RESEARCH PAPER

Development and Evaluation of a Phytoconstituent-Based Phytosomal Drug Delivery System from Erythrina variegata Linn.

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HIGHLIGHTS

- Phytosomes of Erythrina variegata enhanced bioavailability and stability
- HPTLC confirmed diverse phytoconstituents in bark extract
- Strong antioxidant activity (61.72% inhibition; IC_{50} = 401 µg/mL)
- Chloroform fraction showed potent AChE/BChE inhibition
- Optimized phytosome (206 nm, 72.8% EE) showed sustained release

ABSTRACT

The present study aimed to develop and evaluate a phytosomal drug delivery system of Erythrina variegata L. bark extract to overcome limitations such as poor bioavailability and rapid metabolism. Pharmacognostic evaluation revealed total ash (10.48% w/w), acid-insoluble ash (1.36% w/w), water-soluble extractive (21.7% w/w), alcohol-soluble extractive (5.58% w/w), and loss on drying (15.29% w/w), confirming the quality and purity of the crude drug. Phytochemical screening indicated the presence of alkaloids, flavonoids, phenolics, and saponins. HPTLC profiling demonstrated multiple peaks with distinct Rf values, confirming chemical diversity. The methanolic extract exhibited dose-dependent antioxidant activity, with a maximum DPPH inhibition of 61.72% at 500 µg/mL and an IC_{50} value of 401 ± 1.06 µg/mL, compared to ascorbic acid (68.86% inhibition; IC_{50} = 301 ± 0.77 µg/mL). In-vitro anticholinesterase studies revealed that the chloroform fraction showed potent inhibition of AChE (IC_{50} = 39.04 ± 1.99 µg/mL) and BChE (IC_{50} = 21.68 ± 2.79 µg/mL), comparable to donepezil (IC_{50} = 2.52 ± 0.08 µg/mL for AChE and 22.41 ± 0.58 µg/mL for BChE). The isolated compound, eriodictyol, was confirmed through MS, NMR, and FTIR analysis, and molecular docking indicated favorable binding interactions. Phytosomal formulations were optimized using a 3^2 factorial design. The optimized batch (E6) exhibited particle size of 206.4 nm, polydispersity index of 0.312, and entrapment efficiency of 72.8 ± 6.5%. SEM analysis confirmed spherical unilamellar vesicles. The formulation showed sustained drug release and remained stable under refrigerated conditions (2–8 °C) over three months. Overall, the phytosomal system significantly enhanced the physicochemical stability and therapeutic potential of Erythrina variegata, indicating its promise for advanced herbal drug delivery applications.

Keywords: Phytosome; Erythrina variegata; Anticholinesterase activity; Eriodictyol; Drug delivery

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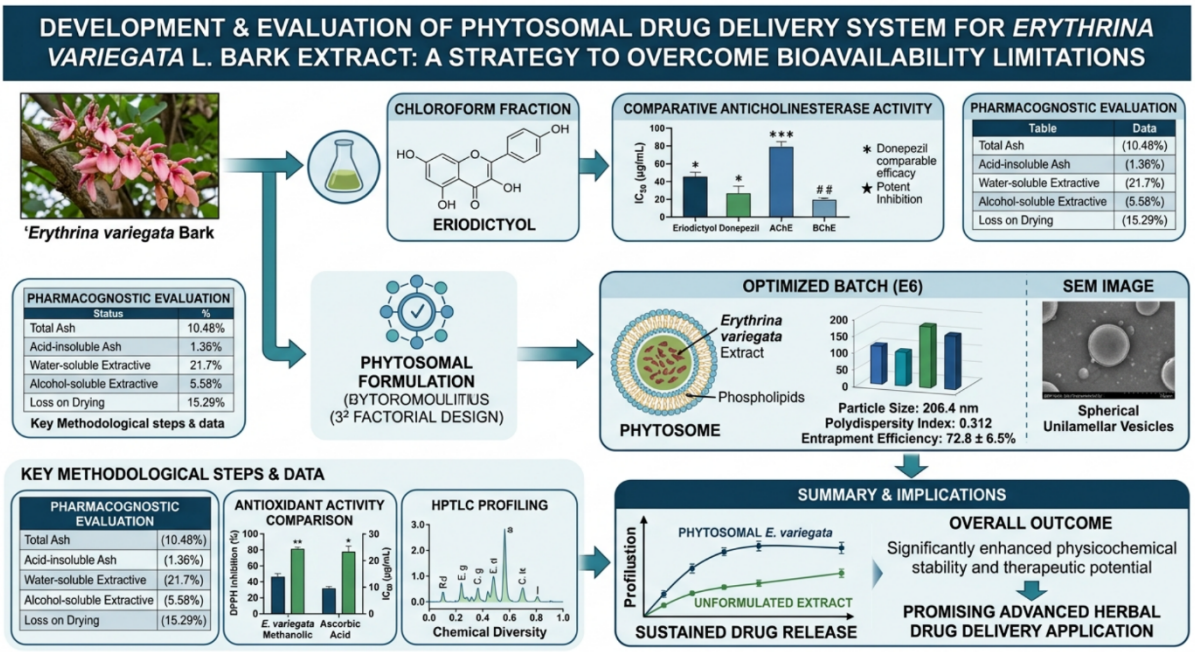
Graphical Abstract



1. INTRODUCTION

The medicinal plant Erythrina variegata Linn., belonging to the Fabaceae family, has a wide range of distribution in tropical and subtropical regions of the world, including India, Southeast Asia, and East Africa [1]. This plant is commonly known as the "coral tree" or "Indian coral tree" (or "Parijata") and is used in traditional Ayurvedic medicine and other folk medicines to treat many inflammatory diseases (e.g., arthritis), parasitic infections, asthma, epilepsy, and various nervous system disorders[2]. The analgesic, anxiolytic, antiepileptic, and anti-inflammatory properties of both leaves and bark of this plant are well established[3].

Erythrina variegata contains multiple types of bioactive compounds based on phytochemical studies, which have been classified into groups according to their pharmacological action; they include alkaloids, flavonoids, and isoflavonoids[2]. The bioactive compounds found in Erythrina variegata function as secondary metabolites, and this group of compounds is also responsible for the many pharmacological effects associated with the use of this plant (e.g., anti-oxidant, hepatoprotective, neuroprotective)[4].

While the bioactive phytoconstituents in Erythrina variegata have tremendous therapeutic potential, many of these compounds are poorly soluble in water and large in molecular size, creating difficulty for them to penetrate through cell membranes and affect physiologic targets in the body[5]. These compounds typically have very low oral bioavailability and are usually eliminated rapidly from the systemic circulation, creating significant barriers for clinical use of these bioactive compounds[6].

Research in the past few years is beginning to identify phytosome technology as a potential means to improve the bioavailability of bioactive compounds from plants by evolving vesicular systems. Phytosomes enhance the lipid solubility, membrane permeability, and systemic absorption of phytoconstituents when phosphatidylcholine is used to form a complex with these compounds, thereby facilitating the incorporation of these bioactive phytoconstituents into the phytosomal vesicle.

Therefore, the aim of this study is to extract bioactive phytoconstituents from the bark of Erythrina variegata so that a phytosome drug delivery system can be developed to improve their bioavailability and therapeutic activity. The study will extract and characterise phytochemicals, develop optimal formulations, and evaluate the phytosome systems that will facilitate extraction of the bioactive phytoconstituents found within the bark of Erythrina variegata.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Bark from Erythrina variegata (Linn.) was gathered in the Satara district of Maharashtra, India. The sample was authenticated by a qualified botanist

who prepared and preserved a voucher specimen. The collected bark was washed thoroughly in running water to remove any impurities adhering to the surface. It was then shade dried under controlled conditions and milled into a coarse powder for use in subsequent experiments.

2.2 Preparation of Extract

2.2.1 Soxhlet Extraction Procedure

The powdered plant material was extracted by using a Soxhlet method, utilizing solvents of increasing polarity: petroleum ether (n=3), chloroform (n=3), ethyl acetate (n=3), ethanol (n=3), and methanol (n=3). After immersing the sample in petroleum ether, for example, extraction of the compound continued until the solvent in the siphon tube no longer contained any color. Generally, the solvents used during extraction are returned to the original flask and continuously reused until colorless. Following extraction, the resulting extracts were filtered when hot and then concentrated using a rotary evaporator under reduced pressure, giving rise to semi-solid residues (after evaporation of the solvent) [7].

2.2.2 Maceration Method

For comparative purposes, a sample of the powdered bark was extracted through maceration by soaking the sample for 7 days and shaking intermittently in 70% (v/v) ethanol. After the extraction period, the extract was filtered and concentrated under reduced pressure to provide a crude extract that was stored in light and air-tight containers at controlled temperatures [8].

2.2.3 Methanolic Extract Preparation

The methanol extract was prepared separately by soaking 50 g of the powdered bark in 250 mL methanol for 72 hours and shaking intermittently. After this time period, the extract was filtered and concentrated at 40°C, followed by storage at 4°C for further evaluation [9].

2.3 Fractionation of Extract

Fractionation of the crude extract involved the solvent-solvent partitioning of the crude extract (obtained above) into n-hexane (3 x 150 mL), ethyl acetate (3 x 150 mL), and chloroform (3 x 150 mL) to produce different fractions of varying phytochemical classes. Each of the fractions was concentrated and stored in airtight containers for later evaluation [10].

2.4 Sample Extraction for Sequential Solvent Extraction

Sequential extracts of 50 g of powdered plant material were performed by using sequential extraction methods and sequentially increasing polarity of solvents (i.e. petroleum ether, chloroform, ethyl acetate, ethanol and water) with 250 mL of solvent used per extraction cycle over a 16-hour period. The extracts were concentrated using a rotary evaporator and all residues were thoroughly dried before proceeding to the next solvent. The aqueous extract, which exhibited the greatest amount of secondary metabolite concentration, was chosen for further evaluation [10].

2.5 Phytochemical Screening

2.5.1 Qualitative Analysis

Preliminary phytochemical screening was performed using standard procedures to detect the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, triterpenoids, glycosides, and carbohydrates.

2.5.2 Quantitative Estimation

Quantitative estimation of bioactive constituents, including alkaloids, flavonoids, and total phenolic content, was carried out using established spectrophotometric methods such as Harborne's method and Folin–Ciocalteu assay.

2.6 Chromatographic Analysis

2.6.1 Thin Layer Chromatography (TLC)

TLC analysis was performed on pre-coated silica gel 60 F254 plates using appropriate solvent systems. The developed plates were visualized under UV light at 254 nm and 366 nm, followed by derivatization using suitable reagents to detect phytoconstituents.

2.6.2 High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis was conducted using CAMAG LINOMAT 5 applicator and silica gel plates. Samples and standards were applied as bands, developed in optimized mobile phases, and analyzed under UV light. The plates were scanned using a TLC scanner, and Rf values along with peak areas were recorded using WINCATS software.

2.7 Isolation of Bioactive Compound

Isolation of phytoconstituents was carried out using column chromatography with silica gel as the stationary phase. Elution was performed using solvent gradients of increasing polarity. Fractions were collected and monitored using TLC, and similar fractions were pooled. The isolated compound was further purified and subjected to structural characterization [11].

2.8 Characterization of Isolated Compound

The isolated compound was characterized using advanced spectroscopic techniques, including mass spectrometry (MS), nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR), and Fourier transform infrared spectroscopy (FTIR) to confirm its chemical structure.

2.9 Pharmacognostic Evaluation

Pharmacognostic parameters such as total ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, and loss on drying were determined according to standard pharmacopeial methods to ensure quality and purity of the plant material [11].

2.10 Herbarium Authentication Procedure

Authentication of plant material involved morphological identification, taxonomic verification, microscopic analysis, and chemical profiling. A herbarium specimen was prepared, documented, and deposited in a recognized repository for future reference.

2.11 In-vitro Biological Evaluation

2.11.1 Antioxidant Activity

The antioxidant activity was evaluated using DPPH radical scavenging assay and reducing power assay. The percentage inhibition and IC50 values were calculated to assess free radical scavenging potential [11].

2.11.2 Anticholinesterase Activity

The inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was evaluated using Ellman's method. The IC50 values were calculated based on enzyme inhibition kinetics [12].

2.12 Preformulation Studies

2.12.1 Solubility Studies

Solubility of the extract was evaluated in different solvents such as ethanol, methanol, and acetone to determine suitable formulation conditions.

2.12.2 Drug–Excipient Compatibility Study

Compatibility between the extract and excipients was assessed using FTIR spectroscopy to identify any potential chemical interactions.

2.13 Experimental Animals and Ethical Approval

Albino Wistar male rats weighing 100–150 g were procured from the institutional animal facility. The animals were housed under controlled environmental conditions, maintaining a temperature of 25 ± 2 °C, relative humidity of 75 ± 5%, and a 12 h light/dark cycle. Standard laboratory feed and water were provided ad libitum throughout the study period.

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of YSPM's YTC Faculty of Pharmacy, Wadhe, Satara, India. The study was conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

The project proposal **(YSPM/YTC/PHARMA/Ph.D/2024–2025)** was approved in the IAEC meeting held on 22/03/2025, and the use of Albino Wistar rats was duly sanctioned for the experimental study

2.14 Pharmacological Evaluation of Erythrina variegata Extract Using In-Vivo Testing Methods

2.14.1 Inhibition of Inflammation & Pain Relief

Inhibition of inflammation (anti-inflammatory activity) was established by placing rats into an experiment whereby carrageenan was injected into the right hind paw and then measuring the amount of swelling that occurred within a defined period after injection. Pain relief (analgesic activity) was evaluated using two different methods: acetic acid induced writhing method and hot plate method to evaluate pain perception in the same rat that had been used for the swelling test previously. All experimental groups (with the extract tested) showed a dose-dependent inhibition of inflammation and pain responses similar to that seen with the standard drugs used (diclofenac and morphine).

2.14.2 Test for Anxiolytic & Sedative Properties

The anxiolytic and sedative properties of Erythrina variegata extract were evaluated using various behavioral tests including elevated plus maze test to test for anxiety, hole-board test to test for exploratory behavior and locomotor activity test to evaluate sedative effects of the extract using mice. All experimental animals showed significant anxiolytic activity as measured by an increased amount of time spent in the open arms of the elevated plus maze, whereas performances on the locomotor activity test indicated a sedative effect similar to that seen in diazepam-treated mice.

2.14.3 Evaluation of Hepatoprotective Potential

The hepatoprotective potential of Erythrina variegata extract was evaluated using rats for liver damage due to carbon tetrachloride (CCl_4) administration and measurement of serum biomarkers (ALT, AST, ALP, Bilirubin) and examination of liver tissue sections for improved

histopathology. After receiving treatment with the extract, all serum biomarkers measured were significantly reduced, and improvements were also noted in all sections of liver tissue examined histologically.

2.14.4 Testing for Antioxidant Activity

Antioxidant activity of Erythrina variegata extract was tested in-vivo using carbon tetrachloride (CCl_4) or hydrogen peroxide (H_2O_2) to behave like oxidants, and the biochemical markers used to evaluate antioxidant activity. All experimental animals showed statistically significant reductions of lipid peroxidation (malondialdehyde) and increased activity of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) when compared to the control group.

2.14.5 Antidiabetic Testing

Experimental rats were induced to have type 2 diabetes by administration of streptozotocin (STZ) and treated with Erythrina variegata extract. The extract produced significant reductions in blood glucose levels of the diabetic rats and induced regeneration of pancreatic β-cells.

2.15 Separation of Phytoconstituents via Column Chromatography

Phytoconstituents were separated by column chromatography using silica gel (60–120 mesh) as the stationary phase, and for more polar phytoconstituents by reverse-phase C18 silica. The mobile phase consisted of progressively polar solvents beginning with non-polar hexane followed by gradient elution (ethyl acetate and methanol).

All columns were approximately 2.5 cm in diameter and 20–40 cm tall, and processed 1–5 g of crude extract. Fractions were collected in 10–20 mL amounts, and each fraction was tested via TLC for evidence of similar chromatographic profiles. Fractions that were identified as having similar chromatographic profiles were pooled, and further purified if necessary, and then characterized by various spectroscopy techniques including NMR, MS, and HPLC.

2.16 Quantification of Bioactive Constituents

Quantitative estimation of major phytoconstituents, including alkaloids, flavonoids, and phenolic compounds, was carried out using established standard methods.

Total alkaloid content was determined using a modified Harborne method (1973), while total flavonoid content and total phenolic content were estimated using methods described by Singleton and Rossi (1965) and Orton et al. (2006), respectively.

2.17 Identification and Profiling of Bioactive Constituents

2.17.1 HPTLC Analysis of Alkaloids, Flavonoids, and Glycosides

HPTLC analysis was performed using CAMAG LINOMAT 5 applicator. Samples and standards were applied as 5 mm bands on silica gel 60 F254 TLC plates using a Hamilton syringe. The plates were developed in a twin-trough chamber pre-saturated with the respective mobile phase; Mobile phase composition and spray reagents **(Table 1).**

The chromatograms were developed up to a distance of 90 mm, dried using hot air, and visualized under UV light at 254 nm and 366 nm. Post-derivatization was carried out using appropriate spray reagents to enhance detection of phytoconstituents.

Densitometric scanning was performed using a CAMAG TLC scanner, and Rf values, peak areas, and retention characteristics were recorded using WINCATS software.

Table 1: Mobile phase and spray reagents of HPTLC profile

Profile	Mobile phase	Spray reagent
Alkaloids	Ethyl acetate: Methanol: Water (10:1.35:1)	Dragendroff's reagent followed by 10 % ethanolic sulphuric acid reagent.
Flavonoids	Toluene: Acetone: Formic acid (4.5:4.5:1).	1 % Ethanolic aluminium chloride reagent.
Glycosides	Ethyl acetate: Ethanol: Water (8:2:1.2)	Lieberman Burchard reagent.

After chromatographic development, the TLC plates were dried using a stream of hot air to remove residual solvents. The dried plates were initially visualized and documented under visible light and ultraviolet (UV) light at 254 nm and 366 nm using a CAMAG REPROSTAR 3 photo-documentation system.

Subsequently, the plates were derivatized with appropriate spray reagents and dried in a hot air oven at 100 °C to enhance the visualization of separated phytoconstituents. Post-derivatization,

the plates were again documented under daylight and UV (366 nm) conditions.

Densitometric analysis was performed using a CAMAG TLC Scanner 3 at a wavelength of 254 nm prior to derivatization. Chromatographic fingerprint data, including retention factor (Rf) values and peak characteristics, were recorded and analyzed using WINCATS software.

Product final Volume-

Final yields of the extract in various solvents

1. Solvent Yield (g)
2. Hexane 3.542
3. Ethyl acetate 6.534
4. Ethanol 18.315
5. Chloroform 2–5
6. Methanol 8–12

2.18 In-vitro Antioxidant Activity

2.18.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of the methanolic extract of *Erythrina variegata* L. was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following the method described by Blois (1958). The decrease in absorbance due to the reduction of DPPH radicals was measured spectrophotometrically, and the percentage inhibition was calculated to assess antioxidant potential.

2.18.2 Reducing Power Assay

The reducing power of the extract was determined according to the method described by Oyaizu (1986). This assay is based on the ability of the extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), indicating its electron-donating capacity and potential antioxidant activity Table 2. Anticholinesterase phytoconstituents from Erythrina variegata L.

Table 2. Anticholinesterase phytoconstituents from *Erythrina variegata* L.

Phytoconstituent	Type	Mechanism of Action	In-vitro Evidence
Erythraline	Alkaloid	Competitive acetylcholinesterase (AChE) inhibitor	Demonstrates inhibition of AChE activity in Ellman's assay using both crude and purified extracts
Erysodine	Alkaloid	Competitive acetylcholinesterase (AChE) inhibitor	Exhibits moderate cholinesterase inhibition, particularly in crude alkaloid fractions obtained from bark and seeds
Erythrinins	Isoflavonoids	Dual AChE and BuChE inhibitor	Methanolic and ethanolic extracts containing erythrinins show moderate inhibitory activity against both AChE and BuChE

2.19 Pharmacognostic Evaluation

2.19.1 Total Ash Determination

About 2-4 g of oven-dried sample was placed in an already tared and ignited silica crucible. The sample was spread evenly inside the crucible and heated in a furnace at 600 degrees Celsius until there was no visible sign of carbon present in the ash. The crucible was placed in a desiccator for cooling and weighed. If needed, the residue was wet with distilled water or saturated ammonium nitrate, dried and reignited to a constant weight. The total ash content was expressed as a percentage of the initial sample that was air-dried.

2.19.2 Acid Insoluble Ash Determination

The total ash obtained from Step 2.19.1 was treated with 45 mL of dilute HCl (1:5) and gently boiled for 5 minutes. The insoluble part was collected onto ash-less filter paper (Whatman No.41), washed with distilled water and transferred back into the original crucible. After drying, the acidic insoluble fraction was ignited to constant weight, cooled in a desiccator and weighed. The acid-insoluble ash was reported as a percentage of the air-dried sample.

2.19.3 Alcohol Soluble Extractive Value Determination

About 4 g of sample was ground with 100 mL of 95% ethanol for 24 hours, shaking every 6hours during the first 6 hours. The filtered sample solution was evaporated at 105 degrees Celsius to fetch a volume of 25.0 mL. The residue was cooled and weighed, and the alcohol-soluble extractive value calculated.

2.19.4 Water Soluble Extractive Value Determination

About 4 g of sample was ground in a solution of 100 mL chloroform water (95:5) for 24 hours. The filtered sample solution was also evaporated at 105 degrees Celsius to produce a volume of 25 mL. The residue was cooled and weighed, and the water-soluble extractive value calculated.

2.20 Thin Layer Chromatography (TLC) Analysis

Four grams of sample were extracted with 40 mL of ethanol and allowed to stand on a bench overnight, heated for 10 minutes, filtered, and concentrated to a final volume of ten millilitres. Ten microlitres of concentrated extract was applied to pre-coated silica gel 60 F254 plates.

The plates were then developed using the following mobile phase composition—five volumes of ethanol, 1.5 volumes of toluene and 0.5 volume of formic acid. After development, plates were dried and viewed with a 366 nanometre light. Plates were then derivatised using a vanillin-sulphuric acid reagent and heated to obtain chromatographic spots.

2.21 Qualitative Phytochemical Analysis

2.21.1 Test for Alkaloids

Formation of a reddish precipitate upon treatment with Dragendorff's reagent confirmed the presence of alkaloids.

2.21.2 Test for Triterpenoids (Noller's Test)

Development of a purple coloration after treatment with tin and thionyl chloride indicated the presence of triterpenoids.

2.21.3 Test for Steroids (Liebermann–Burchard Test)

Formation of a green coloration upon reaction with acetic anhydride and sulfuric acid confirmed steroids.

2.21.4 Test for Flavonoids (Shinoda Test)

Appearance of magenta coloration indicated the presence of flavonoids.

2.21.5 Test for Carbohydrates

Green coloration with anthrone reagent indicated the presence of carbohydrates.

2.21.6 Test for Quinones

Red coloration upon addition of concentrated sulfuric acid confirmed quinones.

2.21.7 Test for Phenols

Green or blue coloration with ferric chloride indicated phenolic compounds.

2.21.8 Test for Saponins

Formation of stable froth confirmed the presence of saponins.

2.21.9 Test for Tannins

White precipitate with lead acetate indicated the presence of tannins.

2.22 In-vitro Antioxidant Activity

2.22.1 DPPH Radical Scavenging Assay

The antioxidant activity was evaluated using the DPPH assay (Blois, 1958), based on the reduction of DPPH radicals measured spectrophotometrically.

2.22.2 Reducing Power Assay

The reducing power was determined using the method of Oyaizu (1986), based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}).

2.23 Screening of Psychopharmacological Activity

2.23.1 In-vitro Anticholinesterase Assay

The inhibition potency of the crude plant extract of Erythrina variegata Linn. was evaluated towards acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using Ellman's colorimetric method. The substrates for AChE and BChE were acetylthiocholine iodide and butyrylthiocholine iodide, respectively.

A reaction mixture containing 50mM Tris-HCl buffer (pH 8.0), various concentrations of extract, and donepezil (the positive control) as the standard, was prepared, then 20µL of 5,5-dithiobis-(2-nitrobenzoic acid)(DTNB) was added, followed by 10µL of the enzyme solution (either AChE or BChE). This reaction mixture was incubated at room temperature for 15minutes.

The enzymatic reaction developed after this incubation was monitored spectrophotometrically at 411nm for 3minutes, measuring the change in absorbance every 45seconds. The percentage inhibition of the enzyme activity was calculated; the IC50 was determined according to the plot of percentage inhibition versus plant extract concentration.

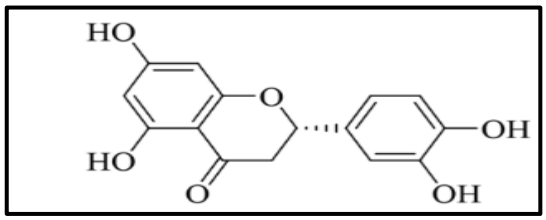
2.24 Preliminary Screening

2.24.1 Solubility Studies

The solubility of *Erythrina variegata* Linn. extract was evaluated in various solvents, including ethanol, methanol, and acetone, to determine its physicochemical characteristics and suitability for formulation development.

2.25 Isolation of Bioactive Compound

Isolation of the bioactive compound from *Erythrina variegata* Linn. extract was carried out using chromatographic techniques, primarily column chromatography. The separated fractions were monitored using TLC, and fractions with similar profiles were pooled and further purified. The isolated compound was subsequently subjected to structural characterization using spectroscopic methods.

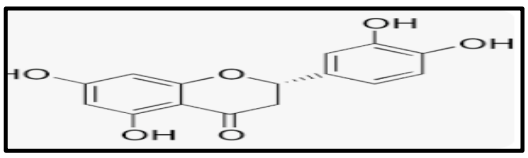


Structure of eriodictoyl

Eriodictoyl

Eriodictyol belongs to the flavanone subclass of flavonoids and has a chemical structure defined by the molecular formula $C_{15}H_{12}O_6$, with a molecular weight of 288.25 g/mol.

Chemical core Structure



Systematic Name: Eriodictiol

TLC Image of Eriodictoyl shown in Figure 1

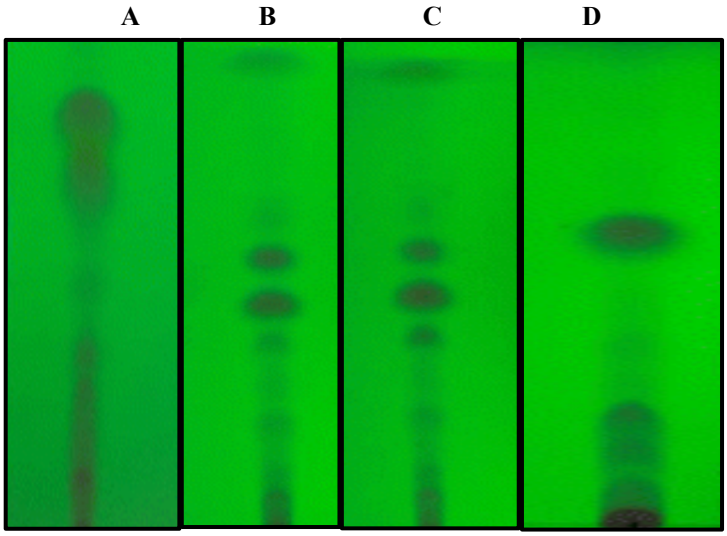


Figure 1 TLC Image of Eriodictoyl

2.26 Mass Spectrometric Analysis of Isolated Compound

An isolated compound identified as eriodictyol (C21H22O11) was analyzed to obtain a mass spectrum. The analysis was conducted using an HPLC system (Agilent Infinity 1290 LC System) which was directly interfaced with an Agilent 6420 Triple Quad Mass Spectrometer via a single quadrupole mass selective detector.

The analysis was completed in positive ion mode under optimized conditions (fragmentor voltage: 100 V, capillary voltage: 2500 V, nebuliser pressure: 30 psi, and dry gas at a temperature of 350 ˚C), scanning a mass range of 100 to 600 Da.

The resulting mass spectra allowed the identification of fragment ions based on their mass/charge (m/z) ratios, thereby providing enough information regarding eriodictyol's structure for the structure to be confirmed as illustrated in figure 2.

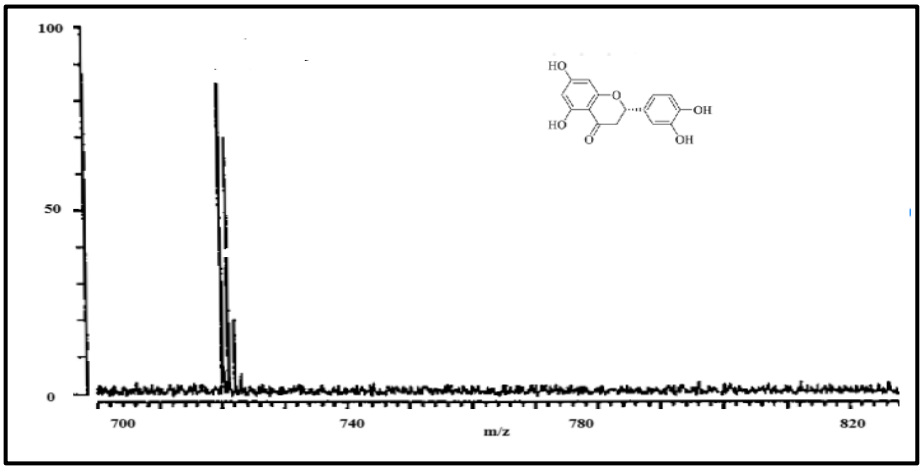


Figure 2: Mass Spectra of isolated compound

2.27 Nuclear Magnetic Resonance (NMR) Analysis

Nuclear Magnetic Resonance was utilized to structurally characterize the isolated compound, eriodictyol. ^1H and ^13C Nuclear Magnetic Resonance spectra were obtained at 500 MHz (protons) and 125 MHz (carbons), respectively, using a Bruker 500 MHz Nuclear Magnetic Resonance spectrometer. The acquired spectra produced detailed information about both the proton and carbon environments, which confirmed the molecular structure of the Isolation Compound. ^1H-Nuclear Magnetic Resonance Spectra of Isolation Compound are shown in Figures(3).

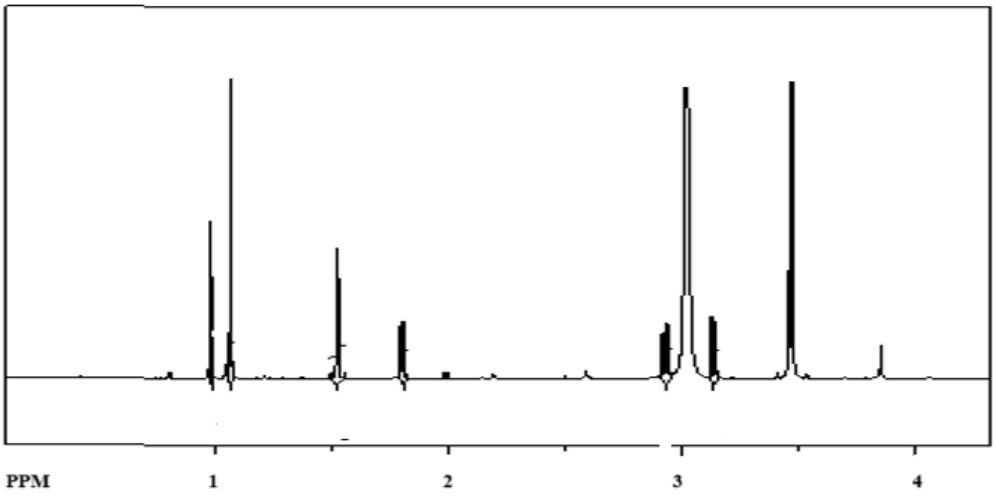


Figure 3: ^{1}H - NMR spectra of isolated compound

2.28 Preliminary Studies

2.28.1 Solubility Studies

The solubility of *Erythrina variegata* Linn. extract was evaluated in various solvents, including ethanol, methanol, and acetone, to determine its physicochemical properties and suitability for formulation development.

2.28.2 Drug–Excipient Compatibility Study

Drug–excipient compatibility was assessed using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of the pure extract, selected excipients, and their physical mixtures were recorded and compared to identify any potential chemical interactions. The absence of significant shifts or disappearance of characteristic peaks confirmed compatibility between the drug and excipients.

2.29 Selection of Lipids and Formulation Strategy

Lipids for phytosome preparation were selected based on criteria such as compatibility with the plant extract, availability, cost-effectiveness, and their influence on vesicle formation and stability. Lecithin, phospholipon 90G, and cholesterol were evaluated for their suitability.

Among the tested lipids, phospholipon 90G demonstrated superior entrapment efficiency and stability, with no observable sedimentation, and

was therefore selected for further formulation development.

2.30 Preparation of Phytosomal Formulation

Phytosomes of *Erythrina variegata* Linn. extract were prepared using a solvent injection method. Ethanol (20–30%) containing varying concentrations of cholesterol (1–3%) was heated to 30 °C and mixed using a magnetic stirrer. Propylene glycol (2%) and the extract–phospholipid complex (2%) were dissolved in the ethanolic phase.

Separately, double-distilled water was heated to 30 °C and maintained under continuous stirring at 700 rpm. The ethanolic phase was slowly injected into the aqueous phase using a hypodermic syringe under constant stirring. Stirring was continued for an additional 30 minutes to ensure uniform vesicle formation.

The formulation was maintained at a constant temperature of 30 °C throughout the process. Sonication was employed, when necessary, to reduce vesicle size and achieve uniform dispersion. The prepared phytosomal formulation was stored at 4 °C for further evaluation shown in Table 3.

Table 3: Different batches of Phytosomal formulation

Batch	Complex of EV Linn. PHL90G	Cholesterol	Propylene Glycol	Ethanol	Water
E1	2 %	1 %	2 %	20 %	Q.S
E2	2 %	2 %	2 %	20 %	
E3	2 %	3 %	2 %	20 %	
E4	2 %	1 %	2 %	30 %	
E5	2 %	2 %	2 %	30 %	
E6	2 %	3 %	2 %	30 %	
E7	2 %	1 %	2 %	40 %	
E8	2 %	2 %	2 %	40 %	
E9	2 %	3 %	2 %	40 %	

2.31 Formulation Optimization

Optimization of pharmaceutical formulations using the conventional one-variable-at-a-time approach is often labor-intensive and fails to account for interaction effects between formulation variables. To overcome these limitations and achieve a robust optimized formulation, a statistical design of experiments (DoE) approach was employed.

2.31.1 Experimental Design

A 3^2 full factorial design was utilized to systematically evaluate the influence of formulation variables on key response parameters. Two independent variables were selected: cholesterol concentration (X_1) and ethanol concentration (X_2). Each variable was studied at three levels (low, medium, and high), as defined in **Table 4.**

The dependent variables (responses) selected for optimization were particle size and percentage entrapment efficiency.

Table 4: Levels of independent variables for 3^2 full factorial design

Batches	Variables level in Coded form		Actual Values of Variables	
	X1	X2	X1 (%)	X2 (%)
E1	-1	-1	1	20
E2	-1	0	1	30
E3	-1	+1	1	40
E4	0	-1	2	20
E5	0	0	2	30
E6	0	+1	2	40
E7	+1	-1	3	20

E8	+1	0	3	30
E9	+1	+1	3	40
Variables Level		Low	Medium	High
Concentration of Cholesterol (%)		1	2	3
Concentration of Ethanol (%)		20	30	40

2.31.2 Statistical Analysis

The effect of independent variables on the selected responses was analyzed using Design Expert software (Version 8.0.4, Stat-Ease Inc., USA). Statistical significance was evaluated using one-way analysis of variance (ANOVA), and response surface methodology was applied to understand the interaction between variables.

The relationship between independent variables and responses was described using the following second-order polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where:

- Y = dependent response (particle size or entrapment efficiency)
- b_0 = intercept representing the average response
- b_1, b_2 = coefficients of main effects
- b_{12} = coefficient of interaction effect
- b_{11}, b_{22} = coefficients of quadratic terms

The main effects (X_1 and X_2) represent the individual contribution of each factor, while the interaction term (X_1X_2) reflects the combined influence of both variables. The quadratic terms (X_1^2 and X_2^2) account for non-linear relationships between variables and responses.

2.31.3 Model Validation

To validate the developed polynomial model, checkpoint formulations were prepared at intermediate levels of the independent variables. The experimentally obtained responses were compared with predicted values, and the model validity was confirmed based on the absence of significant differences between them.

2.32 Evaluation of Phytosomal Formulations

2.32.1 Particle Size and Size Distribution

Particle size and distribution of the phytosomal formulations developed were measured through DLS, using a Malvern Zetasizer and an Argon laser. Measurements were obtained using controlled conditions, and average particle size and PDI were determined as measures of uniformity and stability of the vesicular system.

2.32.2 Entrapment Efficiency (EE%)

Entrapment efficiency of the phytosomal vesicles was determined by ultracentrifugation. The phytosomal suspension was centrifuged at 10,000 rpm for 90 minutes at 4 °C using a REMI high-speed cooling centrifuge. The supernatant and sediment were separated, and the sediment containing the entrapped drug was dissolved in methanol.

The drug content was quantified using UV spectrophotometry at 350 nm. The entrapment efficiency was calculated using the following equation:

$$\% \text{ Entrapment Efficiency} = \left(\frac{\text{Entrapped Drug}}{\text{Total Drug}}\right) \times 100$$

2.32.3 Selection of Optimized Formulation and Checkpoint Analysis

The optimized formulation was selected based on desirability criteria, including minimum particle size and maximum entrapment efficiency. The overall desirability function (D) was calculated by transforming individual responses into desirability values (d_i), ranging from 0 to 1, where 0 represents an unacceptable response and 1 represents the ideal response.

$$D = (d_1 \times d_2 \times \cdots \times d_m)^{1/m}$$

For particle size, individual desirability (d_1) was calculated as:

$$d_1 = \frac{Q}{R_{\max} - R_{\min}}$$

Where:

- **Q** = difference between observed response and extreme value
- **Rmax** = maximum observed response
- **Rmin** = minimum observed response

Similarly, desirability values for other formulation variables were calculated.

A checkpoint formulation (E6) was prepared at intermediate levels of independent variables, and the predicted responses were compared with

experimentally obtained values. Statistical validation using Student's *t*-test indicated no significant difference ($p > 0.05$), confirming the reliability of the optimization model.

2.32.4 In-vitro Drug Release Study

To evaluate the in vitro drug release profile of the optimized phytosomal formulation, a Franz diffusion cell was utilized with pig skin serving as the permeable biological membrane (100%). The phosphate buffer with a pH of 7.4 served as the receiver medium. Prior to mounting the pigskin membrane between both the donor and receiver compartments, it was allowed to pre-soak overnight in the diffusion medium. Once this step was completed, a volume of 10 mL of the phytosomal suspension containing 10 mg of extract was deposited into the donor compartment, which was sealed with paraffin and tape to prevent evaporation. The temperature of the receiver compartment (20 mL) was held at 37°C ± 1°C, and mixing occurred at 100 rpm utilizing magnetic stirring. At specific time points (between 1 and 8 hours), 3 mL samples were withdrawn from the receiver compartment and replaced with additional buffer to maintain ideal sink conditions for the duration of the process. The samples were analyzed for cumulative drug release via a UV spectrophotometer at 350 nm.

2.32.5 Stability Studies

Physical stability and drug retention during the storage of the optimized phytosomal formulation (E6) was evaluated through stability studies. Sealed glass vials containing the formulated product were stored at both room (25°C) and refrigerated (2-8°C) temperatures for three months to simulate typical conditions of handling and shipping.

Samples were taken at regular intervals and analyzed to measure physical changes, including appearance and particle size, as well as the amount of drugs that had leaked out of the container, in order to determine the stability of the formulation.

3. RESULTS AND DISCUSSION

3.1 Pharmacognostic Evaluation

Standardization of pharmacognostic material is fundamental to establishing quality, identity, and purity of crude plant material. In this study on Erythrina variegata bark, the following physicochemical parameters were measured: total ash, acid-insoluble ash, water-soluble extractive value, alcohol-soluble extractive value, and loss on drying.

The total ash value (10.48%), indicates the presence of dust or non-specific inorganic materials. This could be due to the ash from physiological processes as well as contamination from foreign sources. The acid-insoluble ash value (1.36%) suggests very little siliceous materials, like sand and soil are present. The higher water-soluble extractive value (21.7%) versus the alcohol-soluble extractive value (5.589%), indicates that there are significantly more constituents that can be extracted with water than can be extracted with alcohol--water-soluble constituents include phenolics, glycosides, and carbohydrates. The loss on drying value (15.291%) indicates the total moisture content. Both loss on drying and total moisture content are acceptable amounts of moisture content for crude plant materials, as shown in Table 5.

Table 5: Pharmacognostic standardization parameters of *Erythrina variegata* bark

Sr. no.	Parameters	Erythrina variegata
1	Ash (% w/w)	10.48
2	Acid insoluble ash (% W/W)	1.36
3	Water –soluble extractive (% w/w)	21.7
4	Alcohol–soluble extractive (% w/w)	5.58
5	Loss on drying at 105 ºc (% w/w)	15.29

3.2 Phytochemical Screening

A preliminary phytochemical screening of several extracts from different solvents showed a high occurrence of various bioactive secondary metabolites. Alkaloids, phenols and steroids were present in all the solvent extracts and suggest that these compounds are present in high amounts in the bark.

The flavonoids and triterpenoids were primarily observed in the extracts of benzene, ethyl acetate and petroleum ether, indicating that they have moderate polarity. The saponins and phenolic compounds were found to be widely dispersed, thus supporting the possibility of both antioxidant and therapeutic activity for these plants. The lack of quinones and (likely) acids appears to reflect a specific phytochemical profile.

The results of the preliminary screening support the use of Erythrina variegata as a traditional medicine,

which is also evidenced by its potential for pharmacological use as detailed in Table 6.

Table 6: Phytochemical screening of different solvent extracts of *Erythrina variegata* bark

Phytochemicals	Bark inference				
	B	E	EA	M	PE
Steroid	+	+	+	+	+
Triterpenoid	+	-	+	-	+
Flavonoid	+	-	+	-	+
Furan	-	-	+	-	-
Sugar	+	-	-	-	+
Coumarin	-	+	+	+	-
Quinine	-	-	-	-	-
Alkaloid	+	+	+	+	+
Tannin	-	-	+	-	-
Phenol	+	+	+	+	+
Acid	-	-	-	-	-
Saponin	+	+	+	+	-

+ = presence; - = Absence; B = Benzene; E = Ethanol; EA = Ethyl acetate; M = Methanol; PE = Petroleum ether.

3.3 Thin Layer Chromatography (TLC) Analysis

The TLC profile of Erythrina variegata bark extract indicates unique chromatographic patterns observed in both the presence of ultraviolet light (UV 254 nm and UV 366 nm) and following derivatisation after the use of spray reagents. Many spots of differing retention factors (Rf) support there being a diversity of phytochemical constituents.

On exposure to UV 254 nm a number of green-coloured spots were identified, at Rf values of 0.28 and 0.79 indicative of the presence of conjugated systems. In the presence of UV 366 nm several types of fluorescent spots were found (blue and red), indicative of flavonoids and phenolic compounds. Following derivatisation, grey and brown spots with Rf values ranging from 0.16 to 0.92 confirmed the presence of a range of secondary metabolites.

The overall genetic complexity of the extract depicted by TLC fingerprint profile can also be used as an important value-added component for identification and quality control of this plant material as shown in the table below.

Table 7: TLC profile of *Erythrina variegata* bark extract

Sr. no.	UV 254 nm		UV 366 nm		With spray reagent	
	Colour	Rf	Colour	Rf	Colour	Rf
1	Green	0.28	Blue	0.02	Grey	0.16
2	Green	0.79	Red	0.16	Grey	0.38
3			Red	0.20	Grey	0.52
4			Red	0.23	Brown	0.61
5			Red	0.30	Grey	0.67
6			Red	0.58	Grey	0.73
7			Red	0.67	Brown	0.80
8			Red	0.73	Brown	0.83
9			Red	0.80	Brown	0.92
10			Red	0.91		

3.4 HPTLC Analysis of Bioactive Constituents

Thin-Layer Chromatography (TLC) high throughput thin layer chromatography analysis was performed to develop the entire phytochemical profile for the aqueous bark extract of Erythrina variegata. The results allowed us to identify and estimate in semi-quantitative terms the different classes of bioactive compounds that were present

(alkaloids, flavonoids, glycosides) using retention factors (Rf), peak height and peak area as methods of access to this information.

3.4.1 Alkaloid Profiling

Five different peak regions were identified on a HPTLC chromatogram of alkaloids, displaying separate, discrete Rf values that can be quantified between 0.06 and 0.97. Each of these peaks corresponds to an individual type of chemical component found within an alkaloidal constituent. The reference standard used for comparison was colchicine, identified with a Rf value of 0.54.

Many of the peaks detected are clustered together in two major peaks, with the broadest band of activity at lower Rf values (for example, the area of peak at 0.06). This suggests that there are significant quantities of very concentrated alkaloid compounds represented in this sample; additionally, the variety and total number of those patterns represent incredibly diverse and complex structures within the alkaloid fraction of this extract.

These findings confirm that *Erythrina variegata* bark is a rich source of alkaloids, which may contribute to its reported neuropharmacological and anticholinesterase activities shown in Table 8 and Figure 4.

Table 8: HPTLC peak data of alkaloids in aqueous bark extract of *Erythrina variegata*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.54	260.6	8403.3	Colchicine
Sample A	2	0.06	500.8	17840.6	Alkaloid1
Sample A	3	0.12	63.2	1377.5	Unknown
Sample A	4	0.15	41.3	536.8	Unknown
Sample A	5	0.18	21.6	265.1	Unknown
Sample A	6	0.20	57.2	1515.9	Unknown
Sample A	7	0.28	40.6	1066.3	Unknown
Sample A	8	0.30	27.2	541.6	Unknown
Sample A	9	0.34	22.6	391.8	Unknown
Sample A	10	0.37	25.6	320.8	Unknown
Sample A	11	0.43	86.8	3997.2	Unknown
Sample A	12	0.47	66.6	1219.6	Unknown
Sample A	13	0.49	57.8	1357.4	Unknown
Sample A	14	0.60	22.7	571.8	Unknown
Sample A	15	0.67	37.5	587.6	Unknown
Sample A	16	0.68	38.4	782.8	Unknown
Sample A	17	0.74	23.7	485.8	Unknown
Sample A	18	0.80	20.9	485.5	Unknown
Sample A	19	0.89	19.0	426.7	Unknown
Sample A	20	0.97	108.4	469.7	Unknown

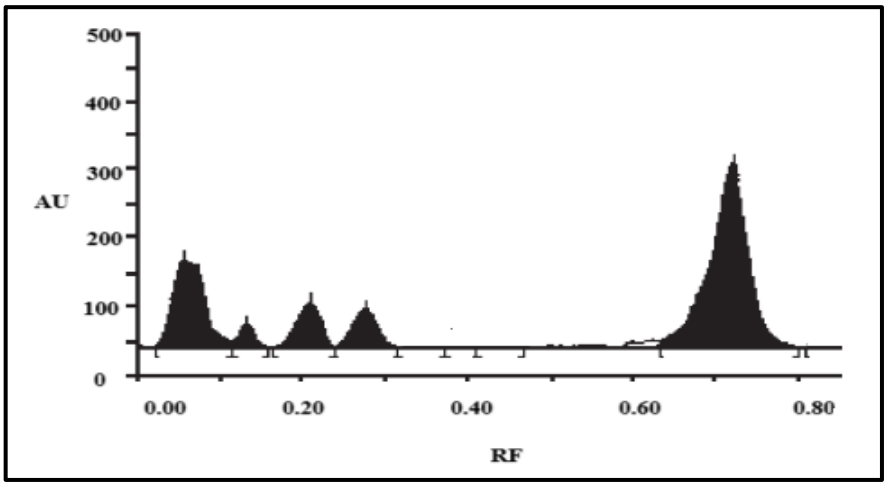


Figure 4: HPTLC chromatogram of alkaloids

3.4.2 Flavonoid Profiling

HPTLC analysis of flavonoids indicated the presence of the flavonoids using a range of Rf values (0.05 – 0.86). The presence of quercetin as the standard flavonoid confirmed this finding as it had an Rf value of 0.79, indicating flavonoid-type compounds are present within the extract. Several of the peaks were representative of the flavonoid peaks (Rf ~0.12, 0.50 and 0.80), which according to their peak areas, were meaningfully contributing to the phytochemical composition of the extract. There were additional peaks that were undetermined or not identifiable indicating that there are several different structural types of the flavonoid moments. As noted above, the biological activity of the extract supports the antioxidant and anti-inflammatory properties attributed to flavonoids.

Peak and RF values, height and area for the flavonoids and unidentified compounds in the aqueous bark extract of Erythrina variegata as found in Table 9 and Figure 5..

Table 9: HPTLC peak data of flavonoids in aqueous bark extract of *Erythrina variegata*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.79	776.9	20263.5	Quercetin
Sample A	2	0.05	633.5	12613.2	Unknown
Sample A	3	0.12	78.4	2046.5	Flavonoid 1
Sample A	4	0.15	57.1	1287.8	Unknown
Sample A	5	0.24	43.1	1274.2	Unknown
Sample A	6	0.34	50.2	1280.1	Unknown
Sample A	7	0.50	28.9	1084.1	Flavonoid 2
Sample A	8	0.60	31.6	1583.9	Unknown
Sample A	9	0.64	24.9	753.7	Unknown
Sample A	10	0.80	85.8	2790.3	Flavonoid 3
Sample A	11	0.86	64.6	2494.4	Unknown

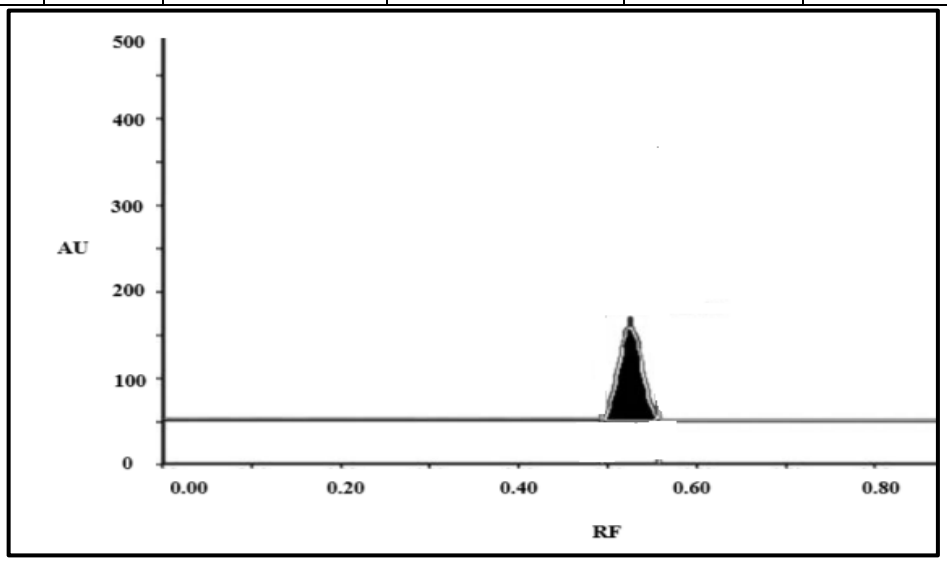


Figure 5: HPTLC chromatogram of flavonoids

3.4.3 Glycoside Profiling

The glycoside fractions demonstrated various distinct peaks with Rf values ranging from 0.02-0.49. The standard swertiamarin, used to establish the Rf for any glycosidic compounds, had an Rf of 0.69. Having a peak at Rf 0.09, which displayed a large area peak indicates that there was likely a single major glycosidic constituent in the extract. The rest of the peaks may be minor glycoside fractions or unidentified compounds.

It is expected that Glycosides exhibit significant pharmacologic activity, while exhibiting cardioprotective & antioxidant effects identified in Table 10 and Figure 6, thus providing additional evidence to the pharmacologic value of this extract.

Table 10: HPTLC peak data of glycosides in aqueous bark extract of *Erythrina variegata*

Track	Peak	Rf	Height	Area	Assigned substance

STD	1	0.69	48.6	1388.1	Swartiamarin
Sample A	2	0.02	15.5	146.1	Unknown
Sample A	3	0.09	478.5	12642.6	Glycoside1
Sample A	4	0.19	224.9	4752.5	Unknown
Sample A	5	0.36	58.5	2007.7	Unknown
Sample A	6	0.49	22.3	737.2	Unknown

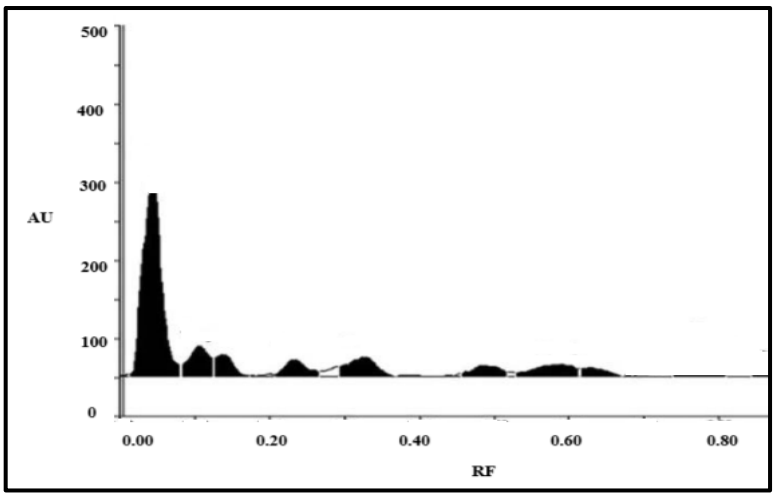


Figure 6: HPTLC chromatogram of glycosides

3.5 Molecular Docking Analysis

The binding interactions of the isolated compound eriodictyol with the target enzyme's active site were investigated through molecular docking studies. The molecular docking simulations were conducted via AutoDock 4.2. The molecular visualization and interaction analysis were conducted via PyMOL, UCSF Chimera, Discovery Studio Visualizer, and MMP Plus.

The docking results showed that eriodictyol has good binding interactions with the active site indicating the formation of a stable ligand-protein complex. Key interaction types (such as hydrogen bonding and hydrophobic interactions) indicate that eriodictyol could demonstrate biological activities by inhibiting enzymes as depicted in Figure 7

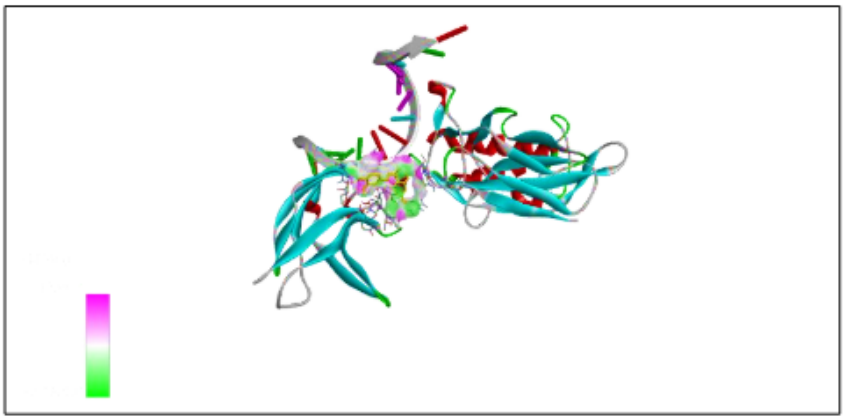


Figure 7: Three-dimensional binding mode of eriodictyol within the active site

3.6 Purity Analysis of Isolated Compound

The purity of the isolated compound was determined from the area and retention times of the chromatographic peaks. The results showed one predominant peak at a retention time of 23.91 minutes (97.53% of the total area), indicating a high purity of the isolated compound.

The other minor peaks with areas less than 1% are interpreted as trace impurities or residual material. Therefore, the high level of purity of the material

provides evidence that the isolation and purification processes described in Table 11 were effective.

Table 11: Peak area and retention time of isolated compound and impurities

Peak	Retention time/min	Area	Percentage of components (%)
1	2.05	2165	0.07
2	12.6	6495	0.08
3	21.52	31256	0.99
4	23.91	346512	97.53

3.7 FTIR Spectral Analysis

Functional groups and chemical structure of the compound that has been isolated were identified as well as confirmed via Fourier Transform Infrared (FTIR) spectroscopy. In the Fourier Transform Infrared (FTIR) spectrum, the characteristic absorption peak at 1599.57 cm^{-1}; which corresponds to the C=O stretching vibration, indicates there are carbonyl functional groups found in compounds of flavonoid nature.

The fact that there are no significant shifts in the characteristic absorption peaks of the compound according to the FTIR spectroscopy shows that the structure of the compound has not suffered any major structural changes and is still intact. There is evidence of intermolecular forces existing between the molecules by way of hydrogen bonding and van der Waals forces as evidenced in Figure 8 and Table 12.

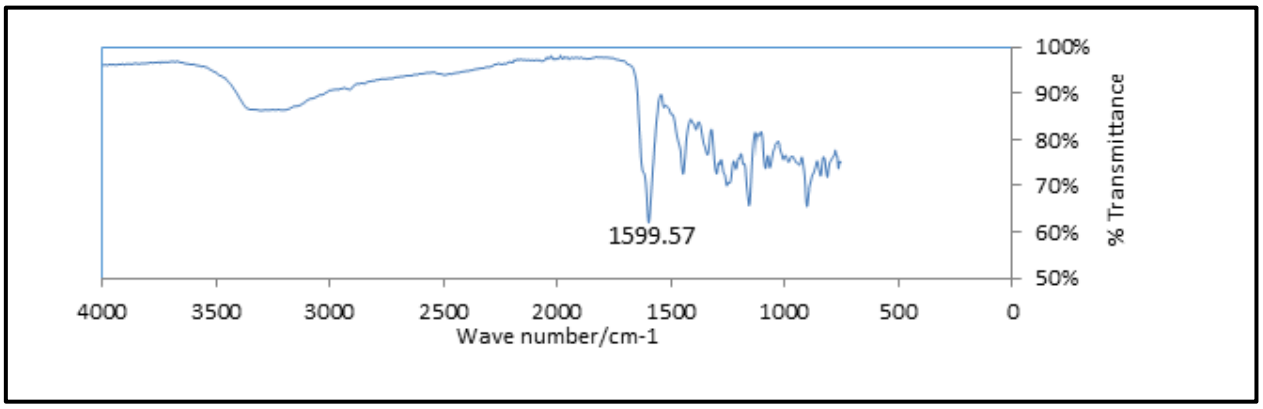


Figure 8: FTIR spectrum of isolated compound

Table 12: FTIR peak assignment of isolated compound

Material	Functional group	Standard IR range	Observed value
Isolated compound	C = O	1900 – 1600	1599.57

3.8 In-vitro Antioxidant Activity

3.8.1 DPPH Radical Scavenging Activity

To assess the methanolic bark extract from Erythrina variegata's ability to scavenge free radicals, a DPPH assay determined the extract's antioxidant activity as a function of concentration across a range between 100-500 µg/mL. The data shows a strong correlation with increasing concentrations of inhibition (%/mg) exhibiting that this bark extract contains strong antioxidative capabilities.

As it relates to the DPPH assay, with the maximum concentration (500 µg/mL) of bark extract was determined to provide 61.72% cumulative inhibition of radical activity. In comparison, the positive control (standard ascorbic acid) provided 68.86% cumulative inhibition of radical activity at the same concentration. Consequently, the IC_{50} (half-maximal inhibition) value of the bark extract (401 ± 0.77 µg/mL) was higher than that of ascorbic acid (301 ± 0.77 µg/mL), suggesting that DPPH radical inhibition by ascorbic acid occurs at a more effective concentration than the bark extract.

The mechanisms by which phytoconstituents exhibit antioxidant activity is that they are able to donate hydrogen atoms and/or electrons to neutralize DPPH (1,1-diphenyl-2-picrylhydrazyl, stable radical) to convert the molecule into a non-radical species. Additionally, the antioxidant activity observed correlates to the presence of

phenolic compounds, flavonoids and additional secondary metabolites which exhibit free radical scavenging activity as demonstrated in figure 9.

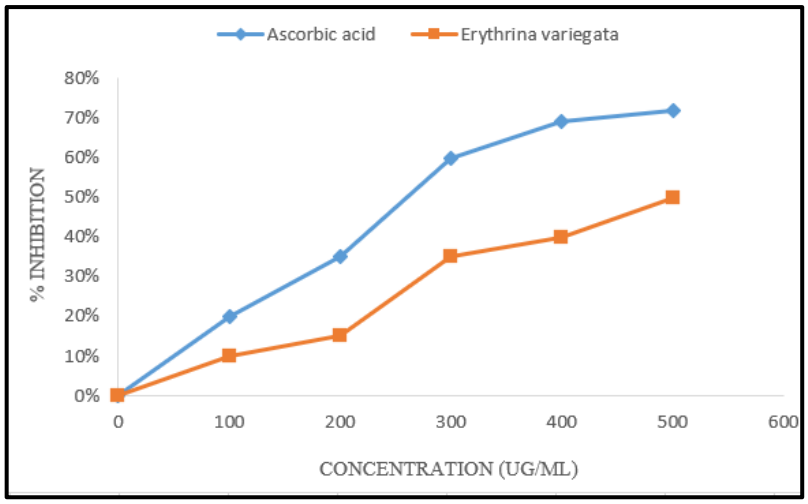


Figure 9: DPPH radical scavenging activity of *Erythrina variegata* bark extract *(Values expressed as Mean ± SD, n = 3)*

A compound's ability to reduce substances may be a key sign of its potential antioxidant action. Figure displays the ascorbic acid and methanolic bark of Erythrina variegata L. standard curves.

3.8.2 Reducing Power Activity

The methanol extracts' reducing power assay confirmed the antioxidant potential of the extract. The reducing power increased progressively with the increasing concentration of the extracts (100–500 µg/mL), reflecting an increased capacity to donate electrons.

The trend for the extracts was almost identical to that of standard ascorbic acid; however, the extracts demonstrated lower activity than ascorbic acid. The reducing power of the extract indicates the ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which can serve as an important parameter for determining antioxidant potential.

The increase in the absorbance with the increasing concentration also indicates the presence of reductants in the extract that have the ability to donate electrons and terminate free radical chain reactions. This activity is consistent with the polyphenols and flavonoids identified in the phytochemical screening conducted shown in Figure 10.

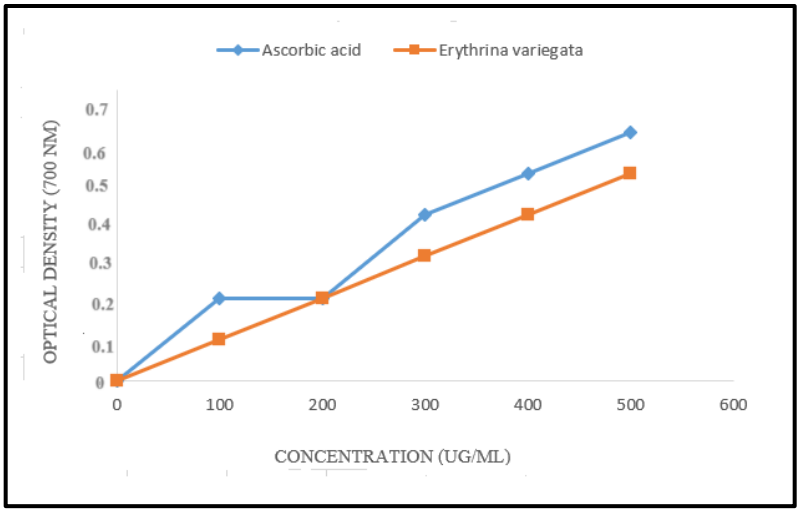


Figure 10: Reducing power activity of *Erythrina variegata* bark extract *(Values expressed as Mean ± SD, n = 3)*

3.9 In-vitro Anticholinesterase Activity

Inhibitory activity of fractions of Erythrina variegata (Inhibitory Activity) against Acetylcholine Esterase (AChE) or Butyrylcholinesterase (BChE) was assessed and compared with donepezil (Standard Drug).

Of all Erythrina variegata fractions, chloroform exhibited potent inhibition of AChE (IC_{50} = 39.04 ± 1.99 µg/mL) and BChE (IC_{50} = 21.68 ± 2.79 µg/mL). In fact, the BChE inhibition of the chloroform fraction is comparable to the BChE inhibition of the standard drug donepezil (IC_{50} = 22.41 ± 0.58 µg/mL), thereby supporting this fraction's selectivity as a cholinesterase inhibitor.

However, moderate inhibition of BChE (IC_{50} = 43.97 ± 5.56 µg/mL) and AChE activity was demonstrated by the n-hexane fraction. Comparatively lower inhibition of BChE and AChE was evidenced by both the ethyl acetate and the crude extracts from Erythrina variegata; therefore, inhibition may be attributed to the elevation of the concentration of the active phytoconstituents, i.e., alkaloids and flavonoids, known to inhibit the cholinesterase enzymes shown in Table 13.

It is suggested that the increased anticholinesterase activity is related to the presence of specific alkaloids (i.e., Erythraline and Erysodine), which are known to inhibit cholinesterase enzymes competitively. These studies indicate that Erythrina variegata may have neuropharmacological applications particularly in the treatment of neurodegenerative diseases, such as Alzheimer's disease.

Table 13: In-vitro acetylcholinesterase and butyrylcholinesterase inhibitory activity of different fractions of *Erythrina variegata*

Extract/fractions	AChE IC50 value (µg/mL)	BChE IC50 value (µg/mL)
Crude extract	194.8 ± 5.569	247.8 ± 6.944
n-Hexane	78.97 ± 5.467	43.97 ± 5.563
Ethyl acetate	119.8 ± 4.668	78.88 ± 7.980
Chloroform	39.04 ± 1.988	21.68 ± 2.795
Donepezil*	2.52 ± 0.080	22.41 ± 0.583

3.10 Evaluation of Phytosomal Formulations

3.10.1 Particle Size and Polydispersity Index

The particle size of phytosomal formulations ranged from 180 nm to 515.9 nm across different batches, indicating successful formation of nano-sized vesicular systems. The optimized batch (E6) exhibited a particle size of 206.4 nm with a relatively low polydispersity index (PDI = 0.312), indicating a uniform and stable vesicle distribution. Lower PDI values (<0.5) observed in most batches suggest homogeneity of the formulation, while the higher PDI in batch E9 indicates possible aggregation or instability.

3.10.2 Entrapment Efficiency

Entrapment efficiency varied significantly across formulations, ranging from 40.9% to 75.3%. The optimized formulation (E6) showed a high entrapment efficiency of 72.8 ± 6.5%, indicating efficient incorporation of the phytoconstituents into the phospholipid matrix.

The increase in entrapment efficiency with optimized lipid composition (phospholipon 90G and cholesterol) suggests improved vesicle stability and drug loading capacity. Although batch E9 showed slightly higher entrapment efficiency (75.3%), its high PDI (2.0) indicates poor stability, making E6 the most suitable optimized formulation shown in **Table 14**.

Table 14: Particle size, polydispersity index, and entrapment efficiency of phytosomal formulations

Batch code	Particle size (nm)	Poly dispersity index	Entrapment efficiency (%) Mean ± SD
E1	464.9	0.694	40.9 ± 3.8
E2	314.5	0.609	50.5 ± 5.4
E3	180	0.078	59.8 ± 3.7
E4	493.4	0.213	52.7 ± 4.6
E5	350.5	0.088	61.5 ± 6.4
E6	206.4	0.312	72.8 ± 6.5
E7	515.9	0.524	63.4 ± 4.3
E8	421.5	0.366	73.9 ± 5.3
E9	317.6	2	75.3± 4.4

3.11 Morphological Analysis (SEM)

Phytosome (E6) structural characteristics and surface morphology were determined using a JSM-

5610 LV scanning electron microscope as depicted in Figure 11. Scanning electron micrographs indicated that the phytosomal vesicles are primarily spherical and possess a unilamellar configuration with a smooth surface morphology. Additionally, the phytosomal vesicles appeared discrete and well-dispersed, indicating low levels of aggregation and high stability of the formulation. The observed morphology agrees with particle size analysis indicated in the dynamic light scattering analysis confirming the successful formation of phytosomes, thus validating the dynamic light scattering particle size analysis results. Having a uniform vesicular configuration benefits drug encapsulation and drug-releasing characteristics.

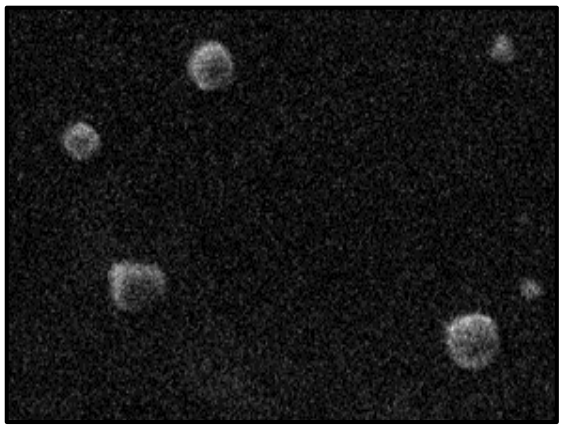


Figure 11: SEM image of optimized phytosomal formulation (E6)

3.12 In-vitro Drug Release Study

Using a Franz diffusion cell, the in vitro drug release profile of the optimized phytosomal formulation (E6) was evaluated over the duration of the study, and the cumulative percentage of released drugs displayed a prolonged and sustained release pattern throughout the duration of the study.

The phytosomal formulation produced a gradual and controlled release rate of Erythrina variegata extract, which can be attributed to the phospholipid bilayer that regulates drug diffusion. The sustained release of the phytosomal formulation also provides for greater retention and longer duration of therapeutic actions than with the conventional extract formulations (shown in Figure 14).

The controlled-release profile allows for increased bioavailability and less frequent dosing. The kinetics of the release demonstrate that the phytosomal formulation is able to regulate the diffusion of drugs across the membranes.

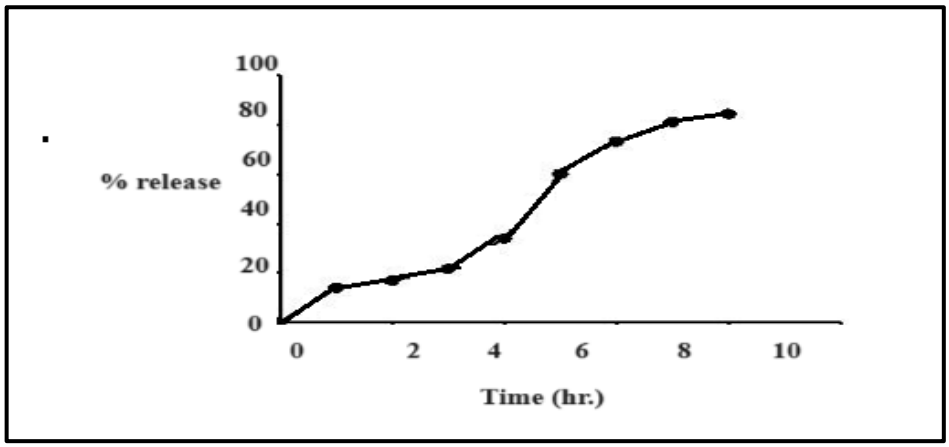


Figure 14: Cumulative percentage drug release profile of optimized phytosomal formulation

3.13 Stability Studies

The stability of the optimized phytosomal formulation (E6) was evaluated over a period of three months under two storage conditions, namely room temperature and refrigerated conditions (2–8 °C). The formulation was assessed for changes in physical appearance, particle size, and entrapment efficiency at predetermined intervals shown in Table 15: Stability study of optimized phytosomal formulation (E6)

Table 15: Stability profile of optimized phytosomal formulation (E6)

Time (Days)	Storage Condition	Physical Appearance	Particle Size (nm)	Entrapment Efficiency (%)

0	Initial	Clear, uniform	206.4	72.8 ± 6.5
30	Room Temp	Slight turbidity	218.7	70.2 ± 5.9
60	Room Temp	Slight aggregation	235.5	67.8 ± 5.3
90	Room Temp	Visible aggregation	258.3	64.1 ± 4.8
30	2–8 °C	Clear	210.2	71.5 ± 5.8
60	2–8 °C	Clear	215.6	70.1 ± 5.2
90	2–8 °C	Slight turbidity	222.4	68.9 ± 4.9

Using a Franz diffusion cell, the in vitro drug release profile of the optimized phytosomal formulation (E6) was evaluated over the duration of the study, and the cumulative percentage of released drugs displayed a prolonged and sustained release pattern throughout the duration of the study.

The phytosomal formulation produced a gradual and controlled release rate of Erythrina variegata extract, which can be attributed to the phospholipid bilayer that regulates drug diffusion. The sustained release of the phytosomal formulation also provides for greater retention and longer duration of therapeutic actions than with the conventional extract formulations (shown in Figure 14).

The controlled-release profile allows for increased bioavailability and less frequent dosing. The kinetics of the release demonstrate that the phytosomal formulation is able to regulate the diffusion of drugs across the membranes.

CONCLUSION

A phytosomal drug delivery system that uses Erythrina variegata L. (E. variegata) bark extract was developed and evaluated through this study. This delivery system must overcome the limitations of traditional herbal formulations in order for this extract to be an effective treatment.

After conducting pharmacognostic standardization and phytochemical screening, it was confirmed that E. variegata contained many different bioactive components (alkaloids, flavonoids, phenolics, glycosides) that give the plant its pharmacological properties. Also, HPTLC fingerprinting provided evidence of the chemical complexity of the extract and supported its authenticity. Antioxidant activity was demonstrated through DPPH and reducing power assays. Conversely, anticholinesterase (AChE) activity was also demonstrated through fractionation studies (the chloroform fraction exhibited the greatest potency), indicating that E. variegata may provide treatment for neurodegenerative diseases.

Eriodictyol was isolated from the bark extract, and its structure and purity were confirmed through mass spectrometry, NMR, and FTIR. In addition to structural characterization, molecular docking studies modelled eriodictyol binding within the active site of the target AChE enzyme, revealing that eriodictyol has great biological potential.

After optimizing the phytosomal formulation (E6) using phospholipon 90G, cholesterol, and ethanol according to a 3^2 factorial design, it was established that the phytosomal formulation could incorporate the drug efficiently and provide uniform vesicle distribution. The optimized formulation demonstrated desirable physicochemical properties, including nanoscale particle size (~206 nm), low polydispersity index, and high entrapment efficiency (~72%). Furthermore, SEM analysis confirmed that the optimized formulation consists of spherical, unilamellar vesicles, and in-vitro drug release demonstrated that the optimized formulation would be appropriate for use as a sustained release system, thereby improving the retention of the drug and controlling how it is delivered.

When subjected to refrigerated storage conditions, the optimized formulation demonstrated minimal changes to particle size and entrapment efficiency. Conversely, after being stored at room temperature, the optimized formulation began to exhibit instability, which was attributed to aggregation and drug leakage from the vesicles.

The phytosomal drug delivery system developed in this study significantly enhances the bioavailability, stability, and clinical efficacy of E. variegata extract. The findings of this study provide a rationale for the use of phytosome-based drug delivery systems as a viable method for improving the clinical relevance of herbal medicines and serve as a basis for conducting future pharmacological and clinical research.

Declarations

Funding

The authors declare that no specific funding was received from any public, commercial, or not-for-profit funding agencies for the conduct of this research.

Conflicts of Interest / Competing Interests

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

All animal experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of YSPM's YTC Faculty of Pharmacy, Wadhe, Satara, India (Approval No.: YSPM/YTC/PHARMA/Ph.D/2024–2025; Date: 22/03/2025). The study was conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

All experimental procedures were performed in compliance with established ethical standards for the care and use of laboratory animals, and efforts were made to minimize animal suffering and reduce the number of animals used.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. Additional data may be made available from the corresponding author upon reasonable request.

Authors' Contributions

All authors contributed equally to the conception, design, execution, and writing of this study. All authors have read and approved the final manuscript.

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Compliance with Ethical Standards

This study complies with all institutional, national, and international guidelines for ethical conduct of research.

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