RESEARCH ARTICLE

Phytochemical Extraction, Analysis, and Characterization of *Madhuca longifolia* Leaf Compounds

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ABSTRACT

Objective: This study aimed to perform the extraction, phytochemical analysis, and compound characterization of *Madhuca longifolia* leaf extracts.

Materials and Methods: Sequential extraction of *M. longifolia* leaves was performed using petroleum ether, chloroform, methanol, ethanol, and water. The yield of the extracts was measured and reported as 1.3 ± 0.10% for petroleum ether, 3.85 ± 0.15% for chloroform, 20.25 ± 1.22% for methanol, 30.1 ± 1.38% for ethanol, and 26.7 ± 0.21% for water. Qualitative phytochemical analysis was conducted to determine the presence of various compounds, including alkaloids, glucose-alkaloids, steroids, tannins, phenols, proteins, and flavonoids. The absence of ethyl and methyl alkalis in petroleum ether and chloroform extracts was noted. Additionally, the presence of saponins and alkaloids was observed only in methanol extracts, with cresol absent from these extracts. The isolated compounds were further characterized using thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), ultraviolet (UV) spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy.

Results: The R_f value of the isolated compound was 0.49 using TLC. HPLC analysis identified major constituents with retention times of 4.576 and 10.461 minutes. These findings highlight the diverse phytochemical composition of *M. longifolia* leaf extracts and provide a detailed analysis of the active compounds present.

Conclusion: This comprehensive study of *M. longifolia* leaf extracts offers valuable insights into the plant's medicinal potential. The identified active compounds could serve as a reference for future medicinal use and research.

Keywords: *Madhuca longifolia*, phytochemical analysis, sequential extraction, HPLC, FTIR spectroscopy.

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INTRODUCTION

Another prime tree species of the Indian subcontinent *Madhuca longifolia* (Mahua), is incorporated to overcome that hurdle. In indigenous medicine, the leaves, flowers, seeds and bark of this tree have traditionally been used because of their therapeutic properties. The leaves of *M. longifolia* possess various bioactive compounds which are responsible for its medicinal activity.[1]

Modern study of phytotherapy was raised with the increasing trend toward natural remedies or products. Among various medicinal plants, *M. longifolia* (Mahua) has been reported to exhibit a wide spectrum of pharmacological activities like- anti-inflammatory, antimicrobial antioxidant and hepatoprotective properties.[2] Although used traditionally, there are few scientific reports indicating the phytochemical composition and biological activities of this plant.[3]

This work provides a protocol for extraction, phytochemical analysis and compound characterization from *M. longifolia* leaf extracts so that the gap might be bridged. The goal is to validate some traditional uses and investigate potential medicinal applications by identifying, then quantitatively the bioactive components in these leaves.[4]

For this purpose, the different polarities of solvent viz, petroleum benzine, chloroform, methanol (MeOH), ethyl acetate and water were used for successive extraction in leaves *M. longifolia*.[5] The extracts were then evaluated for its phytochemical content by conducting standard qualitative color tests. It was further purified and characterized by different analytical techniques, i.e., thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), UV spectroscopy, Fourier-transform infrared spectroscopy (FTIR).[6]

The present study, therefore, provides detailed information on the phytochemical profile of *M. longifolia* leaves along with their probable pharmacological applications. This form of research provides additional support for the medicinal values

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of this plant and suggests avenues to further investigate its pharmacological potential by a combination of traditional knowledge with modern scientific methods.

MATERIALS AND METHODS

Plant Material

Collection and preparation of M. longifolia leaves

The leaves of *M. longifolia* were identified by a Botanist, the Central Ayurveda Research Institute CCRAS (Report No. 020). Leaves of fully developed mature trees in their natural habitats were harvested. The leaves were carefully washed three times in distilled water for the removal of dust and other impurities, followed by air drying. After washing, the leaves were air dried in the shade under an ambient room atmosphere for a few days to complete drying. This was followed by drying the leaves, grinding them to a fine powder with a domestic mechanical grinder and storing it in an air-tight container for future use. This will help to ensure that my leaves are clean, dry and ready for use in the experiment.

Extraction Process

Description of the sequential extraction process

The powdered leaves of *M. longifolia* were extracted with petroleum ether from a Soxhlet apparatus. Extraction took place with various solvents of polarities increases from petroleum ether, chloroform, methanol, ethanol and water. The extraction was performed three times, each on 1 day.[7]

Solvents used and extraction durations

- Petroleum Ether: 1 day
- Chloroform: 1 day
- Methanol: 1 day
- Ethanol: 1 day
- Water: 1 day

The extracts were concentrated by evaporating the solvent under reduced pressure on a rotary vaporizer at 50°C after each extraction. The extracts were dried, weighed to record the extract yields and stored in a sealed container at room temperature until analysis.[8]

Phytochemical Analysis

Qualitative analysis of different phytochemicals

Secondary metabolites present in the sequential extracts were determined using qualitative phytochemical screening.[9] The standard tests conducted:

Alkaloids: Wagner's test

Alkaloids are a group of nitrogenous organic compounds found in plants. They are famous for their extensive pharmacological activities. Wagner test uses iodine in potassium iodide spot-on extract. Light yellow, light orange-red and brownish-orange precipitates indicate the presence of alkaloids.[10]

Carbohydrates: Molisch's test

Carbohydrates are very important biomolecules that play several roles in various biological processes. In this test, alpha-naphthol is added, which turns purple, followed by concentrated sulfuric acid to confirm the presence of carbohydrates. Carbohydrates are present where the solution turns violet at the interface.[11]

Glycosides: Keller-kiliani test

Glycosides are the glycosyl component of a molecule combined with another non-sugar entity. In the Keller-Kiliani test, the extract is treated with glacial acetic acid containing a trace of ferric chloride and then concentrated sulfuric acid. Bluish or green color: glycosides.[12]

Phytosterols: Salkowski's test

Phytosterols are plant-based sterol compounds that have cholesterol-lowering properties. The Salkowski test is to be performed on the extract and chloroform with concentrated sulfuric acid. Phytosterols, which are present at the enzyme: substrate interface, can be visualized by a reddish-brown color.[13]

Tannins: Ferric chloride test

Tannins are polyphenolic compounds that have an astringent taste and possess the ability to precipitate proteins. In the ferric chloride test we add ferric chloride to extract. If you see a blue-black or green-black, tannins are present.[14]

Phenols: Lead acetate test

Phenols are aromatic compounds that have one or more hydroxyl groups directly attached to an aromatic ring. In the lead acetate test, this extract is given a few drops of soluble solution. White precipitate - presence of phenolic compounds.[15]

Proteins: Biuret test

Proteins are large biomolecules, or macromolecules, that provide many essential functions for an organism; these include cellular structure and maintaining cell volume. The extract is analyzed for the protein/groups of proteins status by applying NaOH and an ionic Cu (II) through a Biuret test. The purple or violet color shows the presence of proteins.[16]

Flavonoids: Shinoda test

Flavonoids belong to a class of phytonutrients and are chemically classified as polyphenols with antioxidant activity. Shinoda test; treatment of extract with magnesium turnings and concentrated hydrochloric acid. Red or orange indicates the presence of flavonoids.[17]

Terpenoids: Salkowski test

A broad and diverse class of biological compounds produced by numerous plants are the terpenoids, all based on C5 isoprene molecules. For the Salkowski test, extracts are biraped with chloroform and concentrated sulfuric acid. Terpenoids give the insect itself a reddish-brown color.[18]

Steroids: Liebermann-burchard test

Steroids are a group of organic compounds sharing the same physiological activity. To test for this, the extract was subjected to Liebermann-Burchard reaction using acetic anhydride and concentrated sulfuric acid. Steroids will show up blue or green.[19]

Saponins: Froth test

Saponins are glycosides (a molecule in which a sugar is bonded to each of several hydroxyl groups) that also have soap-like qualities. Froth test: Shake the extract with water and observe frothing. Frothing A stable froth is a telltale sign of saponins.[20]

Isolation and Identification of Compounds

Thin layer chromatography (TLC)

General pointing technique used which is much easier and helped to solve the problems with thin-layer chromatography.[21]

Procedure

The individual compounds were separated and identified by applying the extracts on TLC. The stationary phase was silica gel plates, and the solvent systems of mobile phases were varied. The plates were developed, and individual bands were visualized with UV light or in some cases using specific staining reagents. The R_f values of the identified spots were calculated.[22]

High-Performance Liquid Chromatography (HPLC)

As an additional purification and analysis step to confirm the compounds present, HPLC was conducted on these extracts. Materials and methods: A Shimadzu LC solution HPLC system equipped with a compatible column and mobile phase. Retention times and peak areas were recorded, and compounds identified with known standards using their retention time.[23]

Ultraviolet (UV) Spectroscopy

The UV spectroscopy of such compounds is useful for the analysis of isolated. The UV spectra of synthesized compounds were recorded and certain absorption peaks were observed. These peaks facilitate in the characteristics of excellent groups inside them (pg).[24]

Fourier Transform Infrared Spectroscopy (FTIR)

Advanced analysis using FTIR spectroscopy was deployed to characterize the functional groups existing into isolated compounds. FTIR spectra of the samples at different functional groups were noted and analyzed their absorption bands. This supplied important structural details on the organochloride molecules.[25]

RESULTS

Extraction Yields

Air-dried powdered leaves of *M. longifolia* were sequentially extracted with solvents of increasing polarity, viz., petroleum ether, chloroform, methanol (MeOH), ethanol and water. Here are the quantitative yields from each extraction process:

- Petroleum Ether Extract: 1.3 ± 0.10% (w/w)
- Chloroform Extract: 3.85 ± 0.15% (w/w)
- Methanol Extract: 20.25 ± 1.22% (w/w)
- Ethanol Extract: 30.1 ± 1.38% (w/w)
- Water Extract: 26.7 ± 0.21% (w/w)

The ethanol extract showed highest percentage yield, followed by water and methanol extracts which revealed high solubility of phytochemicals at polar protic (water) level.

Phytochemical Composition

Table 1 Overview of specific qualitative phytochemical constituents in the sequential extracts.

TLC Analysis

The extracts were separated into individual compounds using Thin Layer Chromatography (TLC). As descried below (Figure 1), the R_f value calculated for a major compound is 0.49.

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}} = \frac{9.8\,\text{cm}}{20\,\text{cm}} = 0.49$$

Distance travelled by solvent = 20Cm
Distance travelled by Solute= 9.8

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

R_f value = 9.8/20
= 0.49
Reported R_f value= 0.46

Table 1: Phytochemical constituents of *M. longifolia* leaves in different solvents

Phytochemicals	Petroleum Ether	Chloroform	Methanol	Ethanol	Water
Alkaloids	-	+	+	+	-
Carbohydrates	+	-	+	+	-
Glycosides	-	+	+	+	-
Phytosterols	+	-	-	-	+
Tannins	-	-	-	+	+
Phenols	+	-	+	+	-
Proteins	+	-	+	+	+
Flavonoids	+	-	+	-	-
Terpenoids	+	-	+	+	+
Steroids	-	+	-	+	-
Saponins	+	+	+	+	+

+ = Present; - = Absent

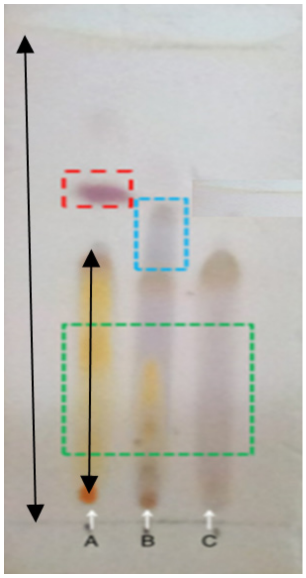


Figure 1: TLC of *M. longifolia* leaves extract

HPLC Analysis

High-Performance Liquid Chromatography (HPLC) analysis was used to purify and identify the molecules present in these extracts (Table 2, Figure 2). The analysis revealed distinct peaks at the retention times towards:

The first peak with 10.461 minutes was the highest (53.70% of total area) demonstrating that this compound is more present within the extract.

UV and FTIR Spectroscopy

UV spectroscopy

After that, the isolated compounds were evaluated by UV spectroscopy and characteristic absorption peaks were observed. These peaks of the UV spectra are characteristics for different functional groups and help to identify compounds (Figure 3).

FTIR spectroscopy

Further characterization of the functional groups present in the isolated compounds were carried out using Fourier-transform infrared (FTIR) (Figure 4). The FTIR spectra showed these main absorption bands.

- O-H Stretch: 3300-3500 cm^{-1}
- C=O Stretch: 1700-1750 cm^{-1}
- C=C Stretch (Aromatic): 1500-1600 cm^{-1}
- C-H Stretch: 2800-3000 cm^{-1}

These hydroxyl, carbonyl and aromatic group showed absorption bands suggesting the intricate nature of phytochemicals in *M. longifolia* extracts.

DISCUSSION

This shows the significant results of comprehensive analysis for *M. longifolia* leaf extracts, indicating that this plant can act as an important therapeutic agent. The extraction with solvent of different polarities led to a significant amount in bioactive compounds and the highest activations were elicited by ethanol

Table 2: Analysis revealed distinct peaks at the retention times

Peak	*Retention time (min)*	*Area*	*Height*	*Area %*	*Height %*
1	4.576	106747	10465	46.29	38.36
2	10.461	123842	16811	53.70	61.63

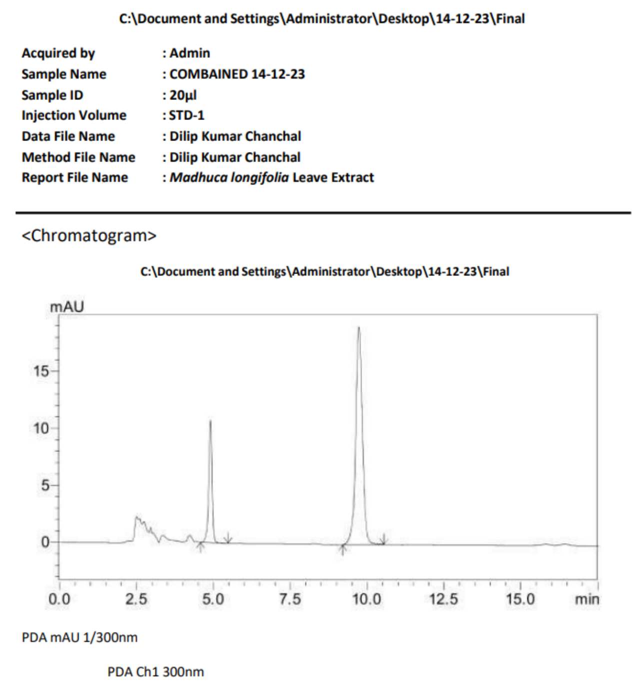
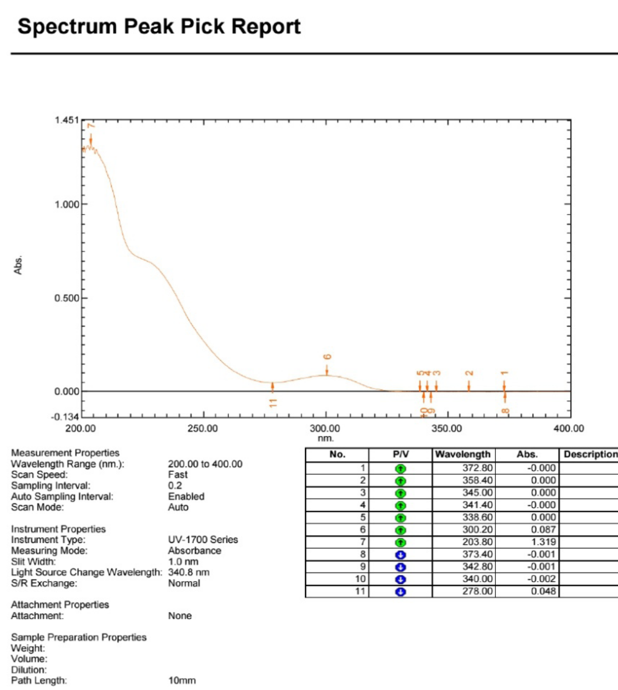


Figure 2: Graph of HPLC analysis of *M. longifolia* leaves extract



No.	P/V	Wavelength	Abs.	Description
1	↑	372.80	-0.000	
2	↑	358.40	0.000	
3	↑	345.00	0.000	
4	↑	341.40	-0.000	
5	↑	338.60	0.000	
6	↑	300.20	0.087	
7	↑	203.80	1.319	
8	↓	373.40	-0.001	
9	↓	342.80	-0.001	
10	↓	340.00	-0.002	
11	↓	278.00	0.048	

Figure 3: Graph of UV Spectroscopy of *M. longifolia* leaves extract

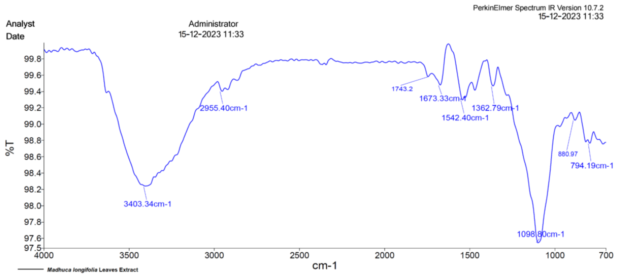


Figure 4: FTIR of *M. longifolia* leaves extract

extract. This commonality indicates ethanol to be an efficient solvent for the extraction of different classes of phytochemicals from *M. longifolia* leaf.

A qualitative phytochemical study of ML showed the existence of alkaloids, carbohydrates, glycosides, tannins, phenols, proteins, flavonoids, phytosterols, terpenes, steroids and saponins. It was quite evident from the high diversity of phytochemicals found in their extracts, especially methanol and ethanol that proved to have therapeutic competencies. These compounds confirmed various reports of traditional medicine regarding the uses claimed for roots and fruit pulp from *M. longifolia*. In fact, alkaloids have both analgesic and anti-inflammatory effects while flavonoids and phenols demonstrate significant antioxidant activities. That is to say, the antimicrobial and immunomodulatory effects of saponins are widespread, which might contribute to understanding why people have used this plant for centuries as an immune booster or in infection prevention.

TLC, HPLC and UV- FTIR-based analysis was used for the isolation and characterization of compounds which provided deep insights about the phytochemical profile in extracts. The Rf value (R_f 0.49) determined for all compounds by TLC analysis were consistent with the separation performance, suggesting specific and sharp peaks in the composition of mobile phase constituents. Initially, major peaks were detected at retention time 4.576 and 10.461 minutes by HPLC analysis which indicates the extracts are rich in phytoconstituents with more purity. The UV and FTIR spectra results had characterized these compounds, where the peaks of the absorption bands can be matched with some functional groups belonging to different types such as hydroxyl, carbonyl or aromatic.

Novel phytochemical constituents and comprehensive characterization of *M. longifolia* leaf extracts may participate in a range of therapeutic applications. The presence of secondary metabolites such as alkaloids, flavonoids, phenols and saponins suggests the possibility that some bioactive compounds might be found for preventive or therapeutic use against inflammations, antioxidantogenicity (effects), and antimicrobial activity into new cellular immunity drugs. The cholesterol-lowering benefits of phytosterols may have implications for cardiovascular health as well. The present study has thus provided a mechanistic basis for the traditional use of *M. longifolia*, and underlines its value in drug discovery. This study is thought to be the largest report on whole analysis of the phytochemical profile and medicinal potential of *M. longifolia* leaf extracts. The results validate the use of this plant for several therapeutic applications and demonstrate its potential in new drug development. Further research should be directed toward the pharmacological validation of these isolated compounds and their mode action, in order explore optimal therapeutic advantages derived from *M. longifolia*.

CONCLUSION

Based on the above information, this study provides a detailed report of extraction techniques along with illustration and identification of compounds from *M. longifolia* leaf extracts. This extraction process also resulted in a relatively large amount of bioactive extracted with the highest yield achieved for ethanol extract. The phytochemical screening of extracts indicated biological active compounds like alkaloids, carbohydrates, glycosides, phytosterols, tannins phenolics agents, proteins and amino acids, flavonoids, Terpenoids, steroids, saponins.

By doing thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) for the extracts of *M. longifolia* and ultraviolet (UV)/Fourier transform infrared (FTIR) Spectroscopy, this study exhibits is comprehensive documentation evidencing the chemical profile *M. longifolia*. The TLC showed consistency in R_f values and the HPLC showed two to three prominent peaks also with characteristic absorption peaks shown by UV spectra, confirming that some specific, well-defined phytoconstituents are present in these extracts.

Based on the rich bioactive content, *M. longifolia* leaf extracts should be useful for diverse therapeutic uses. These phytochemicals have been previously described in several plant species with analgesic, anti-inflammatory antioxidant, antimicrobial and immunomodulatory activities that support the traditional medicinal use of this plant to treat different health disorders. Phytosterols also present with cholesterol-lowering activity, which may be exploitable for stronger cardiovascular health applications.

The phytochemical screening of *M. longifolia* leaves revealed that it is rich in various bioactive molecules, suggesting potential medicinal properties. The results of this study support the claimed traditional uses and provide a scientific base, which could be further pharmacologically evaluated. However, further studies are needed to be conducted in the future, aiming at the detailed pharmacological research of isolated compounds and their mode of action for a better understanding of their therapeutic potential on *M. longifolia*.

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