Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.13.01.Art085

In silico and *In vitro* Study Prediction of the Anti-inflammatory Activities of Identified Bioactive Compounds from *Madhuca indica* Flower Extract

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Abstract

Inflammation is the body's protective response to harmful stimuli such as pathogens, damaged cells, or toxic compounds. However, chronic inflammation can lead to various diseases, including cancer, arthritis and cardiovascular disorders. Natural products have gained attention for their anti-inflammatory properties. This study aims to predict the anti-inflammatory activities of bioactive compounds found in Madhuca indica flower using both in silico and in vitro methods to explore their therapeutic potential in managing inflammatory conditions effectively. A flower extract of M. indica was prepared and analyzed to identify its bioactive components. Phytochemical screening revealed the presence of flavonoids, tannins and saponins. Fourier Transform Infrared Spectroscopy (FTIR) confirmed the functional groups corresponding to these compounds. The extract exhibited significant antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, supporting its free radical scavenging potential and aligning its anti-inflammatory properties. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified specific bioactive compounds, which were further assessed through molecular docking studies using AutoDock. The in silico studies demonstrated strong binding affinities of these compounds toward key inflammatory markers, suggesting their therapeutic potential. In vitro studies confirmed the extract's anti-inflammatory effects by showing significant inhibition of inflammatory mediators. These findings indicate that M. indica flowers possess promising anti-inflammatory properties, attributable to their bioactive compounds providing scientific evidence for their potential in developing natural anti-inflammatory therapeutics. The study highlights the medical significance of M. indica, encouraging further research into its clinical applications for managing inflammatory disorders.

Keywords: *Anti-inflammatory Activity, Bioactive Compounds, Madhuca indica, Molecular Docking, Phytochemical Screening.*

Introduction

Inflammation can be described as a type of protection to pathogens, damaged cells or irritants. It is essential for wound healing and tissue regeneration; however, chronic conditions can result in several

Received: 20.11.2024 ***Accepted: 06.02.2025*** ***Published on: 28.03.2025***

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diseases, such as arthritis, diseases of the heart and circulatory system and cancer [1]. Some drugs that are used to reduce inflammation, such as ibuprofen and aspirin, have proved effective; however, they are associated with dangers, such as the development of ulcers in the stomach and increased cardiovascular risks [2]. This has led to the search for natural and relatively safe remedies.

Using plants for anti-inflammatory purposes has been known for some time now. The active compound obtained from *Curcuma longa* is curcumin which helps in controlling conditions like arthritis due to interference with the prostaglandin synthesizing enzyme COX-2 and Nuclear Factor-kappa B (NF-κB) [3]. Grapes (*Vitis vinifera*) contain resveratrol for its anti-inflammatory effects via NF-κB and COX-2 [4]. Some of the ingredients present in the herbal compound as *Boswellia serrata* contain boswellic acid that helps control 5-lipoxygenase which greatly decreases the level of leukotriene reducing the inflammation characteristics to diseases like asthma and arthritis [5]. Ginger (*Zingiber officinale*) contains gingerols and shogaols that reduce the production of inflammatory cytokines and prostaglandins which are helpful in OA [6].

In this context, *Madhuca indica*, a tree known as 'Mahua', is found in different parts of India and Southeast Asia. The flower contains bioactive components in the form of flavonoids, alkaloids and terpenoids as analyzed through FTIR and GCMS [7]. These compounds have possible therapeutic value, more precisely the anti-inflammatory and antioxidant effects [8]. For instance, flavonoids are well recognized for their effects on TRPV1 mediated pathways involved in inflammation; however, alkaloids and terpenoids also provide general therapeutic value to plants [9].

Oxidative stress is defined as the conditions where the production of free radicals prevails over the levels of antioxidants and is related to inflammation [10]. Some examples of what antioxidants do include: They are responsible for the removal of free radicals and their management of oxidative stress and inflammation [11]. A previous study on the effects of various extracts of *M. indica* flowers reported a high antioxidant property for extracts containing flavonoids and phenolic compounds [7, 12]. Previous studies also stated that these antioxidants are used in the regulation of inflammation following the protection of cells from oxidative damage [13].

In silico tools in drug discovery are a fast and effective way to establish contacts between active compounds and certain proteins, thereby shortening the time required to identify beneficial therapies [14, 15, 16]. This method has been found to yield reasonable estimations that do not always align with the experimental outcomes [17]. Thus, in the case of *M. indica* research for the treatment of diseases such as cancer, there will be a decrease in the number of experimental animals used and *in silico* studies will help to forecast the therapeutic index of the compound of interest [18]. Using molecular modelling computations with traditional phytochemical analyses can better illustrate the therapeutic benefits of natural products [19].

Based on the role that inflammation plays in many diseases, the existence of drugs with anti-inflammatory properties is vital and the *in silico* approach offers a new and efficient way to produce new agents from *M. indica* and other sources [20]. The study aims to explore the anti-inflammatory and antioxidant properties of *M. indica* (Mahua) flowers, focusing on their potential to treat inflammation-

related conditions. By combining traditional methods like phytochemical analysis with modern *in silico* techniques, the research identifies key bioactive compounds in the flower. The goal is to understand how these compounds interact with inflammation-related proteins, their antioxidant effects and their suitability for therapeutic use. Ultimately, the study seeks to contribute to the development of natural treatments for inflammation, offering a safer plant based alternative to conventional drugs.

Materials and Methods

Preparation of Aqueous Extract

The *M. indica* flowers were collected in March from Orissa, India. The samples were authenticated by the Centre for Advanced Studies in Botany at the University of Madras, Chennai, India. The *M. indica* flower powder was kept for drying in the shade for 3 days. Further, the experiment followed the outlined methodology [21].

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an analytical method used to identify organic, polymeric and occasionally inorganic substances. The device quantifies the infrared strength from the wavelength of light. It produces a spectrum that depicts the molecular absorption and transmission, thus providing a distinctive molecular identifier of the sample. After the extraction process, the *M. indica* flower was analyzed by FTIR spectroscopy. This analysis effectively identified functional groups that are present in the *M. indica* flower.

Gas Chromatography-Mass Spectrometry (GCMS)

GCMS is a method of analysis that combines GC with MS to detect and identify various chemicals in *M. indica* flower. It is extensively used in the fields of drug detection, environmental analysis and the identification of unfamiliar substances. This was achieved by separating the chemical mixtures and providing comprehensive molecular data. 100 µL of an aqueous solution of *M. indica* flower was dissolved in 1 mm of methanol. The solution was agitated firmly using a vortex stirrer for 20 sec and then filtered through a 0.2 m membrane filter. Subsequently, this clear extract was used for GC-MS examination [21].

Phytochemical Analysis

Phytochemical analysis refers to the evaluation and identification of different chemical substances generated by plants, which are referred to as phytochemicals. These chemicals frequently possess bioactive characteristics that help in plant defence mechanisms against pests, diseases and environmental pressures. An in depth analysis is essential for comprehending the therapeutic capabilities of plants in both traditional and modern medicine, as well as for uncovering novel pharmaceuticals and health supplements. Phytochemicals encompass a variety of compounds such as flavonoids, alkaloids, tannins, etc. Each of these compounds has distinct characteristics and offers specific advantages for human health. A qualitative analysis of the aqueous extract of *M. indica* flower was conducted using various chemical tests [22].

Antioxidant Assay

Antioxidant activity refers to the capacity of plant derived substances to neutralize the harmful effects of free radicals, which are unstable entities capable of inducing cellular harm via oxidative stress. Plants synthesize a diverse range of antioxidant chemicals,

including flavonoids, phenolic acids and vitamins, to protect cells against oxidative damage. Antioxidants function by transferring electrons to free radicals, thus stabilizing them and inhibiting their potential to cause damage. The antioxidant activity of *M. indica* flower extracts can be quantified using many assays, including the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The presence of plant derived antioxidants is useful in reducing the risk of chronic illnesses, such as cancer by relieving oxidative stress and strengthening the body's immune system [22].

In silico Study

The protein structures were acquired from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (accessed: September 02, 2024: https://www.rcsb.org/) and verified using a build/check/repair model to confirm their integrity. Auto Dock Tools were used to prepare the pdbqt files. The ligands obtained from PubChem were subjected to optimization using the Avogadro software and were subsequently converted for docking. AutoDock4 performed molecular docking using a refined grid, followed by cluster analysis to identify the most favourable binding positions. The analyzing binding interactions and affinities were analyzed using Biovia Discovery Studio (accessed: September 02, 2024: https://www.3ds.com/products/biovia/discovery-studio). Swiss ADMET (accessed: September 02, 2024: http://www.swissadme.ch/) was used to conduct *in silico* ADMET research, which involved evaluating drug likeness according to Lipinski's rule of five. Additionally, key pharmacokinetic parameters such as ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) were assessed. The metabolic stability and drug metabolism profiles were predicted by analyzing cytochrome P450 (CYP) enzyme interactions, gastrointestinal (GI) absorption, blood brain barrier (BBB) permeability and bioavailability scores. This evaluation provided insights into the therapeutic efficacy and safety of the studied compounds by examining interactions with biological components [19, 23, 24]. Figure 1 presents the graphical abstract of the computational investigation conducted on the extract of *M. indica* flowers.

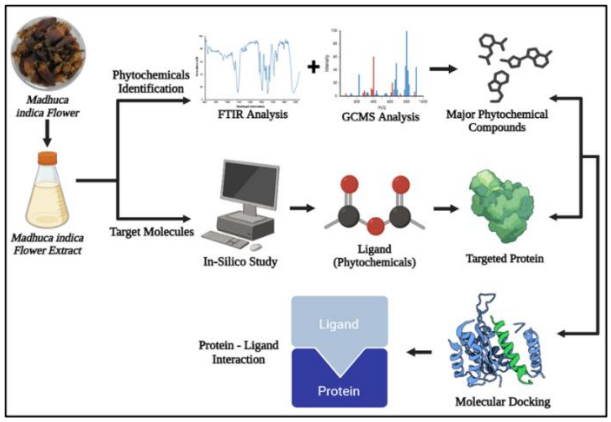

Figure 1. Graphical Abstract of the *In Silico* Study on *Madhuca indica* Flower Extract

Results

Preliminary Phytochemical Screening

M. indica's phytochemical examination reveals several bioactive chemicals as described in Table 1. The extract's pharmacological potential is facilitated by

its modest concentrations of terpenoids, alkaloids, tannins, saponins, proteins and fatty acids. Therapeutic benefits are suggested for saponins, which have immune boosting and anticancer effects and alkaloids, which are known for their analgesic and antibacterial qualities. The high flavonoid content suggests that they have substantial anti-inflammatory and antioxidant properties. Terpenoids have anticancer and antibacterial properties, whereas moderate tannins have astringent, antimicrobial and anti-inflammatory properties. Even in small amounts, phenols have antioxidant activity. Proteins and carbohydrates draw attention to the extract's nutritive value and biological activity, whereas fatty acids promote cellular health and have anti-inflammatory properties. The lack of glycosides suggests that these compounds do not have any particular medicinal benefits. In general, *M. indica's* potential for various medicinal and nutritional uses is highlighted by its moderate to high content of numerous bioactive components.

Table 1. Phytochemical Properties of *Madhuca indica* Flower Extract

Phytochemicals	***Madhuca indica***
Alkaloid	++
Flavonoid	+++
Tannins	++
Saponin	++
Glycoside	-
Terpenoids	++
Phenol	+
Carbohydrates	++
Proteins	++
Fatty acids	++

(-): Indicate Absent

(+): Indicates a weak or low presence.

(++): Indicates a moderate presence.

(+++): Indicates a strong or high presence.

FTIR

When the bioactive component of *M. indica* is analyzed using Fourier Transform Infrared Spectroscopy (FTIR), a diverse variety of functional groups are revealed. Important peaks include a large peak around 3285 cm^{-1} that indicates the presence of hydroxyl groups, a peak for alkane C-H stretching at 2933 cm^{-1}, a peak for carbonyl C=O stretching at 1624 cm^{-1} and peaks for C-O stretching in ethers or esters at 1403, 1226 and 1033 cm^{-1} shown in Figure 2. Furthermore, the 817 cm^{-1} peak indicates the presence of aromatic chemicals. Alcohols, phenols, carboxylic acids, ketones, aldehydes, ethers, esters and aromatic compounds are found in these samples, indicating that the bioactive substance is a complex organic molecule with a variety of functional groups that contribute to its bioactivity.

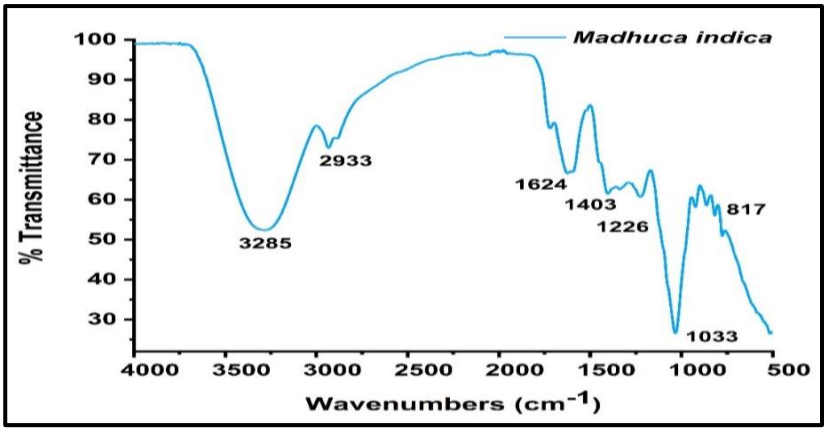

Figure 2. FTIR Spectra of the *Madhuca indica* Flower Extract

GC-MS

The sample contains chemicals with differing abundances, as seen by the multiple peaks at various retention times, according to the GCMS analysis shown in Figure 3. The retention time is plotted on the x-axis and the intensity is plotted on the y-axis. The approximate times of the major peaks are 6.01, 6.64, 7.91, 8.29, 9.86, 11.20, 11.53, 14.66 and 15.00 min. With an intensity of approximately 2,12,680 units, the largest peak at 14.66 min indicates the most abundant component. Less prominent peaks elsewhere indicate the existence of more chemicals. Well defined, sharp peaks indicate effective separation. An overview of sample composition is provided by the total ion chromatogram (TIC). A mass spectral library search and comparison with established standards are recommended to identify certain substances. Based on this investigation, a complex mixture containing multiple minor and one major chemical was suggested.

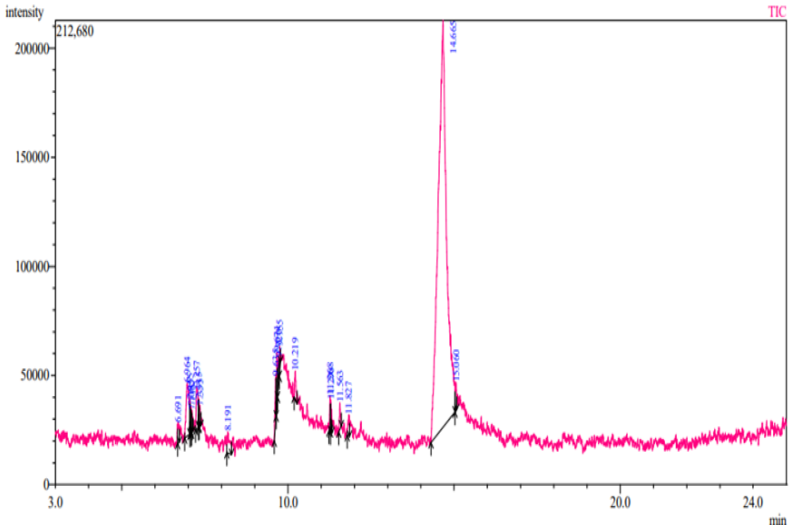

Figure 3. GC-MS Chromatogram of *Madhuca indica* Flower Extract

Antioxidant Activity

The plant extract from *M. indica* exhibited significant free radical scavenging activity, according to the DPPH assay illustrated in Figure 4. The extract exhibits a dose dependent increase in the percentage inhibition at concentrations of 10-50 µg/mL. Values range from 65% to 90%, which is in close

agreement with the 70% to 92% inhibition of typical antioxidants. The standard shows marginally more inhibition at each concentration, but the differences are negligible, suggesting that *M. indica's* antioxidant activity is comparable. The extract inhibited 90% inhibition at 50 µg/mL, highlighting its antioxidant nature. The potential medicinal benefits of *M. indica* are highlighted by its noteworthy antioxidant activity.

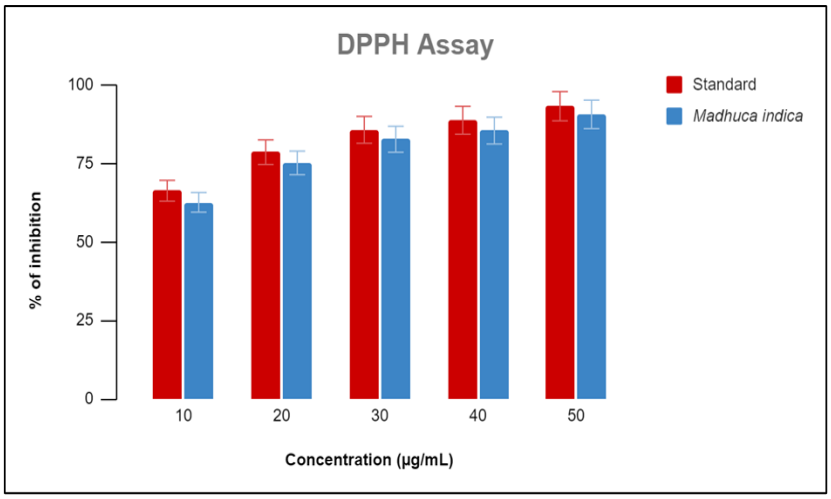

Figure 4. DPPH Assay of *Madhuca indica* Flower Extract

Molecular Docking

The binding affinities and specificities of the *M. indica* plant extract ligands (1EXT with C1, 1EXT with C2 and 1EXT with Catechol) toward a target protein are presented in the molecular docking study presented in Table 2 and Figure 5. While 2D diagrams illustrate simplified depictions of these interactions with protein residues, 3D interactions show spatial arrangements and important interactions, including hydrogen bonds, hydrophobic contacts and π-π stacking within the active site. 1EXT fits well with C1 and C2, demonstrating potential efficacy and substantial binding affinity. Catechol's interactions with hydroxyl groups imply that it might be a lead molecule because of its particular hydrogen bonding. By comparing these ligands, important residues and variations in binding strength can be observed, suggesting that they could serve as lead compounds for additional optimization. Understanding ligand protein interactions is made easier by this research, which will help with future drug discovery and experimental validation to support computer predictions.

Table 2. Molecular Docking Analysis of *Madhuca indica* Flower Extract

S.No	Name of the Ligands	Binding Affinity Value (kcal/mol)	Distance (Å)	Hydrogen Interaction	Amino Acid Residues
1	1EXT with C1	-4.9	2.3(CYS 96), 2.0 (GLU 109), 2.3(TYR 106),	1. Van der Waals 2. Conventional Hydrogen Bond 3. Pi- Donor Bond	1. GLU A: 109, ASN A:110, THR B:94, ASN B:110, PHE B:112 2. ARG B:104, TYR B:106 3. CYS B: 96

			2.3(ARG 104)	4. Pi- Alkyl	4. VAL B:95
2	1EXT with C2	-4.7	2.7(ARG 104)	1. Van der Waals 2. Unfavorable Donor-Donor 3. Pi- Donor Hydrogen Bond 4. Pi- Anion	1. THR B:94, ASN B: 110, PHE B:112, ARG B:104, GLU A:109, ASN A:110 2. TYR B:106 3. CYS B:96 4. VAL B:95
3	1EXT With Catechol	-5.2	2.1 (GLU 149) 2.1 (CYS 150)	1. Van der Waals 2. Unfavorable Donor-Donor 3. Pi- Anion 4. Pi -Alkyl	1. VAL B:151, ASN B:122, SER B:118, CYS B:120, LEU B:119 2. CYS B:150 3. GLU B:149 4. LEU B:121

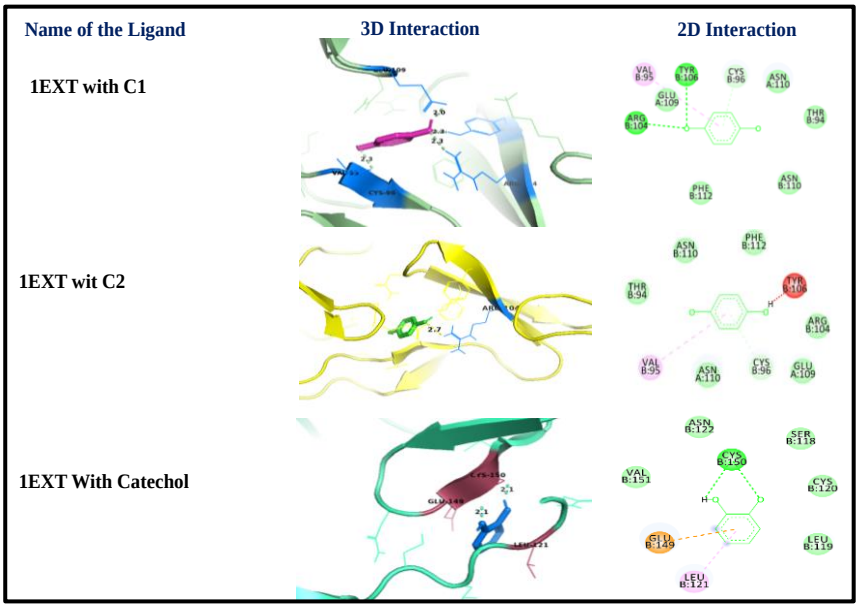

Figure 5. Molecular Docking Analysis of *Madhuca indica* Flower Extract

ADMET Properties of Drug

ADMET analysis revealed some characteristics of a compound that was isolated from the plant *M. indica*, as illustrated in Figure 6. This substance probably contains an ester group with a long hydrocarbon chain and multiple double bonds present in the fatty acid portion of the molecule. While it has high lipid solubility meaning that it can easily pass through cell membranes, its aqueous solubility is very low. For druglike properties, medium size and flexibility are helpful, whereas oral bioavailability is an issue because of its high insolubility and low polarity. The presence of many double bonds, which characterize high unsaturation may affect the stability and reactivity. Some characteristics of ADMET include the drawbacks of the compounds along with their potential use in the development of new drugs. Due to the latter parameter`s low polarity and high non solubility; however, more modifications are needed to increase the compound`s bioavailability and therapeutic effect despite its high lipophilicity factor which enhances the membrane permeability.

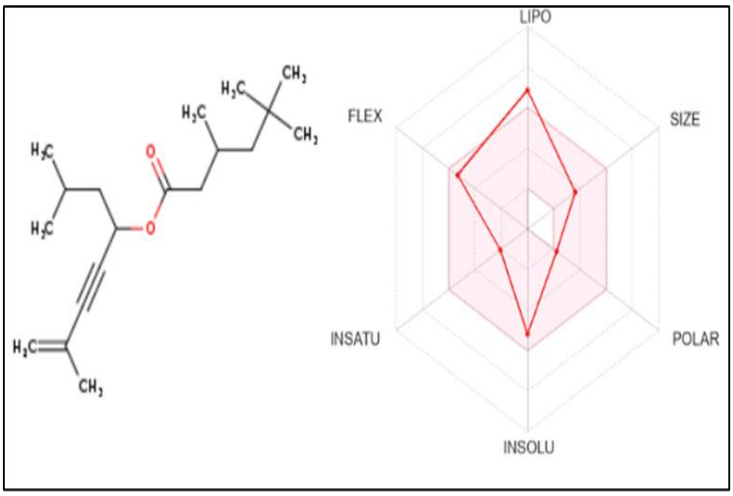

Figure 6. ADMET Assay of *Madhuca indica* Flower Extract

Physiochemical Properties

The extract from the *M. indica* plant has desirable physicochemical characteristics as shown in Table 3 and is suitable for drug formulation; its molecular weight is 194. The molecular weight of 18 g/mol. This indicates a good ADMET profile, drug likeness and oral bioavailability. It has good water solubility which helps in formulation and patient compliance given that the product is orally administered. On the other hand, at a high TPSA of 107. 22 Å² and poor GI absorption indicate inadequate absorption and penetration in the GI tract which impacts bioavailability. Unfortunately, it cannot be used for CNS treatment mainly because it does not cross the blood brain barrier. However, it has several drawbacks and counter helps: Thus, it has a relatively average bioavailability and makes up 0. 55 and a synthetic accessibility score of 3. It is also reasonably achievable to synthesize 52 and demonstrate reasonable efficacy in systemic circulation. Hence, optimization strategies are required to retain desirable features, while enhancing the ability of the product to be absorbed and assimilated into the body.

Table 3. Physiochemical Properties of *Madhuca indica* Flower Extract

Physiochemical Properties	
Mol wt (g/mol)	194.18 g/mol
Formula	C7H14O6
Canonical SMILES	C1=CC(=CC=C1O) O
TPSA	107.22 Å²
BBB permeant	No
GI absorption	Low
Lipinski violations	Yes; 0 violation
Bioavailability Score	0.55
Synthetic Accessibility	3.52
Water solubility	Highly soluble

Discussion

In this study, numerous tests were performed to determine the impact of the *M. indica* plant extract. The results demonstrated that the plant extract yielded significant outcomes, indicating the presence of bioactive compounds that can be utilized in the medical field. FTIR spectroscopy was employed to confirm the presence of functional groups responsible for biological activity [25]. The spectra displayed several characteristic absorption bands, including hydroxyl (-OH), carbonyl (C=O) and alkene (C=C) groups, which are typically associated with flavonoids, phenols and other phytochemicals. These findings align with previous studies demonstrating that functional groups present in medicinal plants contribute to their antioxidant, antimicrobial and anti-inflammatory properties [26]. Such molecular groups play essential roles in interacting with cellular components and enhancing pharmacological activities. The current study supports prior research indicating that the concentration of phytochemicals influences bioactivity and the presence of these functional groups validates the medicinal potential of *M. indica*.

A qualitative analysis using GC-MS was conducted to confirm the extraction of major chemical constituents. The chromatographic profile showed a significant peak at 14.66 minutes, confirming the presence of a key bioactive compound. Additional minor peaks of moderate intensity further suggested the existence of other pharmacologically relevant molecules. Previous studies have confirmed that compounds eluted at similar retention times in GC-MS profiles correspond to essential phytochemicals known for their medicinal properties [27, 28]. The identification of these compounds was cross verified using a mass spectrometry library search and comparison with authentic standards, ensuring the accuracy of the detected constituents. The presence of multiple bioactive compounds indicates that *M. indica* has a complex phytochemical composition, supporting its broad spectrum of biological activities, including antibacterial and antioxidant properties.

The antioxidant activity of *M. indica* extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely accepted method for evaluating free radical inhibition. The extract demonstrated high antioxidant activity, with inhibition percentages ranging from 65% to 90% depending on the concentration. These results are consistent with previous findings on medicinal plants that exhibit strong radical scavenging abilities [29, 30, 31]. The free radical scavenging potential of *M. indica* suggests its effectiveness in reducing oxidative stress, which is a significant factor in the pathogenesis of chronic diseases such as cancer, diabetes and neurodegenerative disorders. The dose dependent antioxidant activity further validates its role as a natural source of antioxidants, emphasizing its potential in therapeutic applications. The findings also indicate that *M. indica* extract functions similarly to standard antioxidants, supporting its pharmacological relevance in preventing oxidative damage.

The phytochemical screening of *M. indica* extract revealed the presence of bioactive compounds such as terpenoids, alkaloids, tannins, saponins, proteins and fatty acids. These compounds contribute to the therapeutic potential of the extract. Flavonoids and phenols, which were identified in the extract, are well known for their antioxidant and anti-inflammatory properties, as confirmed by recent

literature on plant based pharmacology [9, 32]. Additionally, saponins demonstrated immune boosting and anticancer effects, supporting their traditional medicinal applications. Alkaloids were observed to exhibit antibacterial and analgesic activities, reinforcing the ethnopharmacological use of *M. indica* in treating infections and pain management. These phytochemicals play essential roles in the biological activity of the extract and provide a rationale for its continued study in pharmaceutical development.

Molecular docking analysis was performed to understand the binding patterns of *M. indica* ligands with target proteins, revealing high binding affinity and other significant molecular interactions. The visual representation of 3D interactions highlighted the formation of hydrogen bonds, hydrophobic interactions and π-π stacking, which are crucial for the stability and efficacy of potential drug molecules. Among the detected compounds, catechol exhibited the highest hydrogen bonding potential, making it a promising candidate for drug design. This result is consistent with computational studies on plant derived natural products that show similar interaction patterns essential for biological effects [33, 34, 35]. The molecular docking findings emphasize the role of ligand protein interactions in drug discovery and reinforce the potential of *M. indica* in the development of novel therapeutic agents. These results provide a molecular basis for the pharmacological effects of the extract and highlight its value in future drug development initiatives.

An analysis of the ADMET properties of the isolated compounds revealed both advantages and limitations in their potential use in drug development. The lipophilic nature of the compound suggested favourable membrane penetration and diffusion, which are essential for bioavailability. However, the low aqueous solubility and high polarity presented challenges for oral absorption. These results align with studies on natural compound pharmacokinetics, where structural modifications are often required to enhance bioavailability [36, 37]. The physicochemical properties further indicated that the molecular weight of the compounds was within the acceptable range for drug likeness, suggesting their suitability for pharmaceutical applications. However, the low gastrointestinal (GI) absorption and high Topological Polar Surface Area (TPSA) values suggest that further modifications are necessary to optimize the absorption and therapeutic efficacy of the compounds. Formulation strategies such as nanoencapsulation or chemical modifications could help overcome these limitations and improve the drug potential of *M. indica* derived compounds.

Based on the findings of this study, it can be concluded that the *M. indica* plant extract exhibits multiple therapeutic properties, including antioxidant, anti-inflammatory, antibacterial and anticancer activities. These pharmacological properties are supported by phytochemical screening, molecular docking studies and ADMET analysis. The presence of bioactive compounds with high medicinal value warrants further investigation into their mechanisms of action and potential applications in modern medicine. Future research should focus on optimizing the pharmacokinetics of these compounds through formulation techniques and conducting *in vivo* studies to validate their therapeutic efficacy. Additionally, further identification of secondary metabolites and their synergistic effects could enhance the understanding of *M. indica's*

pharmacological potential. The results of this study contribute to the growing body of evidence supporting the use of traditional medicinal plants in drug discovery and development, promoting their integration into modern pharmaceutical formulations. With continued research and innovation, *M. indica* holds significant promise for contributing to the development of new bioactive compounds for medical and food production applications.

Conclusion

This present study successfully integrated *in silico* and *in vitro* approaches to predict and validate the anti-inflammatory potential of bioactive compounds extracted from *M. indica*. The computational analyses, including molecular docking and ADMET profiling, identified several key compounds with strong binding affinity to inflammatory targets, suggesting their potential as therapeutic agents. Subsequent *in vitro* assays corroborated these findings, demonstrating significant anti-inflammatory activity that aligned with *in silico* predictions. This dual approach not only highlights the potential of *M. indica* as a source of natural anti-inflammatory agents and emphasizes the utility of combining computational and experimental methods in the early stages of drug discovery. Future studies will focus on further characterizing these bioactive compounds, exploring their mechanisms of action and evaluating their efficacy *in vivo*, paving the way for the development of novel anti-inflammatory therapies.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

The authors have no relevant financial or non-financial interests to disclose.

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