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Visit Journal at <https://www.jacsdirectory.com/jacs>Isolation and Characterization of Phytochemicals Present in *Morus nigra* L. LeavesIndira V. Rajput^{1,*}, Komal L. Patil¹, S.S. Rajput²¹Department of Chemistry, Vasantrao Naik Arts, Science and Commerce College, Shahada, Nandurbar – 425 412, Maharashtra, India.²Department of Chemistry, S.P.D.M. College, Shirpur, Nandurbar – 425 405, Maharashtra, India.

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ABSTRACT

Morus nigra L., a widely distributed medicinal plant, is increasingly recognized for its rich phytochemical profile and potential therapeutic applications. The present study focuses on the systematic isolation, characterisation, and biological evaluation of phytochemicals present in the leaves of *Morus nigra* L. Crude extracts were prepared using solvents of varying polarity, followed by fractionation through chromatographic techniques such as column chromatography and TLC. Major classes of secondary metabolites, including proteins, carbohydrates, phenolics, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, alkaloids, were identified. Structural characterisation of isolated compounds was accomplished using spectroscopic methods, including FTIR, ¹H NMR, ¹³C NMR and GC–MS. Overall, the findings highlight *Morus nigra* L. as a promising natural source of bioactive compounds.

1. Introduction

Natural products derived from medicinal plants have been a major source of therapeutic agents for centuries and continue to play a vital role in modern drug discovery. Phytochemicals, the biologically active secondary metabolites present in plants, are known to exhibit a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects [1, 2]. The increasing prevalence of chronic diseases and the limitations associated with synthetic drugs have intensified the search for plant-based bioactive compounds that are effective, safe, and environmentally sustainable.

Morus nigra L. (black mulberry), a member of the family Moraceae, is a deciduous tree widely cultivated in Asia, Europe, and the Middle East. The plant has a long history of use in traditional medicine, where its leaves, fruits, bark, and roots are employed to treat various ailments such as diabetes, hypertension, fever, microbial infections, and inflammatory disorders. The fruits of *Morus nigra* are particularly valued for their nutritional importance and are rich in vitamins, minerals, and polyphenolic compounds [3, 4].

Previous phytochemical studies have reported that *Morus nigra* contains a diverse range of secondary metabolites, including flavonoids, anthocyanins, phenolic acids, tannins, alkaloids, and glycosides. These compounds are largely responsible for the plant's pharmacological properties. Anthocyanins and flavonoids, which are abundant in the fruits and leaves, have attracted significant attention due to their potent antioxidant and free radical scavenging activities. Additionally, extracts of *Morus nigra* have demonstrated promising antimicrobial, anti-inflammatory, hepatoprotective, and antidiabetic effects in various in vitro and in vivo studies [5, 6].

Despite the documented medicinal potential of *Morus nigra*, comprehensive studies focusing on the systematic isolation and structural characterization of its bioactive constituents remain limited. The application of advanced chromatographic and spectroscopic techniques such as column chromatography, high-performance liquid chromatography (HPLC), ultraviolet-visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) is essential for accurate identification and characterization of these phytochemicals [7, 8].

In this context, the present study aims to isolate and characterize phytochemicals from *Morus nigra* L. The findings of this research are expected to provide scientific evidence supporting the traditional uses of

Morus nigra and contribute to the development of novel natural therapeutic agents.

2. Experimental Methods

2.1 Extraction and Phytochemical Studies

Fresh leaves of *Morus nigra* L. were collected, washed, shade-dried, and powdered. The sample was authenticated by standard herbarium procedures. The powdered plant material was subjected to Soxhlet extraction using solvents of increasing polarity: hexane, ethyl acetate, ethanol, and water. Extracts were concentrated using a rotary evaporator. Qualitative tests were conducted for alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides using standard protocols. Column chromatography using silica gel as stationary phase was performed. Fractions were monitored by TLC using appropriate solvent systems and visualized under UV light and iodine vapours. The extract was tested for the presence of bioactive compounds by using standard methods [2-9].

2.2 Characterization

Isolated compounds were analysed using UV-Vis, FTIR, ¹H NMR, ¹³C NMR, and GC–MS to determine functional groups and molecular structures.

3. Results and Discussion

3.1 Phytochemical Study

Phytochemical analysis conducted on the plant leaves extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [10]. They possess biological properties such as anti-apoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [11].

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids and tocopherols etc., [12]. Tannins bind to proline-rich proteins and interfere with protein synthesis. Flavonoids are hydroxylated

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phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. They also are effective antioxidants and show strong anticancer activities [13–15].

The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [16]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol-binding properties and bitterness. Steroids have been reported to have antibacterial properties [17] and they are very important compounds especially due to their relationship with compounds such as sex hormones. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports [18]. The phytochemical characteristics of *Morus nigra* L. leaves extracts were tested and summarized in Table 1. The results revealed that the presence of medically active compounds in the plant studied. Carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids and terpenoids were present in aqueous and ethanolic extract of leaves of *Morus nigra* L., while proteins and alkaloids were present only in aqueous extract but absent in ethanolic extract of leaves.

Table 1 Phytochemical constituents of *Morus nigra* L. plant (leaves) studied

S.No.	Parameter	Aqueous Extract	Ethanol Extract
1	Proteins	+	+
2	Carbohydrates	+	=
3	Phenols/ Tannins	+	+
4	Flavonoids	+	+
5	Saponins	+	+
6	Glycosides	+	+
7	Steroids	+	+
8	Terpenoids	+	+
9	Alkaloids	+	-

Key + = Present, - = Absent

3.2 Spectroscopic Characterization

3.2.1 FTIR Analysis

FTIR analysis of *Morus nigra* L. leaves powder revealed peaks at 3743, 3674, 3651, 3293, 3011, 2925, 2854, 2372, 2319, 1736, 1715, 1647, 1561, 1458, 1397, 1373, 1242, 1163, 1041, 993, 925, 829, 713, cm^{-1} (Fig. 1). The broad peak present at 3293 cm^{-1} lies within the range of 3500–3200 cm^{-1} indicates; O-H stretching [19], N-H stretching vibration of peptide groups (Amide A) [20], C=O stretching vibrations overtone [21], and shows the presence of alcohols, phenols, carboxylic acid, aliphatic primary amine, hydrogen bonded amines, amino group, ammonium ion [22], and quinone oximes. The sharp peak at 292 cm^{-1} exhibits C-H stretching [23] vibration of methylene group which corresponds to alkanes, lipids and hydroxyl compounds [24]. The C-H asymmetric methylene group is characteristic of aliphatic compounds [25]. Carboxylic group also shows absorbance in this region. This peak also corresponds to O-H stretching and symmetrical bending [26]. The sharp peak obtained at 2849 cm^{-1} denotes symmetrically C-H, stretching vibration, corresponds to aliphatic ethers, aldehydes [27]. The bands between 2800–3000 cm^{-1} are characterized due to asymmetric and symmetric stretching vibrations of CH_2 and CH_3 groups [28].

No peaks were indicated in the region within the range of 2854–1736 cm^{-1} which denotes the absence of cyanide group therefore no toxic substances are found in *Morus nigra* L. leaves [29]. Sharp peak at 1736 cm^{-1} lies in the range of 1750–1715 cm^{-1} and the peak at 1647 cm^{-1} lies in the range of 1650–1700 cm^{-1} corresponds to C-O stretching amide I band [30], and C-C stretching vibration. A heavy element, group with a heavy element directly attached to C-C [31]. The peak obtained around 1561 cm^{-1} , is a characteristic of amide II band with C-N stretching and N-H bending (C-N stretching and N-H bending vibration of amide II band) [32]. The peak at 1561 cm^{-1} corresponds to C-C aromatic. The peak obtained at 1458 cm^{-1} lies between the range of 1380–1500 cm^{-1} wave number region and indicates C-H deformation vibration, stretching vibrations of C-O amide and C-C stretching from phenyl group and corresponds to ketones, acetates, amides etc. [33]. The peak at 1373+ cm^{-1} shows C-H deformation vibration corresponding to amines [34], alkynes, cellulose and Amide III band components of proteins collagen [35]. Two medium peaks found between the range of 1300–1180 cm^{-1} (phosphodiester region), and 1100–1180 cm^{-1} indicate C-N stretching (amine) [36]. Stretching vibrations of

carbonyl C-O (alkyl aryl ether) or O-H bending [37], corresponding to amine group and phosphate ion [38]. The peak at 1041 cm^{-1} corresponds to C-C, C-OH, C-H.

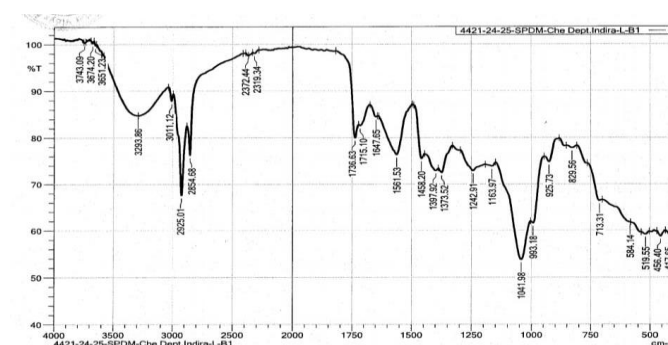


Fig. 1 FTIR of *Morus nigra* L. (Sample L. B1)

3.2.2 ^1H NMR Spectroscopic Analysis

The spectrum was recorded in MeOD (deuterated methanol). Key regions and chemical shifts (δ in ppm) from Fig. 2 are:

Aromatic/Vinyl Region (δ 5.0 - 5.4 ppm): Signals around δ 5.302, 5.293, 5.251, 5.240, and 5.228 ppm suggest the presence of protons attached to double bonds (e.g., vinylic or aromatic protons). The integral of 2.10 in this region corresponds to approximately 2 protons [39].

Highly Deshielded Region (δ 4.0 - 4.8 ppm): A sharp singlet at δ 4.796 ppm is likely an exchangeable proton (e.g., an OH proton or residual solvent signal in MeOD). Signals around δ 4.020–3.991 ppm (integral 2.78 $\approx$ 3 protons) are characteristic of protons on a carbon bearing an oxygen atom (CH-O), such as those in an alcohol or sugar moiety.

Sugar/Hydroxymethyl Region (δ 3.0 - 4.0 ppm): A dense cluster of signals between δ 3.0 and 4.0 ppm (integral 6.22 $\approx$ 6 protons) is highly indicative of sugar protons (H-2' to H-6') which typically resonate in this range [40].

Aliphatic/Methylene Region (δ 1.0 - 3.0 ppm): A complex pattern of signals indicates multiple aliphatic and methylene groups (CH_2 and CH).

- δ 2.709–2.695 ppm (integral 1.70 $\approx$ 2 protons): Methylene or methine next to an electron-withdrawing group.
- δ 1.837 ppm (integral 1.83 $\approx$ 2 protons): Methylene or methane protons.
- δ 1.297, 1.283, etc. (integral 3.34 $\approx$ 3 protons): Likely protons of an isolated methyl group (CH_3).

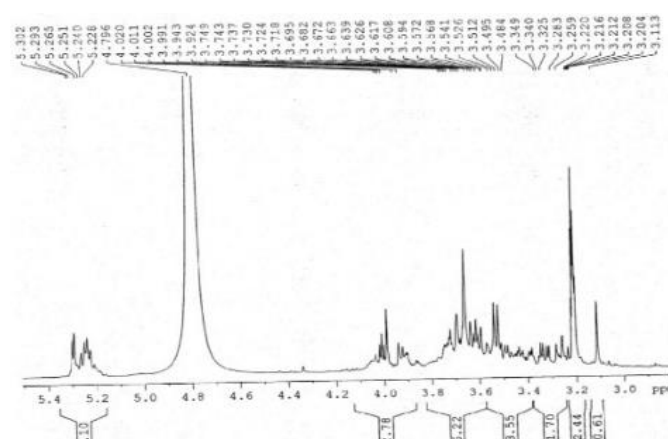


Fig. 2 ^1H -NMR of *Morus nigra* L. (Sample L. B1)

3.2.3 ^{13}C NMR Spectroscopic Analysis

The ^{13}C NMR spectrum in Fig. 3 confirms a complex structure with many carbon atoms. Key regions are:

Aromatic/Vinyl Carbons (δ 120 - 140 ppm): Signals at δ 131.319, 127.810, 127.779, 126.834, and 127.462 ppm confirm the presence of an aromatic or conjugated system.

Ketone/Ester Carbons (Absent): No signals were observed above δ 170 ppm, suggesting the absence of typical ester, carboxylic acid, or ketone carbonyl carbons.

Oxygenated Aliphatic/Acetal Carbons (δ 60 - 110 ppm): Signals in the δ 90–105 ppm range (e.g., δ 103.932 and 92.253 ppm) suggest a glycosidic carbon (C-1 of a sugar) or other acetal/ketal-like structure [40].

A high number of peaks between δ 60 and 90 ppm (e.g., δ 82.358, 77.974, 74.330, 73.213, 73.016, 71.807, 71.142, and 63.006 ppm) are

highly characteristic of the C-2 through C-6 carbons of one or more sugar units.

Aliphatic Carbons (δ 10 - 50 ppm): Multiple aliphatic carbon signals are present, including methyl, methylene, and methine carbons.

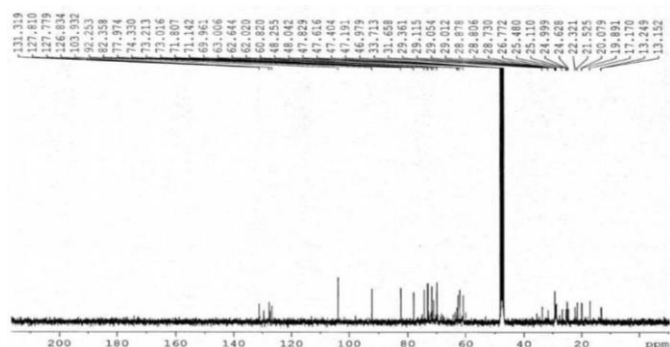


Fig. 3 ^{13}C -NMR of *Morus nigra* L. (Sample L-B1)

3.2.4 Mass Spectrometric Analysis (MS)

The MS spectrum for Sample L (Batch B1) shows a dominant signal (100% relative abundance) at m/z 324.10. This peak is likely the protonated molecular ion $[\text{M}+\text{H}]^+$. The corresponding molecular mass (M) would be ≈ 323 . Other significant peaks are observed at m/z 380.99, 345.03, and 455.02. The peak at m/z 345.03 may be the Na^+ adduct $[\text{M}+\text{Na}]^+$ if the molecular weight is 322.

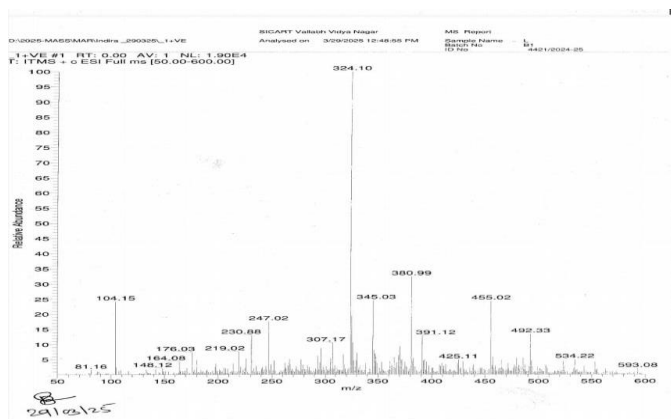


Fig. 4 GC-MS of *Morus nigra* L. (Sample L.B1)

3.3 Possible Structure Interpretation: Sample L (Batch B1)

A molecular weight of 323 Da for a glycoside is relatively small. A common flavonoid aglycone (the non-sugar part) like Kaempferol is 286 Da. A typical hexose sugar (like glucose) is 162 Da. Therefore, the aglycone must be significantly smaller than a standard flavonoid [42]. A possible class of compounds are phenolic glycosides, which often have smaller aglycones. For instance, methyl syringate (an aromatic compound) has an aglycone mass of 198 Da. A structure with $M \approx 323$ Da is consistent with an aglycone having $M \approx 161$ Da (if a single hexose sugar (162 Da) is lost, $323-162=161$).

Table 2 Spectral analysis key data points of *Morus nigra* L. (Leaves)

Technique	Key Data Point	Implication
MS (Positive ESI)	Base Peak at m/z 324.10 $[\text{M}+\text{H}]^+$	Molecular Weight (M) $\approx$ 323 Da
^1H NMR	Signals at δ 5.3 ppm	Vinylic or Aromatic Protons (often H-1 of sugar or H on an aromatic ring)
^{13}C NMR	Signals at δ 103.932, 92.253 ppm	Anomeric carbon (C-1) of a sugar or an acetal
^{13}C NMR	Cluster δ 60–90 ppm (many peaks)	Multiple CH-O carbons, confirming a sugar moiety
^{13}C NMR	Signals δ 126–132 ppm	Aromatic/conjugated system (aglycone)

Based on the interpretation of the spectral data provided in Table 2 (FTIR, ^1H NMR, ^{13}C NMR and mass spectrometry), it is confirmed that the *Morus nigra* L. leaves contains flavonoid derivatives like quercetin, glycoside, kaempferol, quercetin aglycon, and polyphenolic compounds [39-42].

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4. Conclusion

The present study demonstrates that the leaves of *Morus nigra* L. are a rich source of bioactive phytochemicals with significant therapeutic potential. Preliminary phytochemical screening revealed the presence of important secondary metabolites such as proteins, carbohydrates, flavonoids, phenolic compounds, tannins, alkaloids, glycosides, steroids, terpenoids and saponins. These phytochemicals are known to contribute to various pharmacological activities and validate the medicinal importance of the plant.

Spectroscopic characterization using FTIR, ^1H NMR, ^{13}C NMR, and mass spectrometric techniques confirmed the polyphenolic nature of the isolated compounds. The presence of characteristic functional groups, aromatic systems, and glycosidic linkages indicated that the major constituents belong to flavonoid derivatives like quercetin, glycosides, kaempferol, quercetin aglycon, and polyphenolic compounds. The combined spectroscopic data provided reliable structural insights into the isolated phytochemicals and supported their classification as bioactive natural compounds.

Overall, the findings of this study provide scientific validation for the traditional medicinal uses of *Morus nigra* L. leaves and highlight their potential as a valuable source of natural therapeutic agents. Further studies involving purification of individual compounds, in vivo evaluations, and mechanistic investigations are recommended to explore their clinical relevance and pharmaceutical applications.

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