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FORMULATION, OPTIMIZATION, AND CHARACTERIZATION OF PHOSPHOLIPIDS ENCAPSULATED MANGIFERIN TO IMPROVE ITS ANTI-DIABETIC ACTIVITY

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Abstract

Diabetes has emerged as the world's most significant issue in the modern era. The metabolisms could be affected. This imbalance may impair insulin secretion and function. In order to increase the solubility of Mangiferin extracts' phytoconstituents, the current work set out to create and characterise phytosomes of those extracts. Its oral absorption and bioavailability are constrained by the phytoconstituents' poor solubility and rapid rate of dissolution. The solvent evaporation approach was utilised to create phytosomes using the standardised extracts of mangiferin. The optimised phytosomes were evaluated for a number of parameters.

Keyword: *Mangiferin*, Phytosomes, *in-vitro* study

1. INTRODUCTION

Diabetes is a persistent illness. Hyperglycemia could be one of its characteristics. These could be useful in treating both insulin action and insulin secretion abnormalities. Inadequate insulin secretion and tissue size brought on result in anomalies in the metabolism of protein, carbohydrates, and lipids. These could result in modifications or elevations in blood glucose levels. Numerous bodily systems, including blood vessels and nerves, could be harmed by them. The survey found that between 0.5 and 3 percent of people suffered from these disorders. It has increased to about 7% these days. There are between 200 and 300 million impacted people, and in the upcoming years, that number should increase or triple.[1,2]

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1.1 Types of diabetes

1.1.1 Type 1 diabetes mellitus (T1DM) or Juvenile Onset Diabetes

Diabetes mellitus that is insulin dependent is another name for it (IDDM). 5 to 10 percent of the population resides there. These might result in normal glucose production and might lower the sugar level. The Langerhans islet is what this is known as. Blood glucose levels are used to regulate blood sugar levels and kill cancerous cells. The islet antibiotic cell, insulin to autoantibodies, GAD to antibodies, tyrosine phosphate, and IA-2 may all fall within this category.[5,6]

1.1.2 Adult-Onset Diabetes, also known as Type 2 Diabetes Mellitus (T2DM),

Non-insulin dependent diabetic mellitus is another name for this. Up to 98 percent of the population could be affected by this disease. This can be related to the modern style element. It was typical in adults. This might lessen the disease's symptoms. 7,8 The insulin activity may decrease as a result. It has a variety of disorders including gradual deterioration, insulin resistance, and malfunction of pancreatic beta cells. Obesity, advanced age, and a history of diabetes may all be risk factors for this condition.[9,10]

The family Anacardiaceae includes the sizable evergreen tree *Mangiferin*. The leaves are reddish and thinly flaccid when they are newly produced, and when they are crushed, they release a pleasant odour.[11,12]

2. MATERIAL AND METHODS

2.1 Collection of material

The plants' raw materials were gathered from regional farmers in Rajasthan, India.

2.2 Preparation of extract

For the removal of all contamination, leaves were washed with water. Then leaves were washed with 80% ethanol. Then these leaves are put into the oven with 50^{o}C temperature for 24 to 30h. then these leaves are grounded for fine powder. 50gm of the samples are dissolved into 200ml of solvents. Keep this for 48 to 50h under shaking of 150RPM with 40^{0}C temperature. Solvents used were petroleum ether, chloroform, ethyl acetate, n-butanol, and water. After that the sample is transferred into the rotatory vacuum evaporator with 40^{0}C temperature until the solvent has been evaporated. After that these sample were kept into the refrigerator at 4^{0}C. these are then used for the phytochemical analysis. Sample has been weight and stock solution were prepared. These

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solutions are used for the further analysis.

2.3 Calibration curve of *mangiferin*

2µL *mangiferin* solution was drip-fed onto a TLC plate at concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL and 600 µg/mL. The plate was subsequently developed in the chamber using a chosen mobile phase (ethyl acetate: distilled water: formic acid). The plate was then examined using a TLC scanner set to its maximum wavelength. Plotting the relationship between area and concentration yields the calibration curve.

2.4 Physico-Chemical Evaluation

2.4.1 Ash Value Determination

a) Value of Ash Calculation

A 3gm powder sample was weighed and put into a silica crucible. It was desiccated and chilled. A dried air sample was used to calculate the ash value and %.

% ASH = ((ashed wt.)- (crucible wt.)) x 100/ ((crucible and sample wt.)- (crucible wt.))

b) Calculating the value of acid-insoluble ash

After being recovered from the previous, the entire amount of ash was dissolved in 30 ml of HCl for 5 to 6 minutes. The insoluble material was then collected on ashless paper. The paper went through a hot water washing process, a crucible fire, cooling, and desiccation. The insoluble ash value was then calculated after it had been weighed.

c) Calculating the value of water-soluble ash

Five minutes are spent boiling about 30ml of water. Insoluble material is collected using filter paper. After that, wash it with hot water for 15 minutes before lighting it. The weight of insoluble materials serves as the substrate for the total ash.

2.4.2 Loss on drying

Weigh the sample and tared the dish and it was completely dried by the use of heating at 105^0C. the loss of amount of sample is calculated by the weight loss of sample

2.4.3 Extractive values

100ml of solvent were used to macerate 3gm of dry extract. After being placed in a flask, it was kept there for 24 hours. After being shaken for the first six hours, the sample was left for 18 hours.

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2.5 Study on Phytochemicals

2.5.1 Chemical quality tests

2.5.1.1Tests for carbohydrates

A. Molisch test: Purple ring appears after adding 1 ml of test to 2-3 drops of -naphthol and concentrated sulfuric acid.

B. Fehling's test: Brickred precipitate forms when Fehling's solutions A and B are added to 1 ml of the test sample and heated.

C. Benedict's test: A similar amount of Benedict's reagent was added and heated with 1 ml of test sample. Carbohydrates show due to the presence of red colour precipitate.

2.5.1.2Test for alkaloids

After adding Dil. HCL to the extracts, it is filtered.

A. Mayer's test: 1 ml of filtrate, Mayer's reagent, and cream precipitate

B. Hager's test: Filtrate and yellow Hager's reagent precipitate in 1 mL of the filtrate.

C. Wagner's test: Wagner's reagent in 1 ml of the filtrate, resulting in a reddish-brown precipitate

2.5.1.3 Terpenoid testing

A. Salkowski test: Using, the chloroform solution was an equivalent volume of concentrated sulfuric acid. The presence of steroid components is shown by the colour red.

B. Libermann - burchard test: Chloroform was applied the test sample, up to 2 ml before being followed by a few drops of addition of acetic anhydride and concentrated sulfuric acid. Steroids are visible by their bluish colour.

2.5.1.4 Tests for flavonoids

A. Lead acetate test: The extract was combined with a lead acetate solution. Flavonoid was demonstrated by the appearance of a yellow precipitate.

B. Alkaline reagent test: A yellow-coloured precipitate formed when One millilitre of the test sample was added to diluted sodium hydroxide solution, which was then dissolved.

2.5.1.5 Tests for phenolic compounds and tannins

A. 5% $FeCl_3$ solution: Just a little bit of the 5 percent solution of FeCl3 was combined with the extract.. a combination of the colours deep blue and black.

B. 10% lead acetate solution: A white precipitate formed when a little amount of extract a 10

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percent concentration lead acetate solution was added to the mixture.

C. Gelatin test: When 2ml of gelatin solution is added to an extract that has been dissolved in water, a white precipitate appears that indicates phenolic compounds' presence.

2.5.1.6 Tests for saponins

A. Froth test*:* Persistent foam formed after 15 minutes of stirring after adding 20ml of distilled water and 1 ml of the test sample.

2.5.1.7 Test for proteins and amino acids

A. Ninhydrin test: The colour was assessed after boiling 3 ml of the test solution for 10 minutes with 3 drops of 5 percent ninhydrin.

B. Biuret test: A violet or pink colour was observed after the same volume of treatment was applied to the test sample of a solution containing 4% sodium hydroxide and 1% copper sulphate.

C. Million's test: A brick red colour is produced when extract is combined with Million's reagent, indicating the presence of protein.

2.5.1.8 Tests for glycosides:

A. Borntrager's test: The extract solution is heated after dilute H_2SO_4 is added. The remedy was chilled and filtered. Chloroform was added in the same quantity while stirring the mixture. The layer of organic solvent received ammonia. The anthraquinone-type glycosides were validated when the colour of the ammoniacal layer changed to a pinkish red hue.

B. Legal test: A sample is mixed with a solution of sodium nitroprusside and pyridine. It shows the colour changes.

C. Keller killani test: An extract solution is augmented with ferric chloride and glacial acetic acid. After that, the solution is poured into a beaker containing condensed H_2SO_4.

2.5.1.9 Tests for fats and oils

A. Spot test: A tiny amount of extract was dropped on some filter paper, and it was left to stand for a few minutes to let the solvent evaporate. A little amount of oils is discovered after a few minutes, which denotes the presence of fixed oil.

2.6 Phytosome preparation

By using some techniques, the preparation of Phytosme is done. Techniques are as follow

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2.6.1 Technique for antisolvent precipitation

The extract was dissolved in methanol and lecitin. For dissolving cholesterol DCM is used. At 60^0C Temperature this mixture was stored for refluxed. It creates precipitated. It was stored in vacuum desiccators. After completion this mixture was stored it must be kept in a refrigerator and is presented in amber-coloured glass.

2.6.2 Rotating evaporation method

Tetrahydrofuran (THF) was used to dissolve the precise quantity of plant extract and soy lecithin, which was then stirred for four hours at a temperature of no more than 40^0C. The sample was reduced to a thin film before n-hexane was added and swirled continuously with a magnetic stirrer. The precipitate was collected, put in an amber-coloured glass bottle, and refrigerated for storage.

2.6.3 Method of solvent evaporation

A mixture of acetone and the precise proportions for soy lecithin and extract was refluxed at 50 to 60^0C. it was filtered. The extract was dried and stored into glass container and stored in refrigerator.

2.7 Characterization of Phytosome

2.7.1 Entrapment efficiency

The centrifugation technique was used to calculate the Phytosomes' entrapment efficiency. Methanol was used to dilute the phytosome, which was subsequently centrifuged using a high-speed cooling centrifuge at 10,000 rpm for 30 minutes at -4^0C. The amount of free extract was calculated using a UV visible spectrophotometer after the supernatant was collected.

2.7.2 Particle Size Distribution and Mean Particle Size

The Zeta sizer Nano (Malvern instrument) was used to measure particle size and size distribution. In glass cuvette, sample is added. The span parameter uses for distribution of size. If the span is small the distribution of size is also small. This process is repeated 5times.

2.7.3 Visualization

Scanning electron microscopy was used to visualise phytosomes in this study. SEM has been used to determine the complex's surface shape, appropriation estimation, and particle size. For the auxiliary electron emissive SEM before being examined for morphology at 15.0 kV.

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2.7.4 Zeta Potential

Zeta potential is the main element influencing the physical stability of phytosomes.

2.7.5 Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a well-established technique for analysing the thermal conduct of a variety of materials. The melting, degradation, interaction, stability, and other relevant qualities of test materials can all be determined by measuring changes in material properties as a result of controlled temperature changes. These progressions can be seen in DSC thermograms as changes in enthalpy, peaks that develop or disappear, and variations in peak start time, shape, and relative area. It also provides information on the creation of novel substances and the compatibility of drug excipients. The purge gas was dried nitrogen. As per customary practise, indium was used to calibrate the apparatus for heat flow and heat capacity. Each sample was heated to a temperature between 25^0C and 300^0C at a rate of 5^0C per minute. The flow of nitrogen was kept at 5 ml per minute.

2.7.6 Transmission Electron Microscopy (TEM)

Distilled water was added to powdered phospholipid complexes to prepare the samples, which were then swirled for three minutes. The phospholipid complex dispersions that were created were applied as a drop onto a copper grid that had been coated with carbon, leaving a thin liquid layer behind. On a transmission electron microscope, the stained films were afterwards examined and captured on camera.

2.7.7 Fourier Transform Infrared (FTIR) Analysis

FTIR spectrophotometer is use for the scanning of prepared extract. This was useful in the interaction of formulation. The wavelength of 3500 to 1000 cm^{-1} is recorded for extract, optimized formulation, soy lecithin, cholesterol.

2.7.8 X-Ray Diffraction Analysis

The X-ray diffractometer captured the results of the XRD study. Two range in the spectrum from 10-80Å was obtained when the sample was scanned at a rate of 0.3 seconds per degree for Cu-K radiation.

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2.8 *In Vitro* Release Study

In vitro release was measured using dialysis method. Formulation was dispersed in release medium (phosphate buffered saline (PBS), pH 7.5 to form a suspension. The suspension was filled in a cellulose dialysis membrane(15µm), it's both ends were tied. It was then suspended in a glass vial containing 10 ml of release medium. The vial was shaken horizontally using water bath shaker at 37°C. *In vitro* drug release was assessed by intermittently sampling the release medium at predetermined time intervals and was replaced with fresh medium to maintain sink condition. The amount of extract released in each sample was determined using a calibration plot; the reported values are average of three replicates. Results of *in vitro* drug release studies obtained are shown graphically as cumulative percentage drug release versus time. Various kinetic models were used to analyses the *in vitro* study.

3. RESULT AND DISCUSSION

3.1 Calibration curve of *Mangiferin*

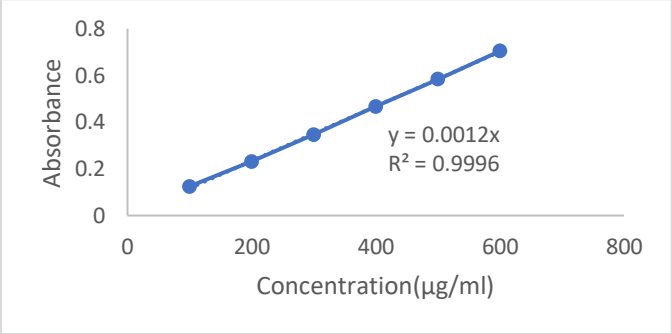

Figure 1. Calibration curve of *Mangiferin*

The result from this is *mangiferin* maximum absorption wavelength is 257 nm. The obtained value of the correlation coefficient (r) is 0.9997. The relationship between the concentration variable and the *mangiferin* area may be seen to be linearly significant in this instance.

3.2 Physical-chemical assessment

3.2.1. Determination of foreign matter

Mangiferin of choice had the most information.

S.no	***Mangiferin*** (Mean±SD)

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1	1.6±0.75g

Table 1. foreign substances

3.2.2 Ash value determination

Mangiferin exhibit the highest quantities of ash.

Plant name	Total ash (% by weight)	Water soluble	Acid insoluble	Sulphated
Mangiferin	3.15±1.24	1.8±0.48	0.55±0.21	0.8±0.55

Table 2. Calculation of the Ash value

3.2.3 Extractive value

The extractive value that is water soluble show a significant role. A reduced extractive value may indicate the use of expired components, adulteration, or incorrect drying, storing, or formulation methods. An individual leaf extract's extractive qualities were determined.

Plants	Alcoholic (% w/w)	Aqueous (% w/w)
Mangiferin (Mean±SD)	19.6±0.68ml	19.1±0.42ml

Table 3. Extractive value of *mangiferin*

3.2.4 Loss on drying (LOD)

This test method is widely used to determine the moisture content of a sample. An established LOD for a specific plant extract.

Plants	***Mangiferin*** (Mean±SD)
LOD	6.51±1.52ng/mL

Table 4. LOD (Loss on drying)

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3.2.5 Extraction

After removing every component, the yield percentage was calculated.

Plants	Percentage yield(w/w)
Mangiferin (Mean±SD)	3.1%

Table 5. Ratio of yield

3.3 Phytochemical screening

S.no	**Phytochemicals**	**Presence (+)/absence (-)**
1	Flavonoids	+
2	Glycosides	-
3	Phytosterols	+
4	Alkaloids	+
5	Terpenoids	-
6	Saponins	-
7	Tannins	+

Table 6. Results of qualitative phytochemical screening of pure *Mangiferin*

3.3 Preparation of Phytosome

Solvent evaporation, rotary evaporation, and antisolvent precipitation were utilised separately to make the phytosomes based on the outcomes of various methods. Antisolvent precipitation was chosen after analysing the results of the three procedures because it produced the smallest particle size and had the best entrapment effectiveness.

	Mangiferin

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Method	Particle size (nm) (Mean±SD)	Entrapment efficiency (%)
Antisolvent precipitation	452.88 ± 0.5	91.6 ± 0.63
Rotary Evaporation	845.71 ± 0.58	89.55 ± 0.75
Solvent Evaporation	763.76± 0.23	76.89 ± 0.11

Table 7. Preparation of Phytosome

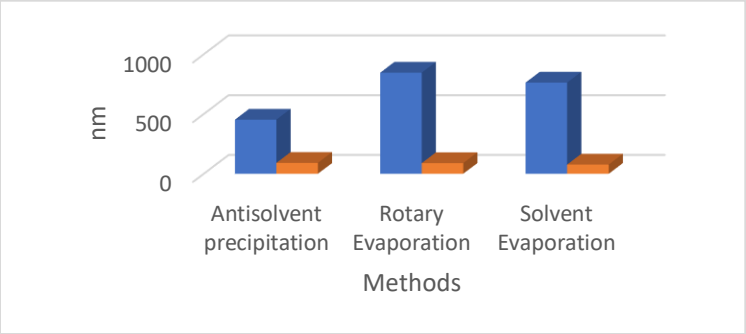

Figure 2. Preparation of Phytosome

3.4 Characterization of Phytosome

To confirm and validate phytosomes' size, shape, and morphology, a variety of procedures are needed for phytosome characterization. The formulation of the phytosome that had been created and optimised was characterised physically, chemically, and functionally. The following procedures are all a part of physicochemical characterisation: zeta potential measurement, DSC, FTIR, XRD analysis, visualisation (structural investigations), and entrapment efficiency assessment. Studies of release in vitro and antidiabetic efficacy *in vivo* are two examples of functional characterisation.

3.4.1 Entrapment efficiency

A high-speed cooling centrifuge was utilised to evaluate the effectiveness of entrapment. The enhanced entrapment efficiency seen when the extract/soya lecithin ratio rose may be attributed due to the higher polymer content, which was thought to improve entrapment effectiveness by

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allowing the drug to be incorporated into more space. 82.43% entrapment efficiency for the *mangiferin* enhanced formulation was discovered.

3.4.2. Mean particle size and size distribution

Mangiferin measurements of the mean particle size and size distribution. The amount of phospholipids in the sample was found to rise along with the mean particle size of the *mangiferin*. There are now more polymeric chains per volume unit of solvent as a result of collisions and the creation of larger nanoparticles.

3.4.3. Visualization

Analysing the phytosome surface form and solid-state properties required seeing the results of SEM. Figure presents an optimised formulation SEM.

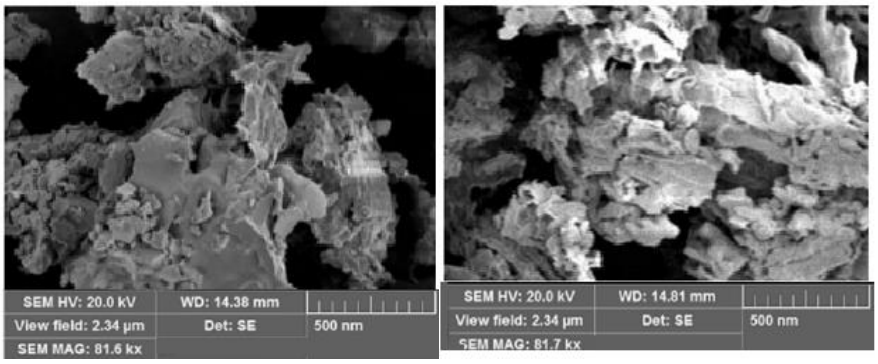

Figure 3. SEM Study

3.4.4 Zeta potential

Numerous colloidal material properties are influenced by zeta potential, which is connected with the charge on the molecule's surface. The literature states that the ideal range for the zeta potential value is -20 to +30 mv. It was found to be -19.40 mv for the modified formulation of *Mangiferin*.

3.4.5 Differential Scanning Calorimetry (DSC)

Two distinct peaks at 87.71^{0}C and 203.91^{0}C can be seen on the soy lecithin thermogram. The second peak represents the transition temperature, whereas the first peak represents melting. The thermogram of cholesterol displays two peaks at 42.870°C and 103.760°C as well as one significant peak at 151.800°C. This steep peak denotes the temperature at which cholesterol dissolves. A single peak at 98.38^{0}C can be seen on the extract thermogram. The thermogram of

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the *Mangiferin* enhanced formulation finally reveals a peak at 74.9^{0}C, indicating the development of a new distinctive component. According to the study, phase transition and isomeric changes in phospholipid structure both take place at the same time, and this is caused by the polar component of phospholipid migrating as temperature increases.

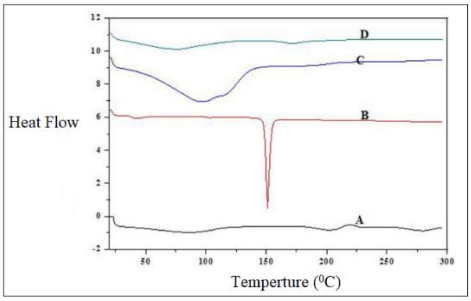

Figure 4. DSC thermogram of an optimised formulation, (A), soy lecithin, (B), cholesterol, (C), and extract (Phytosome)

3.5.6 Fourier Transform Infrared (FTIR) Analysis

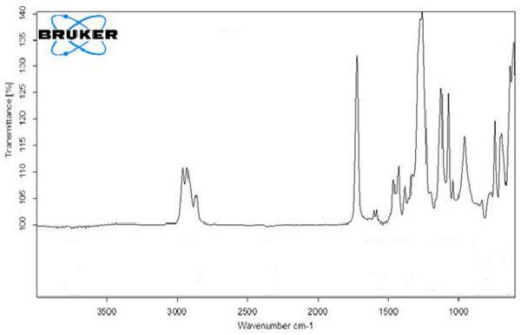

Figure 5. Soya Lecithin

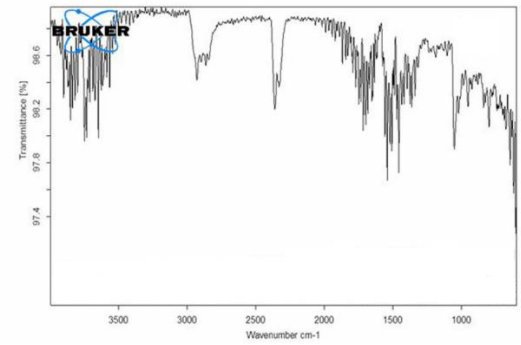

Figure 6. Cholesterol

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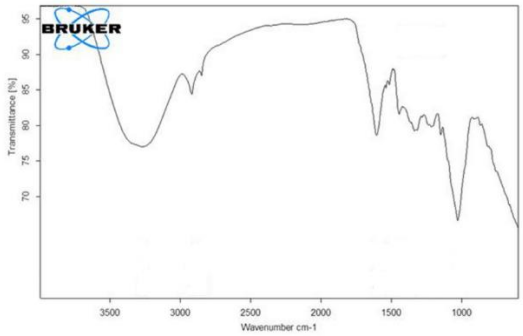

Figure 7. Mixture

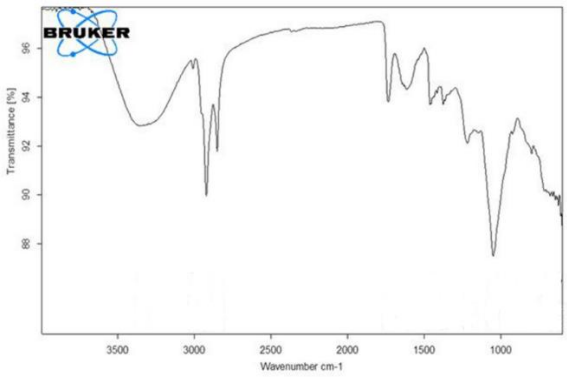

Figure 8. Optimized formulation

3.4.7 X-Ray Diffraction

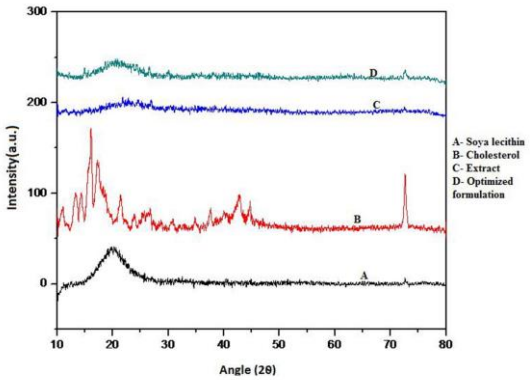

Figure 9. XRD spectra of extracts from (A) soy lecithin (B) cholesterol (C), and (D) an improved formulation

3.5 *In vitro* Release Study

3.5.1 Zero Order Kinetics

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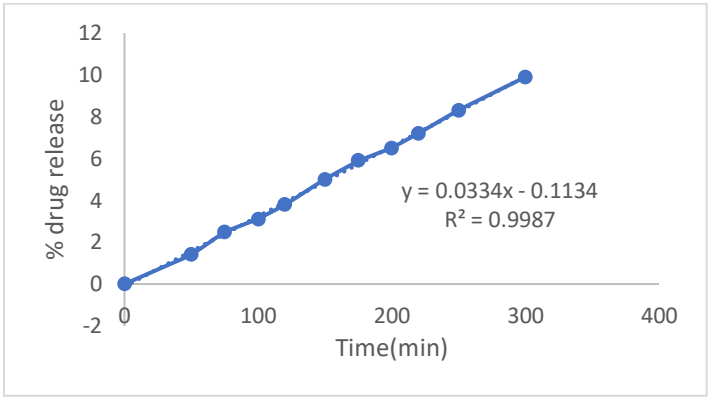

Figure 10. Zero order kinetics

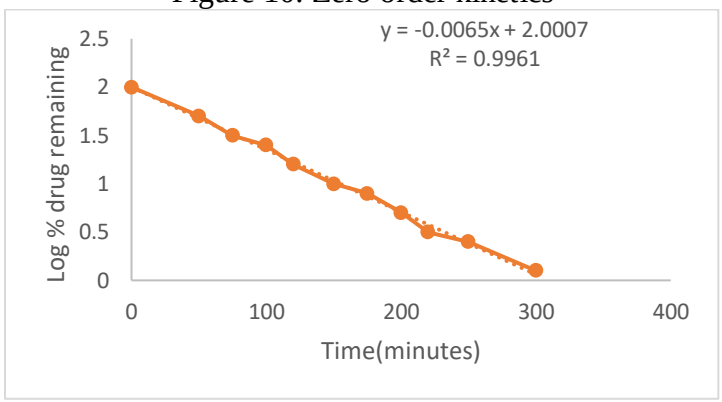

Figure 11. First order kinetics

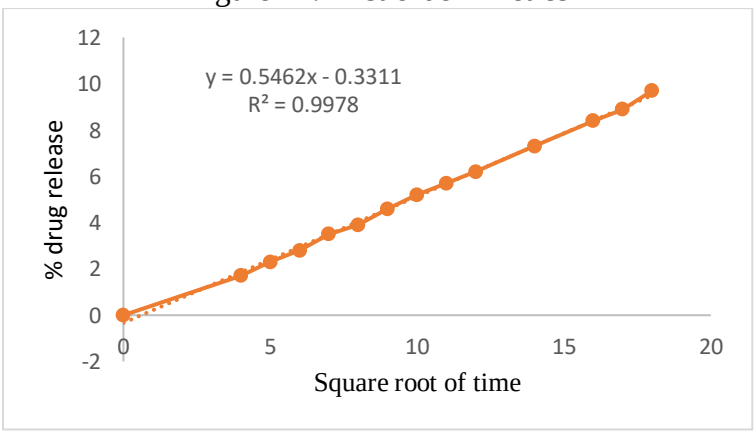

Figure 12. Higuchi model

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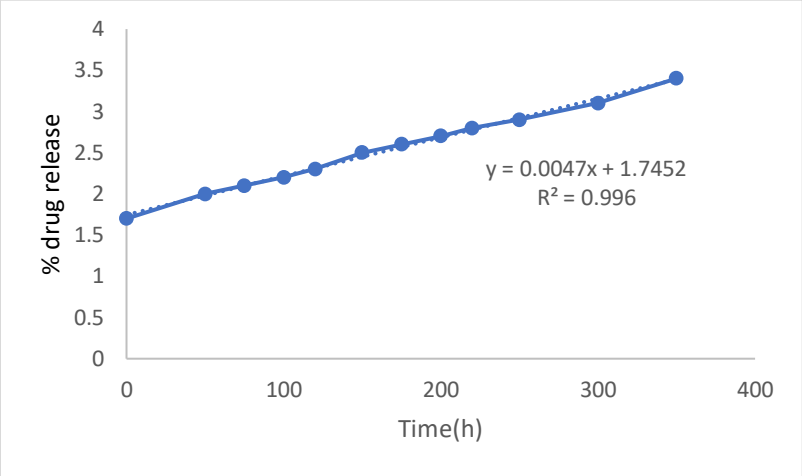

Figure 13. Korsmeyer-peppas model

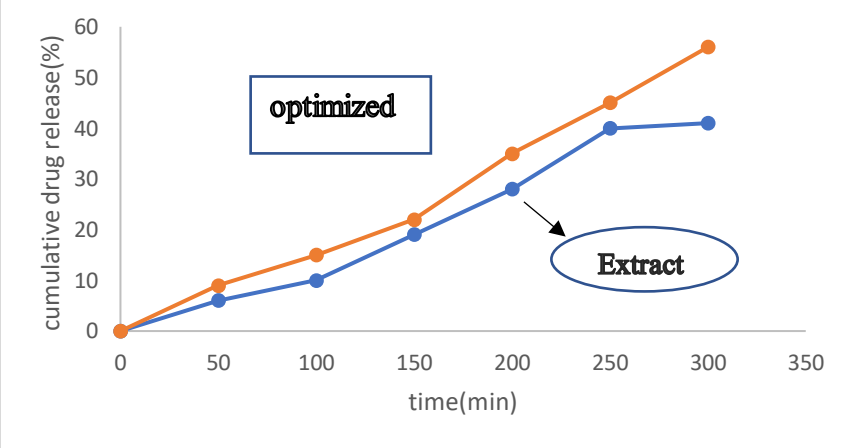

Figure 14. Release profiles of improved formulations compared

4. SUMMARY AND CONCLUSION

Throughout the world, diabetes mellitus, a chronic metabolic condition, continues to be a serious health issue. With 171 million affected, it is one of the largest health issues in the world. By 2025, India is predicted to be the diabetes capital of the globe. Diabetes and its effects are getting worse despite major advancements in therapy. The formulation of these phytoconstituents at the nanoscale as nanoparticles, complexing with a lipophilic carrier to form liposomes or herbosomes/phytosomes, structural modifications, delivery as prodrugs, and the addition of solubility and bioavailability enhancers are just a few of the methods that have been developed to increase their bioavailability. Physical chemical analysis and phytochemical screening were

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completed. Utilizing various methods, including HPLC (marker compound chlorgenic acid), HPTLC, and MS analysis, the plant extract was standardised.

Three separate techniques, rotary evaporation, solvent evaporation technique, and antisolvent precipitation, were used to create phytosomes. In comparison to rotary evaporation (755.62 nm and 87 percent) and solvent evaporation methodology, antisolvent precipitation produced the smallest particle size (345.88 nm) and the highest entrapment efficiency (89 percent) (563.23 nm and 81 percent). Therefore, Phytosomes were created using the antisolvent precipitation method, and 32 factorial designs were used for optimization.

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Physical, chemical, and functional characterisation were performed on the obtained, optimised formulation of the mangoferin extract. Entrapment efficiency assessment, visualisation (structural investigations), zeta potential measurement, DSC, FTIR, and XRD analysis are all components of physicochemical characterisation. Zeta potential readings of -19.35 mV and -16.85 mV show that a stable formulation has formed. The drug release profile was sustained with a low burst release, and the release profile was attributable to the diffusion mechanism. Comparative release profile study comparing of optimised phytosomal preparation revealed that the cumulative percent drug release from phytosome was approximately 50% higher than the cumulative percent drug release obtained by conventional extract, which was 30%.

The interaction with phospholipids causes certain positions in the extract to alter, as shown by FTIR spectra. The improved formulation describes those intermolecular interactions that were produced during phytosome formation by modifying the stretching frequency. According to an X-ray diffraction investigation, the substance's crystal structure was responsible for the sharp peak. The phytosome displayed broader, less intense peaks, indicating a change to an amorphous form.

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