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"Development and Characterization of Phytosomes Loaded with Ethanolic Extract of Mangifera indica Leaves"

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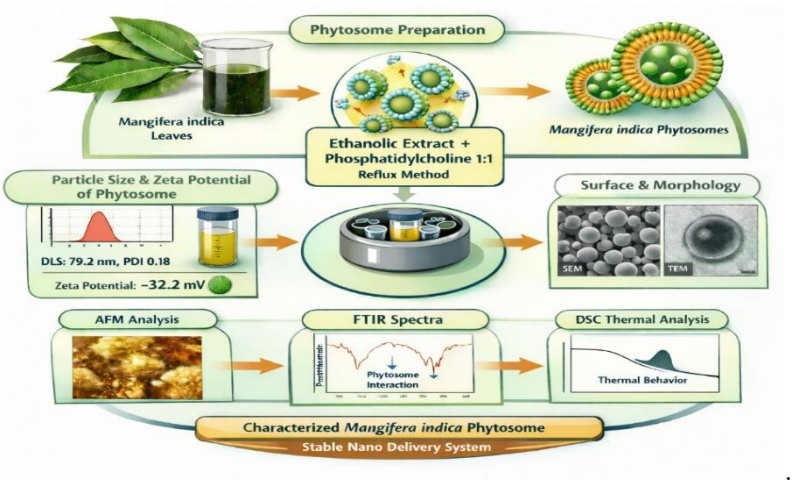
ABSTRACT:

Ethanolic extract of Mangifera indica leaves was prepared and subjected to qualitative and quantitative phytochemical analysis, confirming high levels of phenolics and flavonoids. Phytosomes were formed by complexation of the extract with phosphatidylcholine using a reflux method, in 1:1 ratio of extract: phosphatidylcholine. The resultant vesicles were purified and lyophilized for characterization. Particle size and polydispersity index were measured by dynamic light scattering, yielding uniformly distributed nanosized vesicles with a mean diameter of approximately 79.2 nm and low PDI. Zeta potential analysis indicated good colloidal stability (−32.2 mV). Entrapment efficiency was determined by ultracentrifugation and spectrophotometric quantification, giving 67.24%. Surface and morphological analyses were performed by SEM and TEM, confirming spherical morphology and nanoscale dimensions, while AFM provided topographical details. FTIR spectroscopy showed characteristic interactions between extract phytoconstituents and phosphatidylcholine, indicating complex formation. DSC thermograms revealed changes in thermal behaviour consistent with molecular interaction and partial amorphization of the extract within the phytosomal matrix. The developed phytosome represents a well-characterized, stable delivery system for Mangifera indica phytoconstituents, suitable for further preclinical evaluation.



Keywords: Leaves, Mangifera indica, and Phytosomal Delivery System

INTRODUCTION

Mangifera indica (family: Anacardiaceae), commonly known as mango, is an extensively studied medicinal plant ....

with reported antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities. Its leaves are rich in bioactive phytoconstituents such as mangiferin,

flavonoids, phenolic acids, tannins, and xanthonoids[i]. Mangiferin, a C-glucosyl xanthone, exhibits potent antioxidant and glucose lowering effects through modulation of oxidative stress, insulin signalling pathways, and carbohydrate-metabolizing enzymes. However, the therapeutic application of plant extracts is often limited by poor aqueous solubility, low bioavailability, and inadequate membrane permeability of active phytochemicals.[ii,iii,iv]

To overcome these limitations, lipid-based delivery systems such as phytosomal formulations have emerged as effective strategies to enhance the bioavailability and therapeutic efficacy of herbal extracts. Phytosomal formulations are phospholipid–phytoactive complexes formed by interaction between plant constituents and phosphatidylcholine, resulting in improved solubility, membrane compatibility, and systemic absorption. Compared to conventional extracts, they exhibit enhanced stability, controlled release, and improved pharmacokinetic behaviour.[v,vi,vii]

Based on these considerations, the present study was designed to perform phytochemical evaluation of *Mangifera indica* leaves, develop and characterize a phytosomal formulation

The novelty of this work lies in the development of a phytosomal formulation.

Methodology

Chemicals and Reagents

All chemicals and solvents used were of analytical grade. Ethanol, methanol, petroleum ether (60–80 °C), ethyl acetate, and n-hexane were used for extraction and formulation procedures. Phosphatidylcholine was used as the phytosomal lipid component.

Plant Material and Extraction

Fresh leaves of Mangifera indica were collected from Maharashtra, India, and authenticated at Dr. Babasaheb Ambedkar Marathwada University. A voucher specimen was preserved for future reference. The leaves were shade-dried, powdered, and extracted with 90% ethanol by Soxhlet extraction at 40 °C for 48 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use.

Phytochemical Analysis

Preliminary phytochemical screening of the ethanolic extract was carried out using standard qualitative methods for the detection of flavonoids, phenolics, tannins, glycosides, steroids, carbohydrates, proteins, and saponins.

HPTLC Analysis

High-Performance Thin Layer Chromatography (HPTLC) was carried out to develop the phytochemical fingerprint profiles of the ethanol extracts of *Mangifera indica.* The samples were applied on pre-coated silica gel 60 F254 TLC plates using a CAMAG Linomat V applicator. Chromatographic development was performed in a twin trough chamber pre-saturated with a mobile phase consisting of Toluene: Ethyl acetate: Formic acid (optimized ratio) up to a migration distance of 80 mm. post-development, the plates were air-dried and observed under UV light at 254 nm and 366 nm, followed by derivatization using anisaldehyde-sulfuric acid reagent to visualize the separated compounds under white light.[viii]

HPLC Analysis

Mangiferin in the extract was analyzed by reverse-phase HPLC using a C18 column with methanol: water (60:40v/v) as the mobile phase. Identification and quantification were performed using a standard calibration method.[ix]

Preparation of Phytosomal Formulation

Phytosomes of Mangifera indica extract were prepared by complexing the extract with Phosphatidylcholine in 1:1 ratio. The extract and phospholipid were dissolved in ethyl acetate and subjected to sonication to obtain a uniform dispersion. The mixture was then mildly heated

under controlled conditions to facilitate solvent evaporation and formation of a thin lipid–extract film. The resulting complex was precipitated using n-hexane, followed by filtration and collection. The obtained phytosomal

complex was vacuum-dried to remove residual solvents and ensure complete solidification. The final formulation was stored in a tightly closed container under nitrogen atmosphere at room temperature until further use[x].

Characterization of Phytosomal Formulation

Particle size, polydispersity index (PDI), and zeta potential were measured by dynamic light scattering (DLS). Entrapment efficiency was determined by centrifugation followed by spectrophotometric analysis of unentrapped extract.

FTIR and DSC analyses were performed to evaluate compatibility and thermal behaviour of the formulation[xi, xii,xiii]. Surface morphology and vesicular characteristics were examined using SEM, TEM, and AFM[xiv,xv].

Result

Physicochemical Evaluation

Powdered *Mangifera indica* leaves showed moisture content of 8.34% w/w and loss on drying of 8.9% w/w. Total ash was 2.80% w/w, with acid-insoluble ash (3.88% w/w) and water-soluble ash (4.34% w/w). Extractive values were higher in ethanol (22.43% w/w) than water (18.30% w/w), indicating predominance of ethanol-soluble phytoconstituents.

Preliminary Phytochemical Screening

Qualitative analysis confirmed the presence of glycosides, tannins, flavonoids, steroids, proteins, carbohydrates, fats & oils, phenols, and diterpenes, while alkaloids and saponins were mostly absent. These findings were supported by standard chemical tests. (Table No. 1)

Table No 1 : Phytochemical investigation of Mangifera indica

S.No	Phyto-constituents	Identification Test	Result
1	Alkaloids	Mayer test, Wagner test	-ve
2	Glycosides	Legal test, Salkowski test, Keller killani test	-ve
		Liberman Buchard test	+ve
3	Tannins	Vanillin-HCL test, Gelatin test	+ve
4	Resins	Turbidity test	-ve
		Ferric-Cl test	+ve
5	Flavonoids	Shinoda test, Alkaline test	+ve
		Lead acetate test	-ve
6	Steroids	Salkowski test	+ve
		Liebermann – reaction	-ve
7	Amino acids	Ninhydrin test, Cysteine test	-ve
8	Proteins	Precipitate test, Biuret Test	+ve
9	Carbohydrate	Molish test, Benedict test	+ve
10	Fats & Oil	Sudan red test, Saponification test	+ve
		Spot test	-ve
11	Phenol	Ferric chloride test	+ve
12	Diterpens	Cooper acetate test	-ve
13	Saponins	Forth test	-ve
		Foam test	+ve

Quantitative Phytochemical Screening and Standardization of *Mangifera indica* Extract

Thin Layer Chromatography (TLC) of Ethanolic Extract of *Mangifera indica*

Thin Layer Chromatography of the ethanolic extract of *Mangifera indica* was performed using toluene: ethyl acetate: formic acid as the mobile phase. The chromatogram exhibited multiple well-defined spots under UV light at 254 nm and 366 nm, indicating the presence of diverse phytoconstituents.

The observed Rf values ranged from 0.08 to 0.98, suggesting the presence of compounds with varying polarity,

including phenolics, flavonoids, and xanthone derivatives. The results confirm the chemical complexity of the extract and support its suitability for further chromatographic analysis.

Fig. 1: TLC of Ethanol Extract of *Mangifera indica*

High Performance Thin Layer Chromatography (HPTLC) Analysis of *Mangifera indica*

HPTLC fingerprinting showed several well-resolved bands under white light, UV 254 nm, and UV 366 nm. Fluorescent red bands under UV 366 nm indicated the presence of phenolic and flavonoid compounds. The chromatographic profile confirmed the complexity of the extract and serves as a reliable fingerprint for standardization.

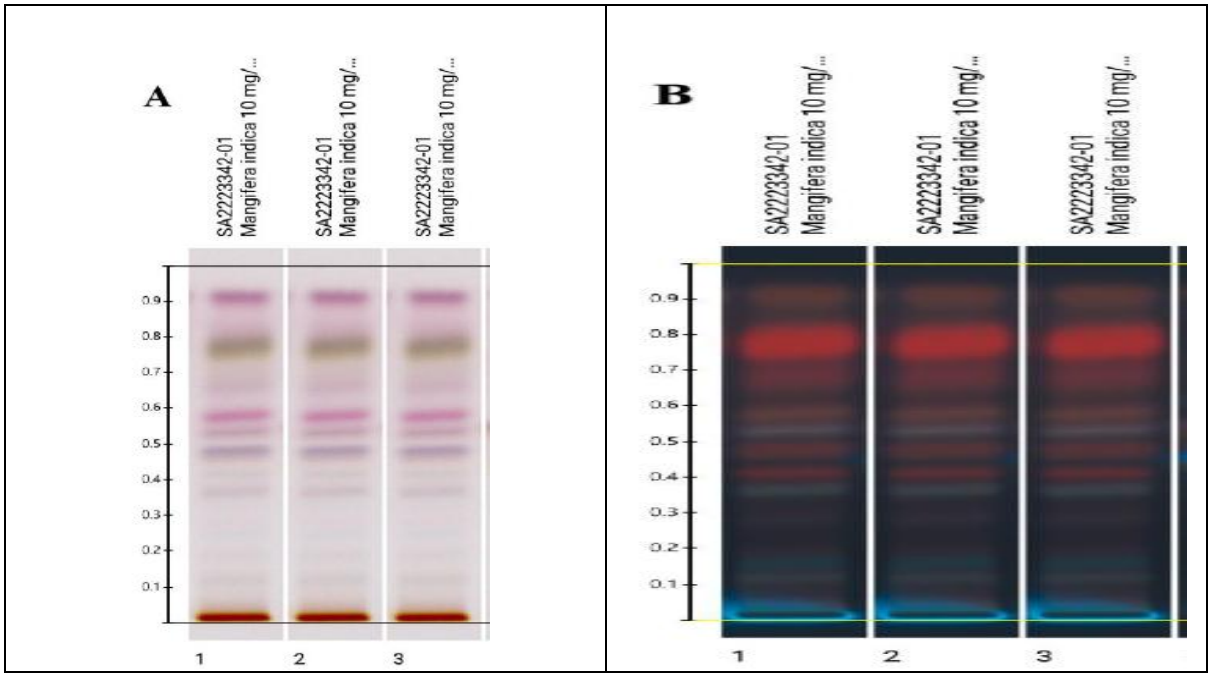


Fig 2: Derivatized HPTLC of ethanolic extracts of MI

HPLC Analysis of Mangiferin in *Mangifera indica*

Reverse-phase HPLC was used to identify mangiferin in the ethanolic extract of *Mangifera indica*. The sample was prepared by dissolving 100 mg extract in 10 mL methanol, followed by sonication and dilution. Separation was performed on an Agilent C18 column using methanol: water (60:40 v/v) as the mobile phase (flow rate 0.6

mL/min), with detection at 258 nm, injection volume of 10 µL, and run time of 6 min. A sharp peak was observed at a retention time of 2.154 min, closely matching the standard mangiferin (2.094 min), confirming its presence. The slight variation may be due to matrix effects. The method showed good peak symmetry (tailing factor 1.725) and acceptable column efficiency (2366.22 plates), indicating suitability for mangiferin identification.

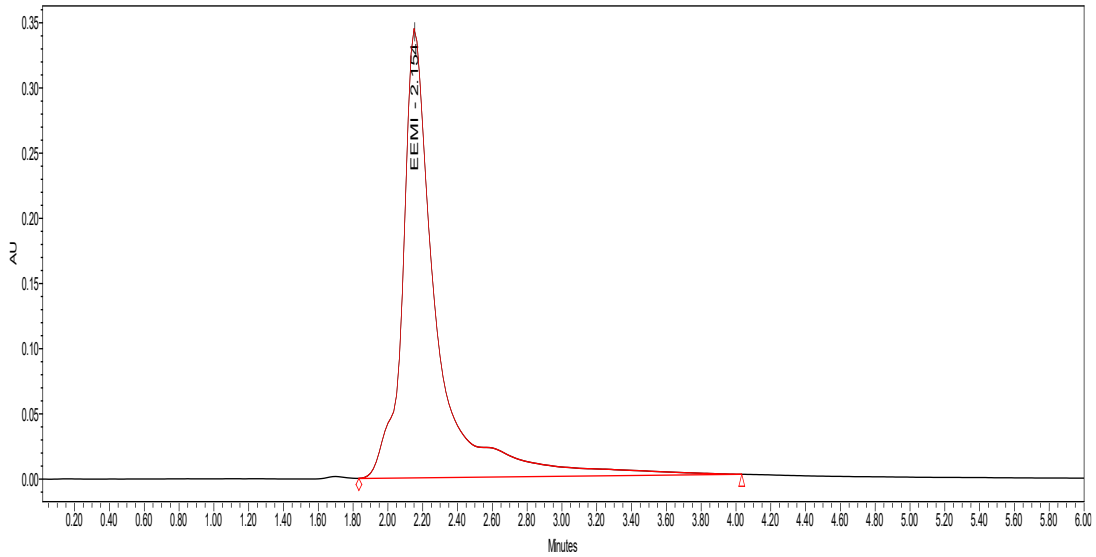


Fig 3. HPLC chromatogram showing overlay of Mangiferin standard and ethanolic extract of Mangifera indica.

High Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) Analysis of *Mangifera indica*

High-resolution LC–MS analysis was carried out to identify major phytoconstituents in the ethanolic extract of *Mangifera indica*. The LC-QTOF chromatogram showed multiple characteristic peaks, indicating a complex phytochemical profile.

A prominent peak at RT 8.535 min with molecular mass 422.0838 confirmed the presence of mangiferin. Several additional peaks observed between 8–16 min correspond to flavonoids, phenolic acids, xanthones, glycosides, and gallotannins. (Table No. 2)

Table No. 2 . HR-LCMS Characterization of *Mangifera indica* Ethanolic Extract

RT (min)	Compound Name	Mass
8.535	Mangiferin	422.0838
9.814	1,2,3,6-Tetragalloylglucose	788.1048
9.938	Eujambolin	520.1221
9.377	Digalloyliriflophenone glucoside	712.1258
8.657	Cicerin derivative	578.1262
8.596	Phenolic glycoside	440.095
16.366	Chromanone derivative	316.0937

The HR-LCMS profile confirms the presence of bioactive compounds such as mangiferin, flavonoids, and phenolic derivatives, which may contribute to the antioxidant and antidiabetic potential of the extract. The multiple peaks further indicate the rich and diverse phytochemical composition of *Mangifera indica*.

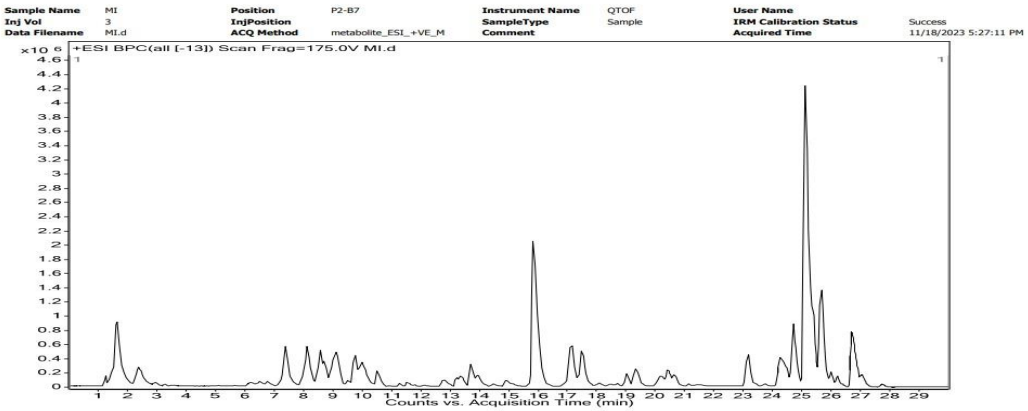


Figure 4: HRLCMS chromatograms of *Mangifera indica*

UV–Visible Spectral Analysis of *Mangifera indica* Extract and Phytosomal Formulation

UV–visible spectra of the ethanolic extract and its phytosomal formulation were recorded in the range of 200–400 nm. The extract showed characteristic absorption peaks attributed to aromatic and conjugated phytoconstituents such as polyphenols and flavonoids. The phytosomal formulation exhibited slight shifts and reduced peak intensity, indicating interaction between phosphatidylcholine and the extract. These spectral changes confirm successful encapsulation within the phytosomal system.

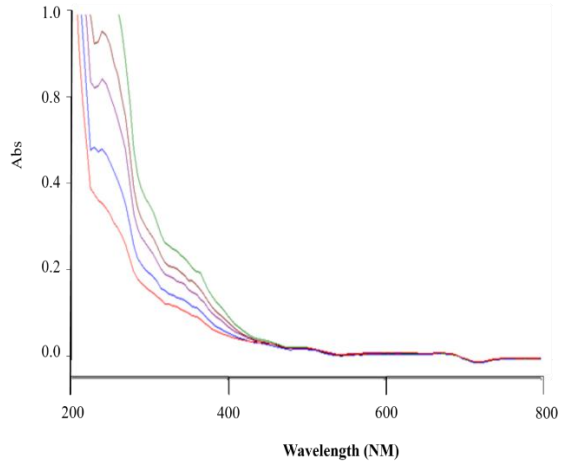


Fig. 5.: UV–visible spectral analysis of herbal extract (*Mangifera indica*) at defined concentrations

Calibration Curve of *Mangifera indica* Extract

The calibration curve was constructed at λmax 265 nm over a concentration range of 100–500 ppm. The data showed excellent linearity with the regression equation:

$y = 0.002x - 0.0025$, with a correlation coefficient $R^2 = 0.9999$.

This indicates high accuracy and reliability of the method for quantitative estimation of the extract.

Entrapment Efficiency of *Mangifera indica* Phytosomal formulation

Entrapment efficiency was determined to evaluate the effectiveness of phytosome formation using the standard formula. The formulation showed an entrapment efficiency of 67.245%, indicating efficient incorporation of the extract into the phospholipid matrix and successful phytosomal development.

Nanoparticle Characterization of *Mangifera indica* Phytosomal formulation

Particle Size Analysis

Particle size analysis revealed nanosized vesicles with good homogeneity. The formulation showed a Z-average size of 82.6 nm, with a peak size of 79.2 nm and PDI of 0.196, indicating a narrow size distribution. The high intercept value (0.901) and good result quality confirm reliability of the measurement. The nanoscale size suggests enhanced surface area, improved bioavailability, and better absorption characteristics of the phytosomal formulation.

Table No. 3. Particle Size Distribution of *Mangifera indica* Phytosomal formulation

Parameter	Result
Peak Size	79.2 nm
Intensity	100%
Z-average	82.6 nm
PDI	0.196
Intercept	0.901
Result Quality	Good

Zeta Potential Analysis of *Mangifera indica* Phytosomal formulation

The zeta potential of the formulation was found to be −32.2 ± 17.4 mV, with conductivity of 0.0403 mS/cm. The negative charge indicates electrostatic repulsion between particles, contributing to colloidal stability and reduced aggregation. Overall, the formulation exhibits moderate to good stability.

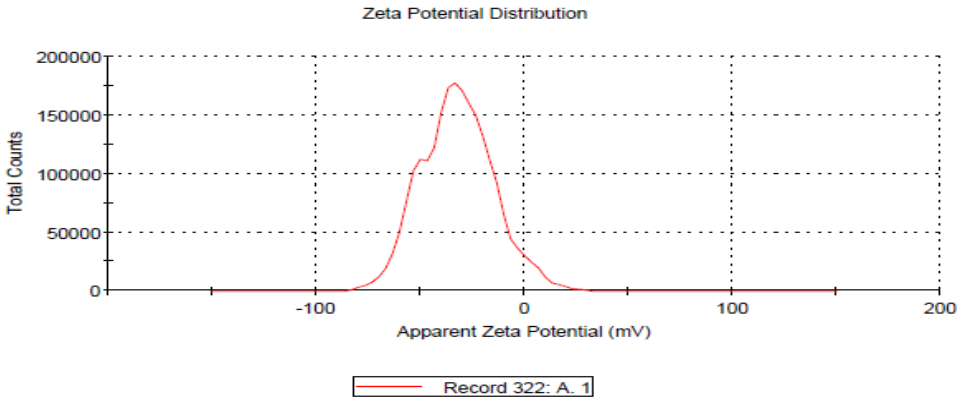


Fig.6.: Zeta Potential Distribution of Phytosomal Formulation MI

Differential Scanning Calorimetry (DSC) Analysis of *Mangifera indica* Phytosomal formulation

DSC Analysis of *Mangifera indica* Ethanolic Extract (EEMI)

The DSC thermogram of EEMI showed broad, low-intensity endothermic peaks at 113.66 °C and 159.07 °C, indicating the amorphous and heterogeneous nature of the phytoconstituents. The absence of sharp peaks suggested a predominantly amorphous profile.

DSC Analysis of Phosphatidylcholine

Phosphatidylcholine exhibited a major endothermic transition at 144.93 °C, corresponding to phospholipid phase transition, followed by degradation at 274.68 °C.

DSC Analysis of Physical Mixture (EEMI + Phosphatidylcholine)

The physical mixture showed endothermic peaks at 123.60 °C, 188.58 °C, and 233.80 °C, representing combined thermal behaviour of both components. Retention of individual peaks suggested weak interaction and absence of major structural modification.

DSC Analysis of Phytosomal Formulation

The phytosomal formulation exhibited broadened and shifted peaks at 135.38 °C, 157.05 °C, 192.46 °C, and

276.89 °C, indicating altered thermal behaviour. Peak broadening and reduced intensity suggested decreased crystallinity and improved molecular dispersion within the phospholipid matrix, indicating possible complex formation.

DSC Overlay Interpretation

The DSC overlay showed clear differences among EEMI, phosphatidylcholine, physical mixture, and phytosomal formulation. The physical mixture retained characteristic peaks, indicating limited interaction, whereas the phytosomal formulation exhibited shifted and broadened peaks with reduced intensity, suggesting molecular interaction and reduced crystallinity. However, DSC alone is not conclusive and should be supported by complementary techniques.

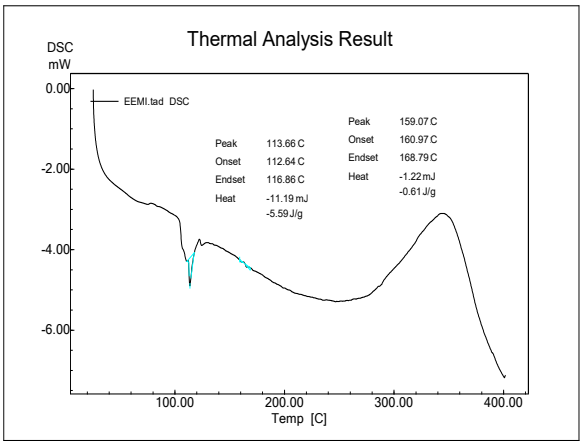


Fig.7 DSC of *Mangifera indica* Ethanolic Extract

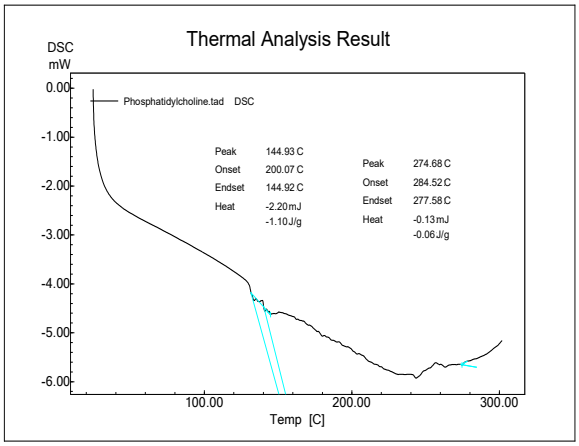


Fig.8 DSC of Phosphatidylcholine

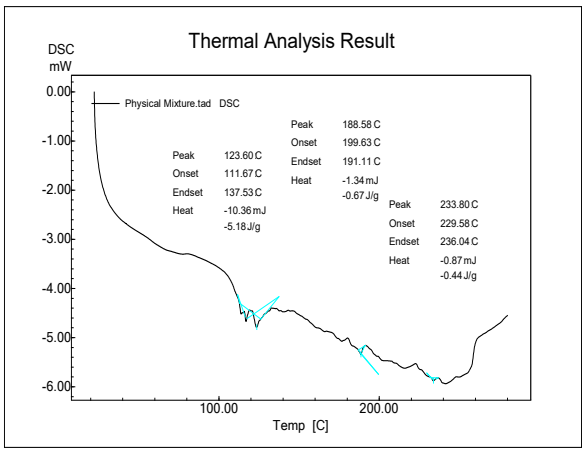


Fig.9 DSC of Physical Mixture (EEMI + PC)

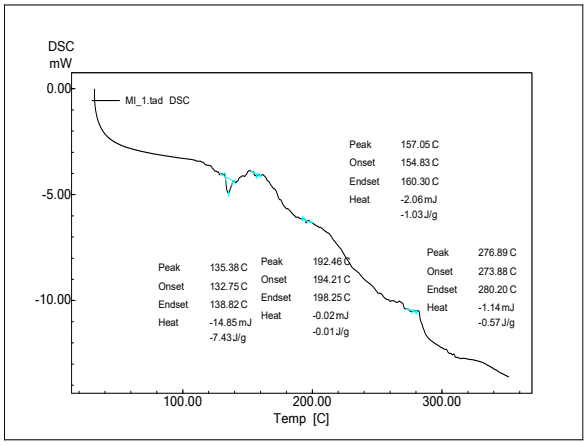


Fig.10 DSC of Phytosome

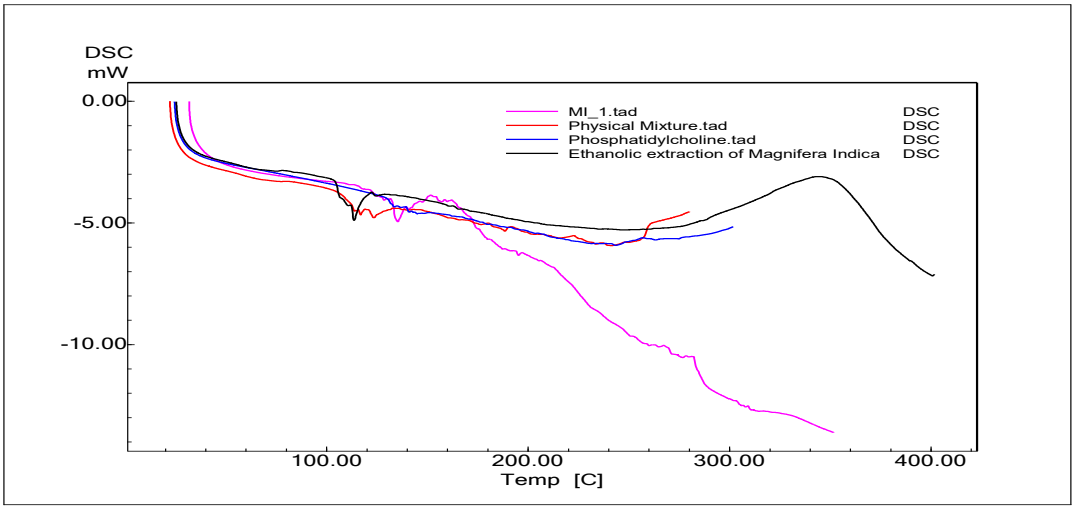


Fig 11: DSC overlay of EEMI, phosphatidylcholine, physical mixture, and *Mangifera indica* phytosomal formulation

FTIR Analysis of *Mangifera indica* Phytosomal formulation

The FTIR spectrum of the ethanolic extract of *Mangifera indica* showed characteristic peaks corresponding to phenolic and aromatic compounds, including O–H, C–H, C=O, and C–O stretching vibrations. Phosphatidylcholine exhibited typical lipid and phosphate group peaks.

The phytosomal formulation retained major characteristic peaks with slight shifting and broadening, indicating interaction between the extract and phosphatidylcholine. These spectral changes confirm successful formation of the phytosomal complex.

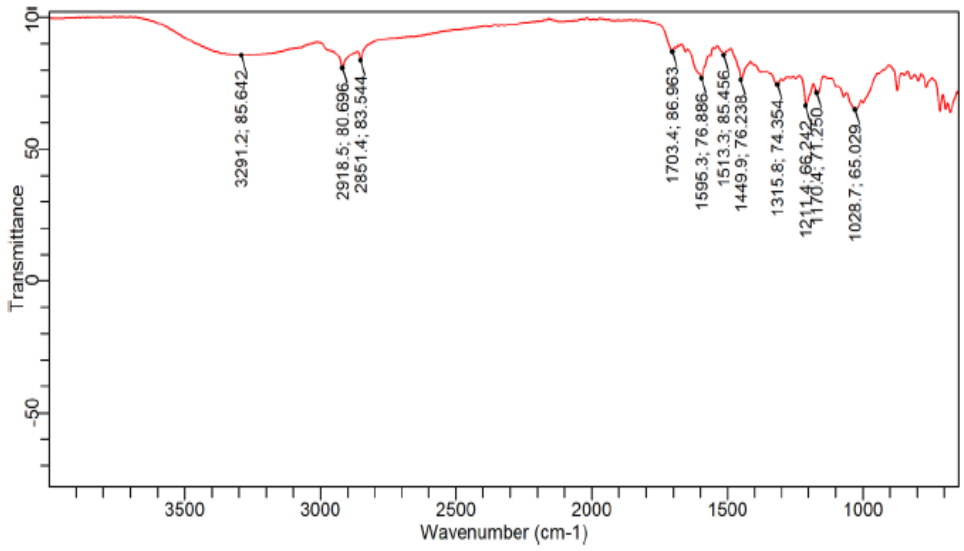


Fig.12: FTIR Spectrum of *Mangifera indica* EE

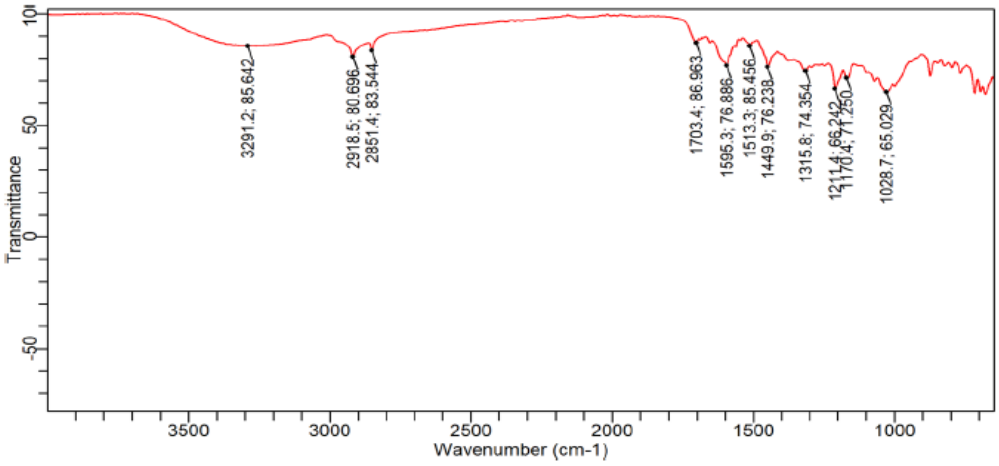


Fig. 13: FTIR of Phosphatidyl Choline

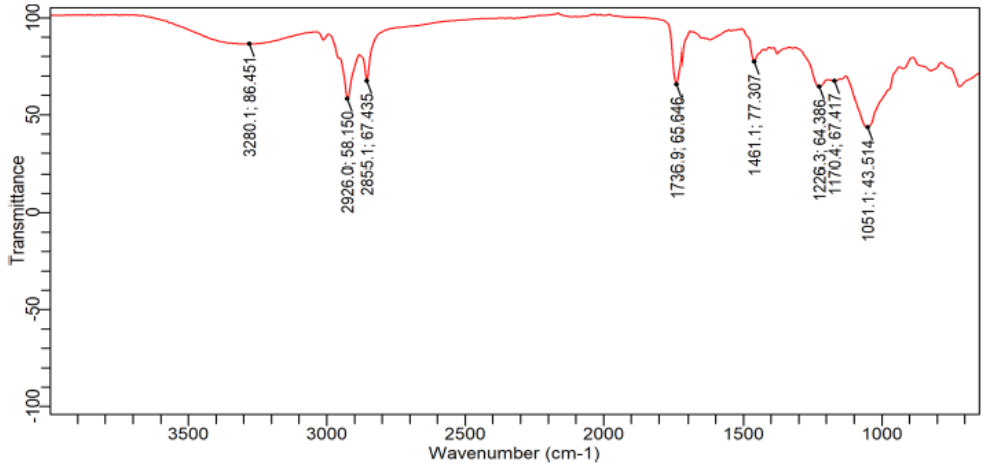


Fig. 14: FTIR of Phytosomes MI

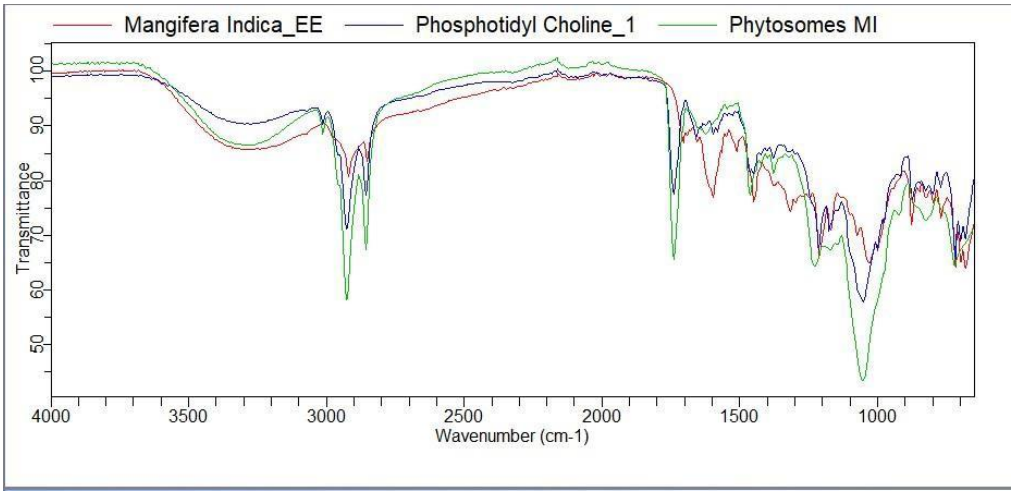


Fig. 15: Overlay FTIR Spectrum of Mangifera indica and Phytosomal Formulation

Morphological Characterization of *Mangifera indica* Phytosomal formulation

Scanning Electron Microscopy (SEM)

SEM analysis revealed predominantly spherical phytosomal particles with a relatively smooth surface and uniform distribution. The particles were well-defined with minimal aggregation, indicating successful formulation.

Higher magnification images showed characteristic vesicular structures with minor surface irregularities and uneven lipid packing, which are typical of phytosomal systems and may reflect phospholipid organization around the extract. These findings correlate with particle size analysis and confirm structural integrity of the formulation.

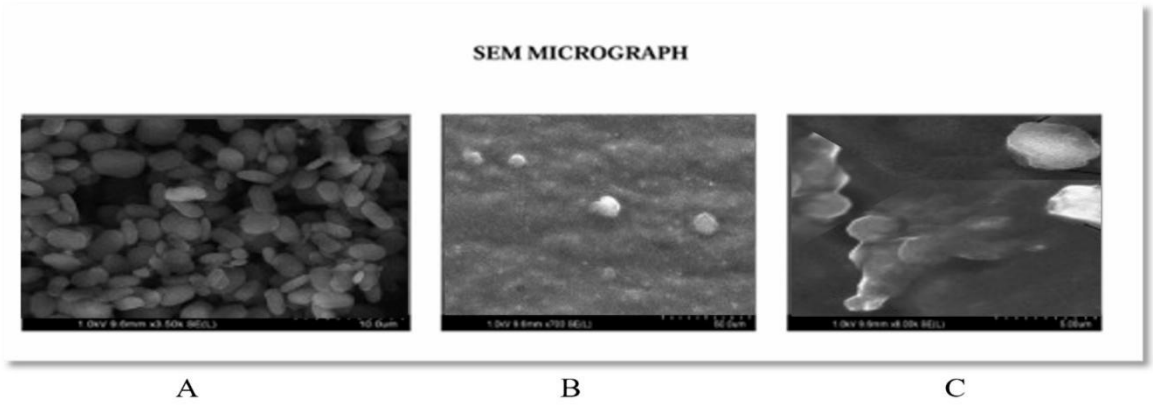


Fig. 16. SEM micrographs showing the surface morphology of the *Mangifera indica* (MI) phytosome formulation at different magnifications: (a) at 10 µm scale (low magnification) showing overall surface morphology; (b) at 5 µm scale (higher magnification) depicting pore structure; and (c) at 1 µm scale revealing uneven lipid packing of the phytosomal formulation particles.

Transmission Electron Microscopy (TEM) Analysis

TEM analysis confirmed the presence of predominantly spherical and vesicle-like structures typical of lipid-based systems. The micrographs showed organized lipid domains and dense vesicular morphology, indicating successful incorporation of the extract within the phospholipid matrix. Minor aggregation and clustered vesicles were observed, consistent with phytosomal behavior.

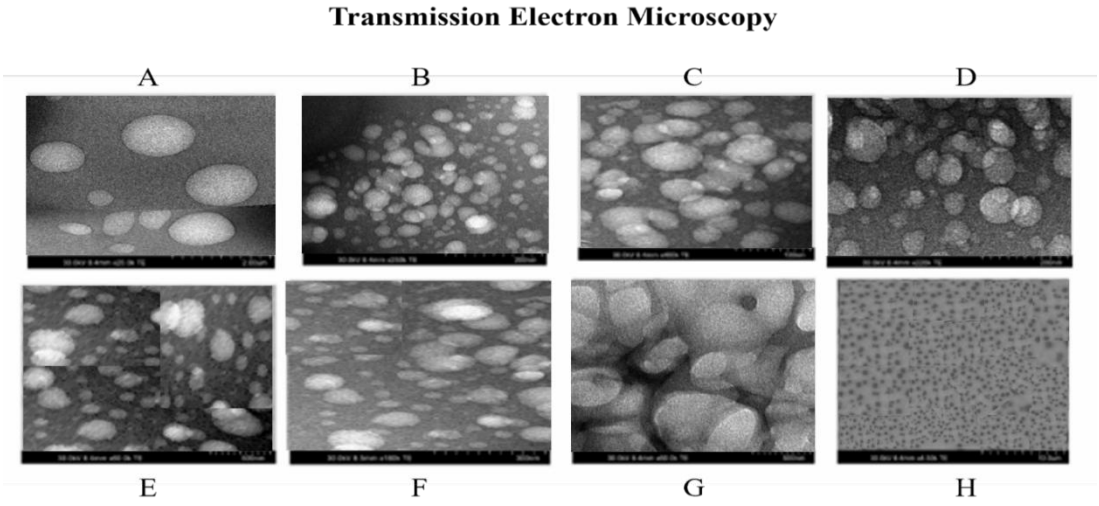


Fig. 17 . TEM images showing the internal structure and lipid bilayer formation ofMI phytosomal formulation particles at different scales: (a) at 2 µm scale – dispersed particles, (b) at 200 nm scale – irregular particle morphology, (c) at 100 nm scale – particle aggregation, (d) at 200 nm scale – clustered particles, (e) at 500 nm scale – spherical particle, (f) at 300 nm scale – dense particle with clear outline, (g) at 500 nm scale – multiple aggregated particles, (h) at 10.0 µm scale – overall particle distribution.

Atomic Force Microscopy (AFM) Analysis

AFM analysis demonstrated nanosized, predominantly spherical particles with relatively smooth surface characteristics and uniform distribution. Three-dimensional images showed variations in surface height and moderate aggregation, consistent with SEM and TEM results. The smooth morphology further confirmed effective phospholipid organization around the phytoconstituents.

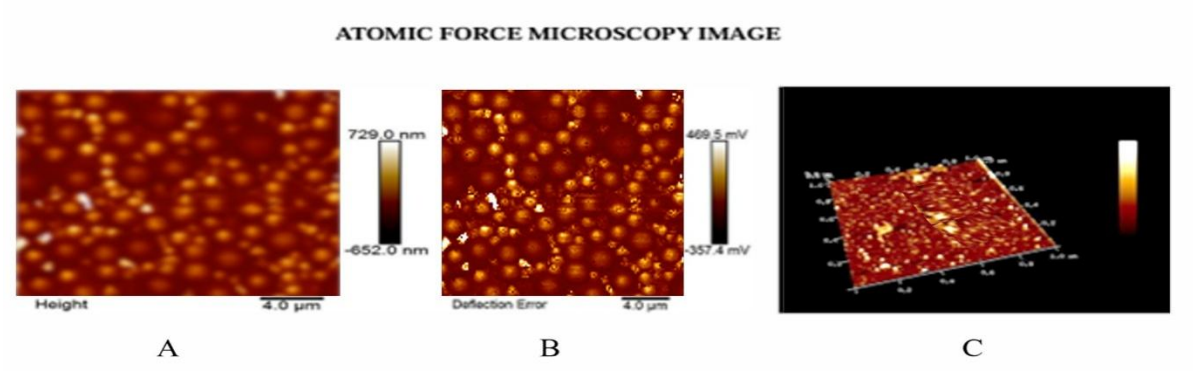


Fig. 18 . AFM images illustrating the topography and morphology of the **MI phytosome formulation:** (a) low-magnification scan showing overall surface morphology; (b) high-magnification scan revealing particle aggregation; (c) 3D topographical image indicating variations in surface height and particle distribution.

DISCUSSION

The present study evaluated the phytochemical profile, phytosomal formulation, and antidiabetic potential of *Mangifera indica* leaf extract. The results demonstrated that complexation with phosphatidylcholine significantly improved physicochemical properties and enhanced biological activity in streptozotocin-induced diabetic rats.

Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, glycosides, steroids, and diterpenes, while alkaloids and saponins were largely absent, consistent with earlier reports on *M. indica* as a rich source of polyphenolic compounds.[xvi,xvii,xviii] HR-LCMS analysis further confirmed mangiferin as a major constituent along with catechins and galloyl derivatives[xix,xxxxi].

Phytosomal formulation showed efficient entrapment efficiency (67.24%) and successful complex formation confirmed by FTIR and DSC analysis. Nanoparticle characterization revealed nanosized particles (~79 nm), low PDI (~0.21), and good zeta potential (−32.2 mV), indicating good stability and improved absorption potential.[xxii,xxiii,xxiv] Lipid-based carriers such as phytosomes are known to enhance membrane permeability and systemic bioavailability of polyphenolic compounds, thereby improving therapeutic efficacy.[xxv,xxvi]

Overall, the findings demonstrate that phytosomal delivery significantly enhances the therapeutic efficacy of *Mangifera indica* by improving solubility, stability, and bioavailability,

CONCLUSION

The present study confirms that *Mangifera indica* leaf extract possesses significant antioxidant and antidiabetic potential due to its rich phenolic and flavonoid content. The phytosomal formulation markedly enhanced solubility, stability, and bioavailability compared to the crude extract.

Acknowledgment

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