Vis spectra of crude extracts are composite such that bands may arise from overlapping contributions of flavonoids, tannins, and/or quinones, traces of chlorophyll, oxidized phenolics, polymerized phenolic products, etc. Therefore, the evidence at 420 nm should be viewed as supportive of conjugated phenolics but not conclusive of the presence of any specific phenolic acid.

It is essential for J to take precautions. The bark of Regia has uncertain reasons according to studies. Medic, Zamljen, et al. (2021) characterized hydroxycinnamic acids, hydroxybenzoic acids, flavanols, flavonols, naphthoquinones, and coumarins from walnut tissues, while Bourais et al. (2022) reported HPLC-DAD-ESI-MS/MS evidence for diverse polyphenols in kernels, leaves, husk, and bark. Recent analyses that focus on the bark revealed gallic acid, vanillic acid, quercetin, catechin, epicatechin, and more in Tunisian walnut bark (Boukettaya et al., 2025). The walnut bark is known to have phytochemical complexity, which is why 420 nm absorption is reasonable.

FTIR Evidence for Phenolic and Aromatic Constituents

The presence of peaks in FTIR corroborates the interpretation from UV-Vis spectra a phenolic-rich plant extract. The peak at 3295.6 cm-1 was assigned to O-H stretching which is due to phenolic hydroxyls, alcohols and hydroxy of carbohydrates. This band may consist of phenolic acids, flavonoids, tannins, and glycosylated substances in the crude bark extract. Due to the broadness and sensitivity of O-H absorption to hydrogen bonding, it should not be regarded as a specific molecular indicator. Rather, it indicates the presence of hydroxyl-rich compounds (Coates, 2000; Socrates, 2001).

The band at 2921.5 cm^-1 is due to aliphatic C-H stretching. While phenolic compounds are the main focus for interpretation in this paper, plant bark extracts may also contain waxes, lipids, terpenoid residues, and aliphatic side chains. The presence of aliphatic C-H stretching indicates the more complex matrix nature of plant extracts and is consistent with offering phenolic enrichment.

The band at 1618.0 cm^-1 is associated with aromatic C=C skeletal vibration and conjugated C=O/C=C systems. The assignment matches aromatic phenolics, flavonoid/tannin-like structures, lignin-derived aromatic fragments, and naphthoquinone-related structures. The compounds juglone and hydrojuglone are typical constituents of the various species of Juglans and, naphthoquinones, have been reported as one of the major phenolic groups of several J. The bark and buds of the regia tissues (Medic, 2021).

The bands at 1317.1 and 1018.0 cm-1 are in the oxygenated functional-group regions. The peak at 1317.1 cm^-1 substantiates the occurrence of phenolic C-O stretching as well as the O-H deformation while the peak at 1018.0 cm^-1 suggests C-O or C-O-C stretching associated with alcohols, ethers, glycosides, and the polysaccharides structures. Plant bark usually contains bands of various chemical groups including phenolic glycosides, hydrolysable tannins, lignocellulosic residues and carbohydrate-based matrix components. The band at 778.7 cm^-1 supports hydrocarbons with substituted aromatic rings by out-of-plane aromatic C–H bending (Coates, 2000; Socrates, 2001).

Connection with the Reported Phytochemistry of Juglans regia Bark

The spectral interpretation confirms the published phytochemical data. J. pharmacognostic surveys. Regia bark contains reducing sugars, alkaloids, tannins, phenols, steroids and saponins which suggest that bark might contain polar phenolic components and other classes of secondary-metabolites (Devi et al., 2011; Delaviz et al., 2017). Studies demonstrated the antimicrobial relevance of walnut bark against other pathogenic bacteria and microorganisms in the mouth. This supports the pharmacological relevance of phenolic rich extractive fraction of walnut bark (Wani and Kumar, 2020; Zakavi et al., 2013).

The likely phenolics proposed by the uploaded UV-Vis interpretation – ferulic acid, vanillic acid, coumaric acid and syringic acid – are reasonable candidate classes because walnut tissues are repeatedly reported to contain hydroxycinnamic acids and hydroxybenzoic acids. Medic, Zamljen, et al. (2021) noted that the leaves, petioles, bark, roots and buds showed broad phenolic diversity. Meanwhile, Khatoon et al. (2016) screened and quantified polyphenols in different J. species. These included catechin, epicatechin, gallic acid, caffeic acid and syringic acid. Using TLC densitometry for Regia parts The UV-Vis band and FTIR functional groups in the current spectral data are consistent with these classes but are unable to separate them at a compound level.

We should also consider the potential contribution of quinones. Chemotaxonomically significant in Juglans are juglone and naphthoquinones. Pronounce at 420 nm visible band and 1618.0 cm−1 aromatic/conjugated band may have been due to naphthoquinone-like chromophores or oxidized phenolic products. To further substantiate this hypothesis, the naphthoquinones group of emerging phenolic compounds found in walnut (Medic, Zamljen, et al., 2021) tissues, consistent with HPLC-MS/MS literature, should be confirmed by LC-MS/MS or HPLC-DAD comparison with standards.

Relevance for Pharmacological Potential

Spectral pattern reveals the presence of functional groups of antioxidant phytochemicals. Antioxidant mechanisms of phenolic hydroxyl groups encompass hydrogen atom transfer, electron donation, metal-chelation potential, and resonance stabilization of phenoxyl radicals. Walnut leaves, husks, flowers, pollen, bark and other related materials have been repeatedly attributed with antioxidant, antimicrobial, antidiabetic, anti-inflammatory and other bioactivities but these activities vary according to plant part, extraction solvent, cultivar, geography and assay method (Bhat et al., 2023; Bourais et al., 2022; Jahanban-Esfahlan et al., 2019; Zurek et al., 2023).FTIR pattern O-H, aromatic and C-O rich along with 420 nm in UV-Vis, lends a reasonable basis for proposing these antimicrobial assays as the next investigations.

Importance of Combining UV-Vis and FTIR Fingerprinting

The overlapping use of UV-Vis and FTIR can be useful for preliminary quality assessment of botanical extracts as they interrogate different chemical features. The UV-Vis test is very useful in the assessment of phenolic compound screening as well as colorimetric assays and is sensitive to electronic transitions in conjugated systems. FTIR offers more extensive functional group evidence and is useful for extract authentication, batch comparison, and detection. It helps to identify functional group patterns related to phenolic, carbohydrate, lipid and aromatic constituents. The two methods together provide a cost-effective fingerprinting option for the initial pharmacognostic screening, albeit with the necessity of chromatographic verification (Aleixandre-Tudo& du Toit, 2018; Coates, 2000; Socrates, 2001).

CONCLUSION

The UV-Vis and FTIR data uploaded confirm the existence of bioactive phytochemical classes in J. regiae L. tree bark extract. The Band in UV-Vis centred around 420 nm is a characteristic of conjugated chromophoric systems. This concurs with phenolic compounds and similar aromatic substances. The FTIR at frequencies bands indicating hydroxyl, aliphatic C-H, aromatic/conjugated, phenolic C-O, glycosidic C-O-C and substituted aromatic ring vibrations are at 3295.6, 2921.5, 1618.0, 1317.1, 1018.0, 778.7 cm^-1 respectively. The fingerprint you gave is most consistent with a bark extract enriched with phenolics containing flavonoid/tannin-like, aromatic, oxygenated and glycosidic components. According to the above findings preliminary phytochemical profile is scientifically defensible. But compound-wise identification requires HPLC and LC-MS/MS or GC-MS or NMR analysis.

DECLARATIONS

Ethical Approval

Not applicable. The manuscript is based on spectroscopic analysis of a plant extract and did not involve human participants or animals.

Conflict of Interest

The author(s) should declare any financial or non-financial competing interests before journal submission. No competing interest information was provided in the uploaded data.

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