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Research Article

Phytosomal Formulation of *Clerodendrum Infortunatum* for Anti-Tubercular Activity and Development of a Therapeutic Suspension

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ABSTRACT

Background: Tuberculosis (TB) continues to pose a significant global health challenge, necessitating the development of novel therapeutic approaches. Clerodendrum infortunatum, a medicinal plant with traditional therapeutic uses, contains bioactive constituents like flavonoids and terpenoids with known anti-tubercular properties. Phytosome technology offers a promising platform to enhance the bioavailability and efficacy of plant-derived compounds. Aim: This study aimed to formulate and evaluate phytosomes prepared from the ethanolic extract of Clerodendrum infortunatum leaves for anti-tubercular activity. Furthermore, the study focused on the development of an anti-TB suspension using the optimized phytosomal formulation. Method: The ethanolic extract of Clerodendrum infortunatum was prepared using Soxhlet extraction. The flavonoidal bio-fraction was isolated via column chromatography and used to formulate phytosomes in a 1:2 ratio (extract: soya lecithin). Phytosomes were characterized using TLC and evaluated for in vitro anti-tubercular activity using the alamarBlue assay, with isoniazid and streptomycin as standards. Anti-TB suspensions (F1, F2, F3) with varying concentrations of phytosomes were developed and assessed for sedimentation volume, viscosity, pH, and stability. Results: The phytosomes exhibited enhanced anti-TB activity compared to the crude extract, with the flavonoidal bio-fraction showing significant efficacy at 1.6 µg/mL. Among the suspensions, F2 (6.35 mg sample) demonstrated optimal parameters, including improved stability and bioactivity. Conclusion: The study highlights the potential of Clerodendrum infortunatum phytosomes as an effective anti-tubercular agent. The developed anti-TB suspension offers a promising formulation for enhanced therapeutic delivery. Further investigations are warranted to explore its clinical application.

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INTRODUCTION

Another earliest recorded forms of healing practices include the use of herbal medicine; the evidence show that it dated even before recorded history. There is sufficient evidence from a variety of sources that links man and his search for drugs in nature to a distant past: from writing, as well as from the first plant remedies[1]. As noted by the WHO, only the conventional medicine is something over 80% of the global population uses as the primary medical remedyIn this context, the use of herbal medicine emerges as a widely accepted component of complementary and alternative medicine systems[2]. Phytotherapy therefore is the science which uses plant based materials in treatment of a certain disease or preventing the occurrence of that particular disease. Herbal medicine is believed to be practiced in many culture to cure various ailments that include cardiovascular diseases, cancer, neurodegenerative diseases, and diabetes mellitus[3]. By and large, plants have served as a very important source of new drugs for a long time and contributed immensely to the medical field. Since about 25% of modern pharmaceuticals are based on plant compounds, their importance cannot be overstated. Plants exhibit a large diversity in secondary metabolites, including alkaloids, flavonoids, and terpenoids, which display multiple kinds of biological activities such as antimicrobial, anti-inflammatory, and anticancer. The age-old ethnobotanical knowledge of indigenous people concerning the medicinal use of plants has led to the discovery of many drugs, including such important drugs as morphine from the opium poppy and quinine from the cinchona tree. Moreover, the growing problem of resistance of pathogens to synthetic drugs underlines the role of novel plant-based compounds in the development of novel therapeutic agents. Since there remains such a wide scope of discovery into plant diversity, the potential for new drugs discovery remains to be great, and thus plants are an exceedingly vital resource for pharmaceutical innovation. Plants represent a crucial source of novel pharmaceuticals, providing a diverse range of bioactive compounds that hold therapeutic promise. Many modern pharmaceuticals, such as paclitaxel for cancer and artemisinin for malaria, have been derived from plant sources, highlighting their significant role in drug discovery[4]. The complexity and versatility of plant biochemistry offers sulky opportunities in the discovery of new disease treatments, including cancers, bacteria, and viruses, and chronic ailments. With new technology, there lies hope that other drugs from plants could be discovered and this means that there is need to conserve plant species and indigenous knowledge on the same[5]. Subsequent studies on these plants may lead to development of other drugs which are way more effective than synthetic drugs and cause little or no side effects[6].

Tuberculosis

Tuberculosis is a viral disease which is very contagious with two thirds of the whole population of the entire world and 40 percent of the Indian population affected by it. However, this concern has arisen due to development of drug resistance of Mycobacterium tuberculosis to both the first and the second line drugs. As a result, MDR and XDR strains of M. tuberculosis are emerging in different parts of the world including India infected it. However, this has become a serious problem as the drug resistance of Mycobacterium tuberculosis has become resistant both against the first line as also the second line drugs. As a result of this, multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of M. tuberculosis have emerged all over the world including India[7]. Anti-TB drugs consist of two

groups: First-line or essential therapy which is mainly used in the ordinary TB patient with ordinary Mycobacterium tuberculosis; second-line or reserve anti-TB drugs which are used with MDR-TB. Compared to first-line anti-TB drugs, second line drugs have several fold more adverse effects [8]. There has been increasing concern in the search for natural products against TB based on the efficiencies of medicinal plants in providing Organization of an alterative mode of handling TB in view of the increasing drug resistance. Quite a number of researchers have investigated the effectiveness of different plants in the control of Mycobacterium tuberculosis. For instance, findings on the indicator plant, Andrographis paniculata, demonstrate that the global plant possesses major anti TB element labeled as andrographolide[9]. In the same manner, Curcuma longa (turmeric) has a potential because of its active ingredient, the curcumin that possess a potent antimycobacterial activity[10]. Such an observation indicates that the isolated administration of the medicinal plants may improve the treatment outcomes if combined with the conventional chemotherapy drugs together with the standard TB drugs especially in the areas where healthcare facilities restrain the availability of the conventional anti-TB medications.

Clerodendrum infortunatum

Clerodendrum infortunatum Linn., belongs to the family Verbanaceae, it is a Terrestrial shrub having blackish, square stem its leaves are simple oppositely decussate petiolate exstipulate, coriaceous and hairy, having very obnoxious smell[11]. Clerodendrum infortunatum is a perennial shrub that typically grows to a height of 2-4 feet. It has a slightly woody nature, with blunt, quadrangular stems and branches. The leaves are usually arranged in groups of three or four at a node, although they may occasionally be opposite, and are oblong or elliptical with serrated edges. The plant produces sweetly fragrant, bluish-purple or white flowers that form in terminal, pyramid-shaped panicles. The fruits are four-lobed, purple drupes that are somewhat succulent, each lobe containing a single pyrene. [12].The Clerodendrum of the family Verbenaceae is a very large family comprising of plants that are distributed mostly in the tropical and sub topic parts of the world with very little penetration into the temperature climate areas. They have estimated the species number five hundred sixty and five hundred eighty. Its origin can be attributed to the year 1753 when Linneus was at last able to set the genus; kind of which is Clerodendrum infortunatum indigenous to India. Later in 1763, Adamson replaced the Latinized form of the family name Clerodendrum to the Greek form of the name Clerodendron. The genus was first described by Linneus for the year 1753 but for the type species that is Clerodendrum infortunatum the descriptions was made from India.The layman's name, Clerodendrum, then posed to him by Linnaeus, was translated by Adanson in 1763 into the Greek equivalent of Clerodendrondrum infortunatum from India. In 1763, Adanson changed the Latinized form Clerodendrum into Greek form Clerodendron. Linnaeus's original s-adopted the Latinized form Moldenke in 1942, However, similar to other taxonomists this practice has been continued[13]. In India and several other countries, Clerodendrum infortunatum has a long history of use in the Ayurvedic system for various ailments. In Assam, the roots of this plant are utilized to treat bronchitis and asthma. The leaves, roots, and flowers are often prepared as a paste or ash for medicinal purposes. Additionally, the leaves and flowers are applied to counteract scorpion stings, while the young shoots are used as a remedy for snake bites.Several researchers have established that different fractions of Clerodendrum infortunatum

exhibit a range of pharmacological properties. These include antimicrobial, anthelmintic, insecticidal, analgesic, antipyretic, hepatoprotective, wound healing, thrombolytic, antidiabetic, cytotoxic, and anti-inflammatory activities[14].

Herbal Nanotechnology

Herbal remedies and natural products are being used to treat the diseases. Unlike the allopathic system that is in common practice today, these herbal remedies are made of thousands of chemical constituents which all act at once, on the diseases. Phyto therapeutics require an understanding where the components have to be delivered in sustained manner so that patient concordance can be enhanced without having to administer the components repeatedly. This can be realized through the development of NDDSs of the herbal constituents. Besides overcoming the need for repeated administration to overcome non-compliance, NDDSs also enhance the therapeutic value by decreasing toxicity and enhancing entrapment, efficacy, penetration and solubility of the drugs. There are certain benefits of incorporating the herbal extracts in the new formulation systems and these include less absorption that can be solved which is the major drawback seen, and the possibility of attracting major drug companies. Nanotechnology is as area of applied science and technology that focuses on the systematic fabrication of devices and dosage forms in the nanometer range (nanoscale) of 1 to 100 nm. The uses of nanotechnology for therapeutic, diagnostic, sensing, and regulating biological systems have been recently described as nanomedicine. The nanocarriers have been prepared from safe materials such as synthetic biodegradable polymers, lipids and polysaccharides[15]. Herbal nanotechnology is an innovative approach that integrates nanotechnology with plant-based products to enhance their therapeutic potential. By using nanoscale delivery systems such as nanoparticles, liposomes, phytosomes, herbal bioactive compounds can achieve improved bioavailability, stability, and targeted delivery. This technology addresses common challenges faced by traditional herbal formulations, such as poor solubility and rapid degradation. In particular, it enhances the absorption of phytoconstituents, allowing for lower dosages with enhanced efficacy and reduced side effects. Herbal nanotechnology is being increasingly explored in various applications, including in the development of nutraceuticals, cosmetics, and pharmaceuticals, making it a promising field for the efficient use of plant biodiversity[16]. In phyto-formulation research, the preparation of nano dosage forms such as phytosomes, liposomes, cubosomes and nanosponges, and many more have numerous benefits on herbal drugs in terms of solubility, bioavailability, reduction in toxicity, improved pharmacological activity, protection against degradation, increased macrophages distribution in tissues, sustained release and protection from physical-chemical degradations. The portable herbal drug delivery of nano size drug delivery systems could possibly create an added future to the enhancement of activity besides challenging difficulties related to plant medicine in the future. The crucial of NDDS found in the traditional medicine system is the incorporation of nanocarrier to treat more chronic ailments like asthma, diabetes, cancer diseases, microbial diseases, among others. Some specific nanocarriers can transport phytochemicals and endocytose them within the expected cells. Liposomes and phytosomes have enhanced the wound healing properties of the phytochemicals. Yet another advantage of co-delivery of phytochemicals and therapeutic agents is the capability of eliminating the probability of failure

or formation of drug resistance. These drug delivery systems are capable of reducing enzymatic degradation in gastrointestinal tract, having bypass effect over the first pass metabolism and reducing toxicity of the off-targeted tissue. More advancements in this particular field may also reveal some concealed opportunities related to liposome and phytosome based delivery of natural products and phytochemicals in therapeutic relevance[17].

Phytosomes

Phytosomes are advanced herbal preparations, which ensure that the bio-actives derived from plants possess high bioavailability in the body. Traditionally used plant extracts have hydrophilic nature, hence possesses low absorption levels in the body. The process of generating phytosome involves complexing phospholipids usually phosphatidylcholine with plant bio actives, thereby producing the lipid-compatible molecular complexes, which helps enhance the solubility and absorption levels of the bioactive compounds across the cell membranes along with their therapeutic efficacy. Phytosomes have been mostly used in the preparations of flavonoids, alkaloids, and other polyphenols. They are most particularly found to be highly useful in chronic treatments wherein improved absorption of the actives of herbal content can lead to better clinical results. Phytosome nanotechnology presents promising therapeutic potential in enhancing drug delivery systems, particularly for bioactive phytochemicals used in topical applications. As lipid-based nanocarriers, phytosomes play a crucial role in influencing the pharmacokinetics and pharmacodynamics of plant-derived polyphenolic compounds. This nanotechnology offers significant potential for advancing the development of topical formulations. Moreover, the penetration of phytochemicals can be enhanced by this nano size delivery system owing to the peculiar physiochemical properties at the biological barriers, and these will enhance its bioavailability[18].

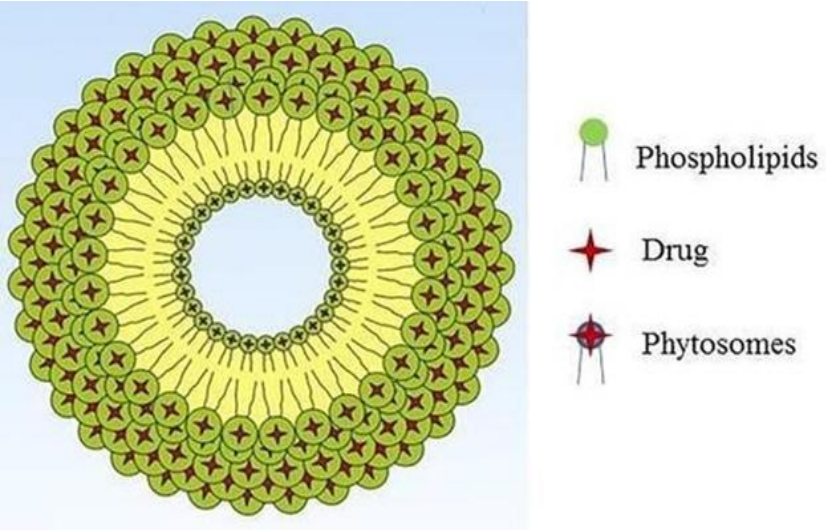

Fig. 1. Diagrammatic representation of a phytosome.

MATERIALS

The leaves of *Clerodendrum infortunatum* were collected from the Thrissur district, Kerala, India, on February 1, 2024. The plant was identified and

authenticated by Dr. Ranjusha A. P., Head of the Department of Botany, NSS College, Ottapalam, Kerala. A voucher specimen was deposited at Nehru College of Pharmacy, Thrissur, for future reference.

METHOD

Isolation Of Flavonoidal Bio-Fraction

The flavonoid bio-fraction from the ethanolic extract of *Clerodendrum infortunatum* leaves was isolated using column chromatography. This technique is employed to separate individual components from a mixture that is dissolved in a fluid, and it falls under the category of chromatography methods.[19]

Preparation And Evaluation of Phytosome

Table 1. The composition and different phytosomes prepared from bio-fraction of *Clerodendrum infortunatum* leaves.

Sl. No.	Phytosomal Formulation Code:	Ratio of Drug: Soya lecithin	Dichloro methane (ml)	n- hexane (ml)	Phosphate buffer solution (ml)
1.	P1	1:1	20ml	15ml	5ml
2.	P2	1:2	20ml	15ml	5ml
3.	P3	1:3	20ml	15ml	5ml

The isolated bio-fraction and soya lecithin were dissolved in dichloromethane at ratios of 1:1, 1:2, and 1:3 using a rotary round-bottom flask. The mixture was stirred for one hour, ensuring the temperature did not exceed 40°C. A thin film of the sample was formed, to which n-hexane was added and stirred continuously until a monolayer was achieved. Following this, phosphate buffer at pH 6.8 was incorporated and stirred to ensure thorough mixing. The final sample was stored in an amber bottle at room temperature[20,21]. The phytosome complexes F1, F2, and F3, which have molar ratios of 1:1, 1:2, and 1:3 of the isolated bio-fraction and soya lecithin, were prepared using the rotary evaporation technique.

Evaluation Of Phytosomes

The behavior of phytosomes is influenced by factors like size, shape, stability, and distribution. Therefore, the phytosomes are characterized by the following evaluation parameters:

Scanning Electron Microscopy (SEM)

A scanning electron microscopy study was conducted to assess the surface morphology, size, and shape of the prepared phytosomes. The freeze-dried phytosomes were analyzed using scanning electron microscopy and photographed.

Measurement of particle size (PS)

The particle size of phytosomes was determined using a particle size analyzer.

Measurement of Zeta potential (ZP)

Zeta potential, which refers to the total charge produced by the medium, plays a crucial role in determining the charge of phytosomes within emulsions. Depending on the composition of the phytosomes, the zeta potential can be negative, positive, or neutral. The zeta potential of the optimized phytosome suspension was measured using a zeta sizer.

Drug entrapment efficiency

Entrapment efficiency (EE is assessed using the ultracentrifugation method. In this process, the

phytosomes were centrifuged at 12,000 rpm for 45 minutes to separate them from any unentrapped drug. The concentration of the free drug in the supernatant was measured by assessing the absorbance at 462 nm with a UV-Visible spectrometer[22,23].

In Vitro Pharmacological Activity

Determination of minimum inhibitory concentration (Resazurin-based 96-well plate microdilution method)

Test microorganism - Mycobaterium smegmatis (MTCC No :6, Incubation 37°C for 24 hours)

Alamar Blue Susceptibility Assay procedure

• Plates were prepared in a sterile environment, and a sterile 96-well plate was appropriately labeled. A volume of 100 µL of the test substance, either dissolved in 10% (v/v) DMSO or sterile water (usually at a stock concentration of 10 mg/mL), was added to the first row of the plate. For the other wells, 50 µL of normal saline was introduced.

• Serial dilutions were performed using a multichannel pipette, with tips discarded after each use to ensure that each well contained 50 µL of the test substance at progressively lower concentrations.

• Following this, 10 µL of resazurin indicator solution was added to each well, and then 30 µL of a 3.3× strength isosensitized broth was pipetted into each well, achieving a final volume of single-strength nutrient broth.

• Lastly, 10 µL of bacterial suspension was added to each well. To prevent the bacteria from dehydrating, each plate was loosely covered with cling film. Each plate included a set of controls: one column containing three antitubercular drugs (isoniazid, pyrazinamide, and

streptomycin) as a positive control, another column with all solutions except the test compound, and a final column with all solutions excluding the bacterial solution, where 10 µL of nutrient broth was added instead.

• The plates were sealed and incubated at 37°C for 24 hours. One of the control wells was treated with Alamar blue dye and monitored for the development of pink coloration.

• If a pink color was observed in the control well, the test samples and standard drugs were applied to the experimental wells at various concentrations, and the plate was incubated for another 24 hours.

• After incubation, Alamar blue dye was added to all experimental wells, followed by further incubation to allow for pink coloration development. Results were recorded through visual observation and by measuring absorbance at 600 nm.

Experimental Group

- B- Culture Medium +Resazurin reagent (sterility control) (no drug, no culture)
- C- Culture Medium + Microbial culture+ Resazurin reagent (culture and reagent control)
- S- Culture Medium + Microbial culture+Standard drugs + Resazurin reagent
- T- Culture Medium + Test sample + Microbial culture + Resazurin reagent [24,25,26]

Formulation Of Anti-Tubercular Suspension

The formulation for preparing a 100 ml suspension of the sample powder is detailed in Table 13. The fine particles of the drugs, sized at 100 mesh, are thoroughly mixed through triturating. The drug

mixture is then blended with water and various additives, including Tween-80, sodium carboxymethyl cellulose (CMC), a sweetener, a flavoring agent, and sodium benzoate, to improve its stability[27,28]

Table 2. Ingredients and their quantities for the formulation of the anti-tubercular suspension in formulations F1, F2, and F3

Sl. No.	Name of Ingredients	Quantity taken		
		F1	F2	F3
1.	Phytosome Complex	0.312mg	0.625mg	1.25mg
2.	Tween 80	0.1 w/v	0.1 w/v	0.1w/v
3.	Sodium CMC	0.5%	0.5%	0.5%
4.	Sodium benzoate	1g	1g	1g
5.	Sorbitol	0.1g	0.1g	0.1g
6.	Lemon oil	1ml	1ml	1ml
7.	Purified water *Q.S.*	100ml	100ml	100ml

Evaluation Of Anti-Tubercular Suspension

 Organoleptic Properties

O Color: Visually inspect the suspension under natural light.

O **Odor:** Smell the suspension and note any characteristic or unusual odor.

O **Taste:** Test a small quantity (with proper consent and safety considerations) to check the taste. This is usually done in trials or on a controlled basis.

O **Texture:** Rub a small amount of the suspension between your fingers or test it on a glass plate to check for grittiness.

 Sedimentation Volume

Sedimentation volume is a measure used to evaluate the stability of suspensions, indicating the volume occupied by sediment after a certain period. To determine sedimentation volume, a known volume of the suspension is placed in a graduated cylinder and allowed to stand undisturbed for a specific time, usually 24 hours. After this period, the height of the sediment is measured, and the sedimentation volume is calculated by dividing the height of the sediment by the total height of the suspension, expressed as a percentage. This procedure helps assess the stability and dispersion characteristics of the formulation, providing insights into its performance and shelf-life.

 Viscosity

O The sample's viscosity was measured at room temperature using a Brookfield viscometer set to 50 rpm.

 pH Measurement

O The suspension's pH was measured using a pH meter.[29,30]

RESULTS

Preparation Of Phytosomes

The phytosomes was prepared by using rotary evaporation method.

Fig. 2. Prepared Phytosomes

Evaluation Of Phytosome

Entrapment efficiency (%)

Table 3. Entrapment efficiency of phytosomes

Sl. No.	Phytosomal Formulation code	Ratio of Drug: Soyalecithin	Entrapment efficiency (%)
1.	P1	1:1	94
2.	P2	1:2	97
3.	P3	1:3	96

The entrapment efficiency of P2 having 1:2 (biofraction: soyalecithin) was found to be higher and hence selected as the optimized phytosome.

Scanning Electron Microscopy (SEM)

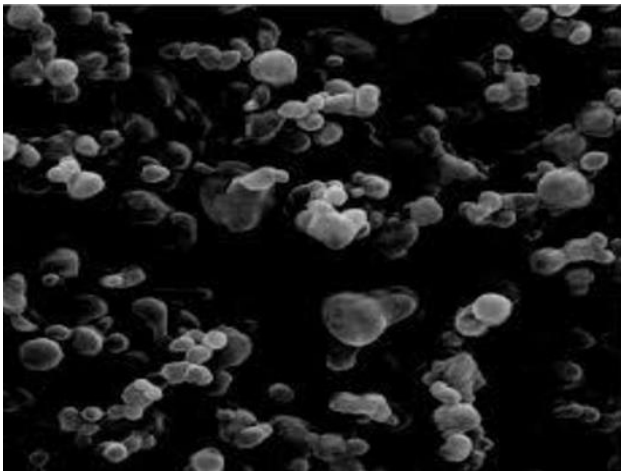

Fig. 3. SEM image of phytosome

The SEM image shows that the optimized phytosome shows spherical shape and uniform size distribution.

Measurement of Zeta potential (ZP)

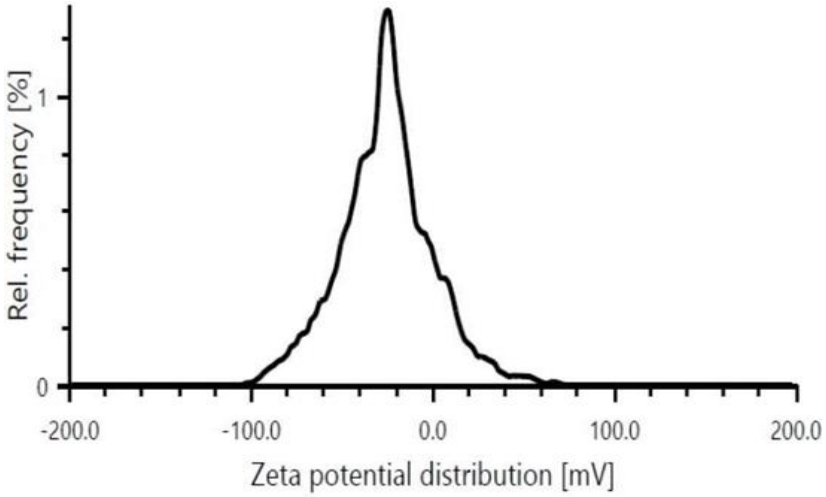

Fig. 4. Zeta potential graph of prepared phytosomes

Zeta potential of the optimized phytosome was found to be -23.6 mv. This indicates that the sample is highly stable and do not form aggregates.

Measurement of particle size (PS)

The particle size of the optimized phytosome was found to be 140.74 nm. This was in accordance with the particle size range of phytosomes. The evaluation of the phytosome formulation revealed promising results, particularly for the optimized phytosome labeled P2, which exhibited a high entrapment efficiency. Scanning Electron Microscopy (SEM) analysis confirmed that the optimized phytosomes possess a spherical shape and a uniform size distribution, essential characteristics for effective drug delivery. Additionally, the zeta potential of the optimized phytosome was measured at -23.6 mV, indicating high stability and minimal tendency to form aggregates. The particle size of the optimized phytosome was found to be 140.74 nm, aligning well with the expected size range for phytosomes.

***In-Vitro* Pharmacological Activity**

Resazurin-based 96-well plate microdilution method

Table 4. Color intensity and Minimum Inhibitory Concentration (MIC) for each concentration of the tested compounds

Concentration	INH	PYZ	STP	BF	PHY
0.8	Deep Pink	Deep Pink	Purple MIC	Deep Pink	Deep Pink
1.6	Purple MIC	Deep Pink	Purple	Purple MIC	Purple MIC
3.12	Purple	Purple MIC	Purple	Purple	Purple
6.25	Purple	Purple	Purple	Purple	Purple
12.5	Blue	Blue	Blue	Blue	Blue

25	Blue	Blue	Blue	Blue	Blue
50	Blue	Blue	Blue	Blue	Blue
100	Blue	Blue	Blue	Blue	Blue

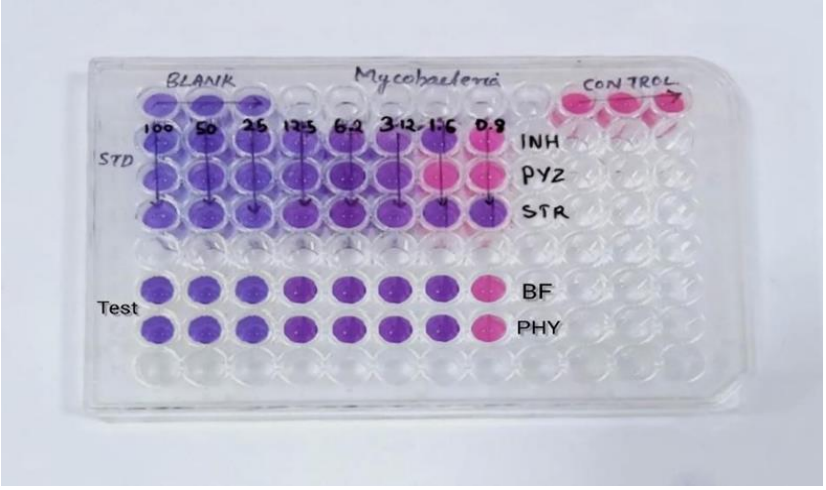

Fig. 6. MABA Assay of Standard drugs and Test samples

Table 5. Average absorbance values at 600 nm for various concentrations of Isoniazid (INH), Pyrazinamide (PYZ), Streptomycin (STP), Biofraction (BF), and Phytosome (PHY).

Absorbance at 600nm					
Blank - 0.056			Control - 1.003		
Test concentration (µg/ml)	Average absorbance at 600nm				
	Isoniazid (INH)	Pyrazinamide (PYZ)	Streptomycin (STP)	Biofraction (BF)	Phytosome (PHY)
0.8	0.533±0.001	0.534±0.001	0.065±0.001	0.543±0.002	0.539±0.002
1.6	0.064±0.002	0.148±0.001	0.063±0.001	0.064±0.001	0.063±0.001
3.12	0.063±0.001	0.06±0.001	0.059±0.001	0.062±0.002	0.063±0.003
6.25	0.059±0.002	0.057±0.002	0.056±0.001	0.058±0.001	0.058±0.002
12.5	0.057±0.003	0.055±0.001	0.055±0.002	0.056±0.001	0.056±0.001
25	0.055±0.001	0.054±0.001	0.053±0.001	0.054±0.003	0.055±0.003
50	0.054±0.002	0.053±0.002	0.051±0.003	0.053±0.003	0.054±0.001
100	0.052±0.001	0.051±0.001	0.05±0.002	0.051±0.001	0.051±0.002

± indicates standard deviation values from six readings taken under similar conditions. The significance of differences was evaluated using a one-way ANOVA test, and statistical differences were considered significant at $p < 0.005$.

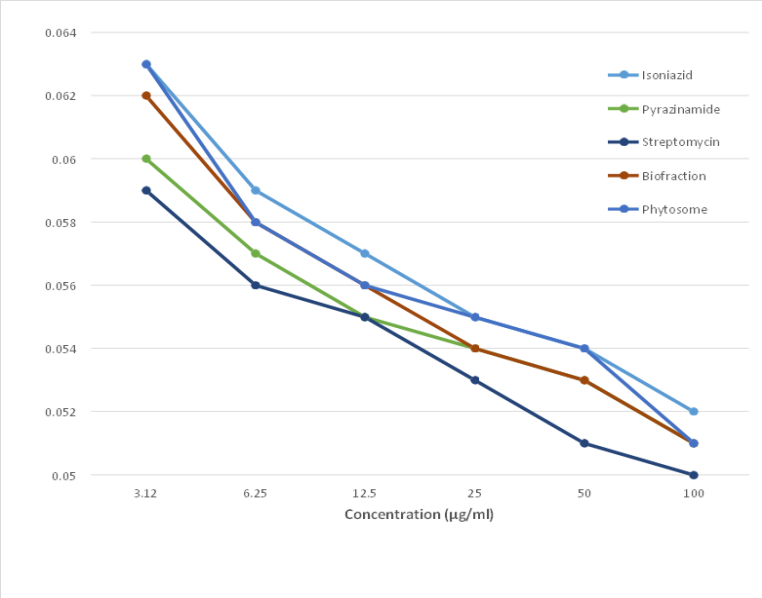

Fig. 7. Graph of anti-tuberculosis activity showing absorbance at 600 nm for varying concentrations of Isoniazid (INH), Pyrazinamide (PYZ), Streptomycin (STP), Biofraction (BF), and Phytosome (PHY).

From MIC data, both the biofraction (BF) and phytosome (PHY) has shown anti- tuberculosis effect at a concentration of 1.6 µg/mL in which the solution turned purple thereby implying the bacterial inhibition. This MIC also confirms that both the BF and PHY contains potent anti-TB active components that can be further developed. Based on this, for the formulation of a phytosome-loaded anti-TB suspension, concentrations of 3.12 µg/mL, 6.25 µg/mL, and 12.5 µg/mL has been selected for evaluation.

Formulation Of Anti-Tubercular Suspension

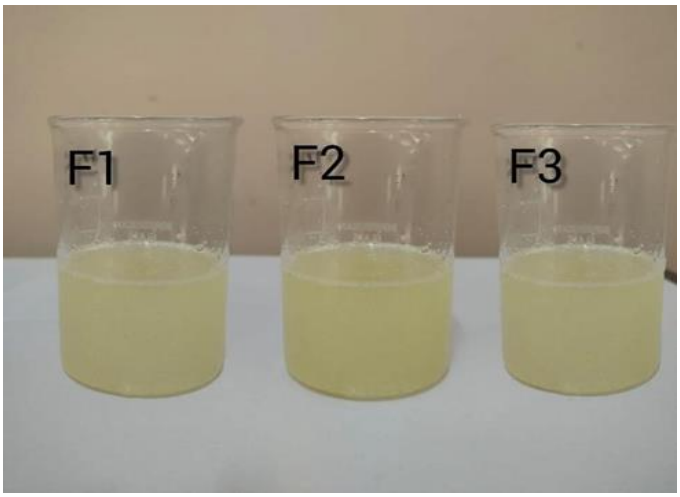

Fig. 8. Phytosome loaded anti- tubercular suspensions

The phytosome loaded anti- tubercular suspensions (100ml) of different formulations (F1, F2, F3) was prepared.

Evaluation Of Anti-Tubercular Suspension

Table 6. Evaluation of phytosome loaded anti-tubercular suspension.

Sl No	Parameters	Observations		
1.	**Organoleptic Properties**			
		F1	**F2**	**F3**
	• Color	Pale Yellow	Pale Yellow	Pale Yellow
	• Odor	Pleasant	Pleasant	Pleasant
	• Taste	Slightly sweet and citrusy	Slightly sweet and citrusy	Slightly sweet and citrusy
	• Texture	Suspension	Suspension	Suspension
2.	Sedimentation volume	1.34	1.02	0.94
3.	Viscosity	98cP	106cP	109cP
4.	pH	6.66	6.65	6.66

The table presents the observations for three formulations (F1, F2, and F3) based on various parameters, including organoleptic properties, sedimentation volume, viscosity, and pH. As the concentration of the phytosomal complex increases, F3 shows better sedimentation volume and viscosity. However, F2 is chosen as the preferred option because it serves as the intermediate formulation.

DISCUSSION

The current study aimed to develop a phytosomal formulation of Clerodendrum infortunatum leaf extract for its potential anti-tubercular activity and subsequently formulate an anti-TB suspension using the optimized phytosome. The findings highlight several significant outcomes regarding the formulation, characterization, and evaluation of these phytosomal suspensions. The phytosomes were prepared using the rotary evaporation method, and among the different formulations (P1, P2, and P3), the 1:2 ratio of biofraction to soya lecithin (P2) demonstrated the highest entrapment efficiency (97%). This suggests that the 1:2 ratio provided an optimal balance between the drug and the lipid component, facilitating better encapsulation of the bioactive compounds. The SEM analysis confirmed that the optimized phytosomes had a spherical shape with uniform size distribution, which is essential for consistent drug delivery. The particle size of the optimized phytosome (P2) was found to be 140.74 nm, aligning with the typical size range for phytosomal formulations. Additionally, the zeta potential was measured at -23.6 mV, indicating good stability of the formulation with minimal aggregation tendency. These characteristics are crucial for maintaining the integrity and efficacy of the phytosome during storage and administration. In vitro anti-tubercular activity was assessed using the alamarBlue assay. The biofraction (BF) and phytosome (PHY) demonstrated a minimum inhibitory concentration (MIC) of 1.6 µg/mL against Mycobacterium smegmatis. This result underscores the potential of Clerodendrum infortunatum flavonoidal biofraction to inhibit Mycobacterium species effectively. The enhanced activity of the phytosome compared to the crude extract can be attributed to improved

bioavailability and stability imparted by the phytosomal complex. Three anti-tubercular suspensions (F1, F2, and F3) were formulated with increasing concentrations of the phytosome complex (0.312 mg, 0.625 mg, and 1.25 mg, respectively). The suspensions were evaluated for organoleptic properties, sedimentation volume, viscosity, and pH. All three formulations exhibited pale yellow color, pleasant odor, and slightly sweet-citrusy taste, ensuring patient compliance. Among the suspensions, F2 (0.625 mg) demonstrated the most balanced parameters, including a sedimentation volume of 1.02 and viscosity of 106 cP. While F3 had a slightly higher viscosity (109 cP) and lower sedimentation volume (0.94), F2 was chosen as the optimal formulation due to its stability and intermediate concentration, offering a balance between efficacy and physical characteristics. The results highlight the potential of phytosomal formulations to enhance the therapeutic efficacy of plant-based anti-tubercular agents. The optimized phytosome-loaded suspension offers a promising alternative to conventional anti-TB therapies, especially in addressing drug-resistant strains. Given the increasing incidence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis, phytosome-based delivery systems could play a crucial role in future TB management strategies. Future studies should focus on in vivo evaluations to confirm the efficacy and safety of the formulated suspension. Additionally, exploring the synergistic potential of phytosomal formulations with standard anti-TB drugs could further enhance treatment outcomes and mitigate resistance development.

CONCLUSION

This study successfully developed and evaluated a phytosomal formulation of Clerodendrum infortunatum leaf extract for anti-tubercular activity. The optimized phytosome (1:2 ratio of biofraction to soya lecithin) exhibited enhanced entrapment efficiency, stability, and uniform particle size. The in vitro anti-tubercular activity demonstrated a minimum inhibitory concentration (MIC) of 1.6 µg/mL, confirming the potential of the flavonoidal biofraction in inhibiting Mycobacterium smegmatis. Among the formulated suspensions (F1, F2, and F3), F2 (0.625 mg) was identified as the optimal formulation based on sedimentation volume, viscosity, and stability parameters. This suspension offers a balanced approach for delivering the anti-tubercular phytosomal complex effectively. The findings underscore the promise of phytosome technology in enhancing the bioavailability and efficacy of plant-based compounds, providing a viable alternative for TB treatment, especially in the context of increasing drug resistance. Further in vivo studies and clinical trials are warranted to establish the full therapeutic potential of this formulation and explore its use in combination with standard anti-TB therapies.

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