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Development and Evaluation of a Polyherbal Dry Powder Inhaler for Pulmonary Delivery in Management of Tuberculosis

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ABSTRACT

Pulmonary tuberculosis remains a major global health challenge, and while conventional oral therapies are effective, they are often limited by systemic toxicity, extensive first-pass metabolism, and poor patient adherence. This study aimed to develop and evaluate a polyherbal dry powder inhaler (DPI) for localized pulmonary delivery using a simple carrier-based blending technique. Standardized extracts of Adhatoda vasica, Curcuma longa, Glycyrrhiza glabra, and Piper nigrum were combined in a fixed ratio and blended with lactose monohydrate to prepare three DPI formulations (F1–F3). Micromeritic evaluation revealed improved flowability and reduced particle size with increasing carrier concentration, with F3 demonstrating the most favorable characteristics. Aerodynamic analysis using the Next Generation Impactor showed that F3 achieved the highest emitted dose and fine particle fraction, along with an MMAD suitable for deep lung deposition. Drug content and uniformity assessments confirmed consistent distribution of phytoconstituents within the carrier matrix, while in vitro cytotoxicity studies on A549 cells indicated good biocompatibility. Stability testing under accelerated conditions demonstrated minimal changes in particle size, drug content, and aerosolization behavior over 90 days. Overall, formulation F3 emerged as the optimized DPI, offering a promising approach for delivering anti-inflammatory and immunomodulatory phytoactives directly to pulmonary tissues. The developed system may serve as a supportive or adjunctive therapy in the management of pulmonary tuberculosis, warranting further in vivo and clinical investigations.

KEYWORDS: Polyherbal DPI; pulmonary drug delivery; carrier-based blending; lactose monohydrate; aerodynamic performance; tuberculosis adjunct therapy..

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INTRODUCTION

Pulmonary tuberculosis (PTB) continued to pose a substantial global health challenge despite advances in diagnosis and chemotherapy. The disease remained highly prevalent in low- and middle-income countries, where overcrowding, delayed detection, socioeconomic barriers, and treatment interruptions contributed to persistent transmission. Although standard anti-tubercular therapy (ATT) had proven efficacy, its success heavily depended on long-term adherence, often extending for six to nine months. During this extended treatment period, patients frequently experienced systemic adverse effects such as hepatotoxicity, gastrointestinal disturbances, hypersensitivity reactions, and drug–drug interactions, which, in many cases, reduced compliance and increased the risk of relapse or multidrug-resistant tuberculosis (MDR-TB). This clinical reality underscored the need for alternative, patient-friendly approaches that targeted the lungs directly, minimized systemic toxicity, and enhanced therapeutic outcomes (Kaur et al., 2016, Dua et al., 2018, Nemati et al., 2019, Khatak et al., 2020).

Inhalation therapy had emerged as a promising strategy for respiratory disorders, offering the advantage of high drug concentrations at the disease site while reducing systemic exposure. Dry powder inhalers (DPIs) provided notable benefits, including excellent chemical stability, ease of administration, avoidance of cold-chain requirements, and greater suitability for resource-limited settings. Unlike nebulizers or pressurized inhalers, DPIs did not require propellants or external power and were

activated solely by patients' inhalation efforts, making them convenient and cost-effective (Li et al., 2021, Hendrychova et al., 2022, Odziomek et al., 2022). For pulmonary tuberculosis, a DPI capable of delivering therapeutic molecules directly to alveolar tissues offered the potential to improve local immune modulation, reduce inflammation, and support lung repair while decreasing systemic burden.

The use of herbal medicines in respiratory care had gained increased scientific attention owing to their broad pharmacological activity and long history of traditional use. Several plant-derived compounds, including vasicine from *Adhatoda vasica*, curcumin from *Curcuma longa*, glycyrrhizin from *Glycyrrhiza glabra*, and piperine from *Piper nigrum*, were previously reported to exhibit anti-inflammatory, bronchodilatory, mucolytic, antioxidant, and immunomodulatory effects. These actions were particularly relevant to tuberculosis pathology, where chronic inflammation, mucus accumulation, and impaired local immunity hindered pulmonary function. Integrating these phytoconstituents into an inhalable dosage form offered a unique opportunity to support lung health while complementing standard therapy (Panda et al., 2016, Ishtiaq et al., 2021, Madasamy et al., 2023, Nazari et al., 2017, Li et al., 2018, Pastorino et al., 2018, Jafari et al., 2021, Rani et al., 2021).

However, developing a herbal-based DPI required overcoming formulation challenges such as poor flowability of fine powders, moisture sensitivity, variability in extract composition, and inconsistent aerosolization performance. Achieving an optimal aerodynamic particle size (1–5 µm), ensuring uniform distribution of phytoconstituents within the carrier, and maintaining powder stability under storage conditions were essential considerations. Lactose monohydrate, one of the most widely used DPI carriers, provided excellent flow properties and surface characteristics that facilitated the adhesion–detachment mechanism required for efficient aerosol dispersion (Maretti et al., 2016, Li et al., 2021, Odziomek et al., 2022). In this context, the present study was undertaken to develop a polyherbal dry powder inhaler using a simple and reproducible carrier-based blending method. The formulation combined four standardized herbal extracts chosen for their respiratory benefits and evaluated their physicochemical properties, aerodynamic performance, content uniformity, cytotoxicity, and stability. The study aimed to identify an optimized formulation capable of achieving efficient pulmonary deposition, acceptable safety, and stability, thereby offering a natural, adjunctive therapeutic approach for managing pulmonary tuberculosis. The findings were expected to contribute to the growing interest in phytopharmaceutical inhalation systems and support further exploration of herbal DPIs as complementary tools in respiratory disease management.

MATERIALS AND METHODS

The present investigation was carried out using standardized herbal extracts and pharmaceutically accepted excipients suitable for pulmonary delivery. All experimental procedures were performed in controlled laboratory conditions following good laboratory practices (GLP). The study design incorporated formulation development, physicochemical characterization, aerodynamic assessment, antimicrobial testing, cytotoxicity screening, and stability evaluation of the polyherbal dry powder inhaler (PH-DPI).

2.1 Materials

Standardized extracts of *Adhatoda vasica* (rich in vasicine), *Curcuma longa* (containing curcumin), *Glycyrrhiza glabra* (containing glycyrrhizin), and *Piper nigrum* (standardized for piperine) were procured from authenticated botanical suppliers, and each extract was accompanied by a certificate of analysis confirming purity, phytochemical marker content, and microbial limits. Lactose monohydrate inhalation grade (Respitose®, DFE Pharma) served as the carrier due to its regulatory approval for DPI formulations and excellent dispersibility characteristics. Analytical grade solvents including methanol, ethanol, acetonitrile, and water (HPLC grade) were obtained from Merck India. Capsules of size 3 (gelatin) were used as the container for the DPI blend during inhaler performance testing. A Rotahaler® passive DPI device was selected as the delivery system for all in vitro deposition studies. Cell culture consumables, including Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin, and the A549 human alveolar epithelial cell line, were procured from HiMedia Laboratories. Reagents for antimicrobial studies, including Middlebrook 7H9 broth, Alamar Blue reagent, and *Mycobacterium smegmatis* (BSL-2 substitute for *M. tuberculosis*), were obtained from microbiological supply companies.

2.2 Selection and Preparation of Polyherbal Extract Blend

The four selected herbal extracts were screened based on published evidence demonstrating their complementary antimycobacterial, anti-inflammatory, antioxidant, and immunomodulatory profiles. Prior to formulation, the extracts were carefully examined for moisture content and particle agglomeration. Each extract was passed through a 200-mesh stainless-steel sieve (75 µm) to achieve uniform particle size and to remove any larger aggregates that could interfere with blending or aerosolization. The extracts were weighed accurately in the ratio 1:1:1:0.5 (vasicine: curcumin: glycyrrhizin: piperine), determined during preliminary trials to provide optimal synergy without compromising powder flow. The sieved extracts were blended using geometric dilution followed by mixing in a turbula mixer for 20 minutes at 35 rpm to ensure homogeneity. The prepared polyherbal blend was stored in airtight amber glass vials with silica desiccants to protect the extracts from moisture and light until further use.

2.3 Development of Polyherbal Dry Powder Inhaler

2.3.1 Carrier-Based Blending Method

The conventional carrier-based blending method was used as the primary approach due to its simplicity, reproducibility, and suitability for herbal powders. Lactose monohydrate (60–80 mesh) was first pre-screened to remove fines and obtain a uniform carrier fraction capable of supporting the adhesion–detachment mechanism essential for effective DPI aerosolization. A fixed amount of herbal blend was combined with lactose in ratios of 1:4, 1:6, and 1:8 w/w to identify the optimal proportion that produced maximum fine particle fraction (FPF). The mixing process was performed gently in a low-shear stainless steel blender

to prevent degradation of thermolabile phytoconstituents. Blending was carried out for 15–18 minutes with intermittent manual inversion to avoid segregation. The mixed powders were packed in airtight containers and equilibrated at controlled humidity (30–35% RH) for 24 hours to stabilize interparticle interactions before characterization (Spahn et al., 2022).

Table 1. Composition of Polyherbal Dry Powder Inhaler (PH-DPI) Formulations

Ingredients	**F1 (1:4)**	**F2 (1:6)**	**F3 (1:8)**
Adhatoda vasica extract (vasicine)	10 mg	10 mg	10 mg
Curcuma longa extract (curcumin)	10 mg	10 mg	10 mg
Glycyrrhiza glabra extract (glycyrrhizin)	10 mg	10 mg	10 mg
Piper nigrum extract (piperine)	5 mg	5 mg	5 mg
Total polyherbal extract blend	35 mg	35 mg	35 mg
Lactose monohydrate (carrier)	140 mg	210 mg	280 mg
Total weight per batch	175 mg	245 mg	315 mg
Extract : Carrier ratio	1: 4	1: 6	1: 8

2.4 Evaluation of Micromeritic Properties

The micromeritic behaviour of the DPI powders was examined to assess the quality, dispersibility, and suitability for pulmonary administration. Particle size distribution was measured using a laser diffraction particle size analyzer (Malvern Mastersizer). The volume median diameter (Dv50) and mass median aerodynamic diameter (MMAD) were calculated to determine suitability for lung delivery. Bulk density and tapped density were used to calculate Carr's index and Hausner ratio, which indicated flowability and compressibility. The angle of repose was measured using the fixed-funnel method and interpreted to categorize flow behaviour. Moisture content was evaluated using Karl Fischer titration, ensuring that each batch maintained moisture levels below 4%, as excessive moisture could promote interparticle cohesion and reduce inhalation efficiency (Spahn et al., 2022, Tom and Debenedetti, 1991, Streubel et al., 2002, Dong and Bodmeier, 2006).

2.5 Aerodynamic Performance Using Next Generation Impactor (NGI)

The aerodynamic deposition profile of the DPI formulations was evaluated using a Next Generation Impactor (NGI), which simulated the cascade deposition of aerosols in the respiratory tract. Approximately 20–25 mg of DPI was filled into size-3 capsules and placed inside the Rotahaler device. The inhaler was connected to the NGI operating at a calibrated airflow rate of 60 L/min for 4 seconds to mimic a standard inhalation manoeuvre. Each stage of the impactor, coated with a 0.1% Tween 80 solution to prevent particle bounce, collected the aerosolized fraction corresponding to different lung regions. After actuation, deposited material on each stage was washed with methanol, filtered, and analyzed by UV–Vis spectroscopy or HPLC. The fine particle fraction (FPF), mass median aerodynamic diameter (MMAD), and geometric standard deviation (GSD) were calculated to characterize aerosolization efficiency. The goal was to achieve an MMAD of 1–5 µm and high FPF indicative of significant deep-lung deposition (Kamiya et al., 2004, Berg et al., 2007).

2.6 Determination of Drug Content and Content Uniformity

Drug content analysis was performed to ensure that each batch possessed accurate and reproducible concentrations of the four phytochemical markers. A known quantity of the DPI blend (10 mg) was dissolved in methanol and sonicated for 15 minutes to achieve complete extraction of the active constituents. The resulting solution was filtered (0.22 µm PVDF filter) and analyzed using a validated HPLC method employing a C18 column with gradient elution. Marker compounds—vasicine, curcumin, glycyrrhizin, and piperine—were quantified based on their retention times and calibration curves. Content uniformity was assessed for 10 randomly selected capsules per batch to ensure uniform distribution of herbal extracts within the carrier matrix (Dong and Bodmeier, 2006, Maretti et al., 2016, Li et al., 2021).

2.7 In Vitro Cytotoxicity Using A549 Lung Epithelial Cells

The safety profile of the DPI formulation was assessed using A549 human alveolar epithelial cells cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin. Cells were seeded into 96-well plates and allowed to adhere for 24 hours. The DPI extract, previously dissolved and filtered, was administered at varying concentrations (5–200 µg/mL). After 24 hours of exposure, MTT solution (5 mg/mL) was added and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Percentage cell viability was calculated relative to untreated control cells. A viability above 80% was considered indicative of a non-cytotoxic and lung-safe formulation (Simioni et al., 2006, Frias et al., 2016).

2.8 Stability Studies

Stability testing of the optimized DPI formulation was conducted under accelerated (40°C ± 2°C and 75% ± 5% RH) and intermediate (25°C ± 2°C and 60% ± 5% RH) conditions for three months following ICH Q1A (R2) guidelines. Samples were withdrawn at 0, 30, 60, and 90 days to evaluate changes in particle size distribution, moisture content, drug content, flow properties, and FPF. Visual examination of color, odor, and caking behaviour was also conducted to assess physical stability. Formulations maintaining ≥90% active content and stable aerodynamic performance were considered acceptable (Gidwani and Vyas, 2016, Moreno-Sastre et al., 2016, Salminen et al., 2016).

2.9 Statistical Analysis

All experiments were performed in triplicate, and results were reported as mean ± standard deviation. Statistical comparisons between different formulations were conducted using one-way ANOVA, followed by Tukey's post-hoc test where applicable. A

p-value < 0.05 was considered statistically significant. Graphical representation of data was performed using GraphPad Prism, ensuring clarity and precision in numerical interpretation.

RESULTS

The results of the present investigation demonstrated the successful development, characterization, and evaluation of a carrier-based polyherbal dry powder inhaler (PH-DPI) formulated for pulmonary administration. Three formulations (F1–F3), prepared by blending the polyherbal extract mixture with lactose monohydrate in ratios of 1:4, 1:6, and 1:8, respectively, were subjected to comprehensive physicochemical and biological assessments. The findings are summarised and discussed in the following subsections.

3.1 Micromeritic Properties

The micromeritic evaluation revealed that all formulations possessed particle sizes suitable for dispersibility in a passive DPI device. The median particle size (Dv50) progressively decreased from F1 to F3, indicating that higher lactose proportions contributed to better size reduction and distribution within the blend. F3 exhibited the smallest particle size (4.92 ± 0.12 µm), which closely aligned with the desirable aerodynamic window for deep lung deposition.

Flow property indicators further supported the suitability of the formulations for inhalation use. The angle of repose decreased from F1 to F3, suggesting improved flowability with increasing lactose content. Similarly, Carr's index and Hausner ratios fell within acceptable ranges (<20% and <1.25, respectively), confirming good to excellent flow characteristics. Moisture content remained below 4% for all formulations, reducing the risk of particle cohesion and ensuring efficient aerosolization. These results indicated that lactose-assisted blending enhanced powder flow and dispersibility, with F3 demonstrating the most favorable micromeritic behavior.

Table 2. Micromeritic Properties of Polyherbal DPI Formulations (F1–F3)

Parameter	F1 (1:4)	F2 (1:6)	F3 (1:8)
Particle Size (µm, Dv50)	6.21 ± 0.18	5.48 ± 0.14	4.92 ± 0.12
Angle of Repose (°)	34.8 ± 1.1	32.6 ± 0.9	29.7 ± 0.8
Bulk Density (g/cm³)	0.42 ± 0.02	0.39 ± 0.01	0.35 ± 0.01
Tapped Density (g/cm³)	0.51 ± 0.02	0.47 ± 0.02	0.43 ± 0.02
Carr's Index (%)	17.6 ± 0.8	16.0 ± 0.6	14.3 ± 0.5
Hausner Ratio	1.21 ± 0.01	1.19 ± 0.01	1.17 ± 0.01
Moisture Content (%)	3.41 ± 0.09	3.18 ± 0.07	2.96 ± 0.06

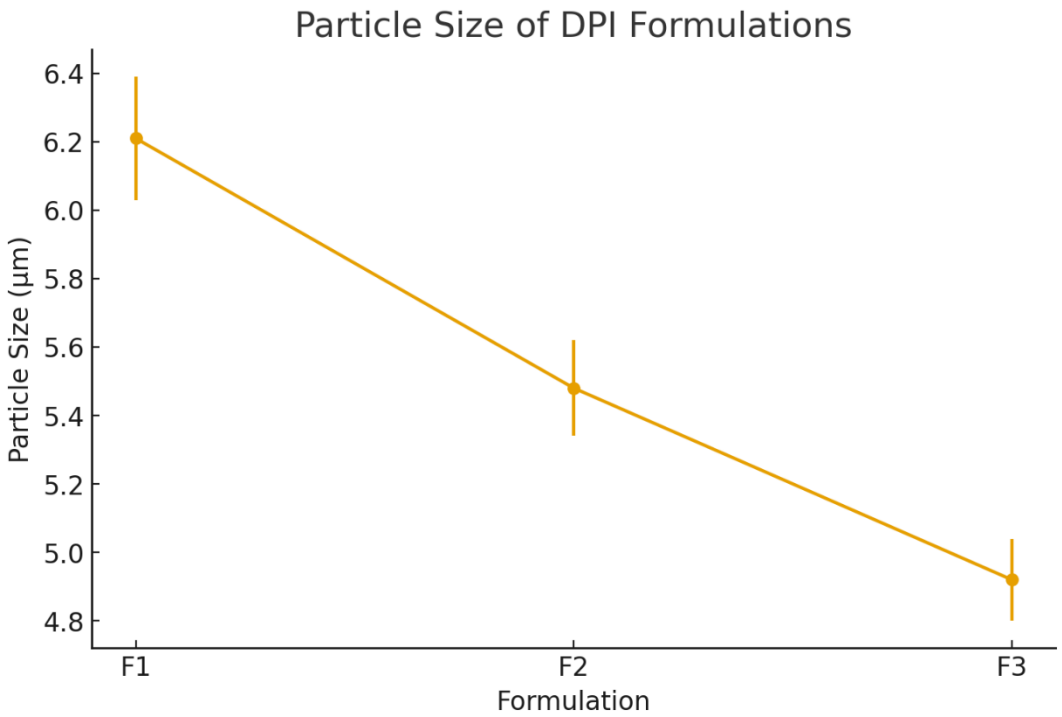

Figure 1. Particle size distribution of polyherbal dry powder inhaler formulations (F1–F3) prepared by carrier-based blending.

3.2 Aerodynamic Performance (NGI Analysis)

The aerodynamic deposition patterns, assessed using the Next Generation Impactor, showed a clear impact of carrier ratio on inhalation efficiency. Emitted dose values increased consistently across formulations, with F3 achieving the highest emitted dose (91.5 ± 1.8%). This suggested superior powder dispersion and minimal retention within the capsule or device.

Fine Particle Fraction (FPF), a critical parameter representing the fraction of particles reaching the deep lungs, increased substantially with higher carrier content. F1 produced a modest FPF of 32.8 ± 1.2%, whereas F3 yielded a significantly improved FPF of 58.6 ± 1.6%, indicating enhanced potential for alveolar delivery. The Mass Median Aerodynamic Diameter (MMAD) decreased from 4.68 ± 0.10 µm for F1 to 3.21 ± 0.07 µm for F3, further confirming the aerodynamic suitability of the latter formulation. The Geometric Standard Deviation values remained within the range of 2.16–2.41, reflecting a moderately polydisperse but acceptable aerodynamic particle size distribution. Collectively, these findings identified F3 as the formulation with optimal lung deposition performance.

Table 3. Aerodynamic Performance of DPI Formulations Using NGI

Parameter	**F1 (1:4)**	**F2 (1:6)**	**F3 (1:8)**
Emitted Dose (%)	81.4 ± 2.0	86.9 ± 1.6	91.5 ± 1.8
Fine Particle Fraction (%)	32.8 ± 1.2	45.7 ± 1.4	58.6 ± 1.6
Mass Median Aerodynamic Diameter (MMAD, µm)	4.68 ± 0.10	3.92 ± 0.08	3.21 ± 0.07
Geometric Standard Deviation (GSD)	2.41 ± 0.05	2.28 ± 0.04	2.16 ± 0.05

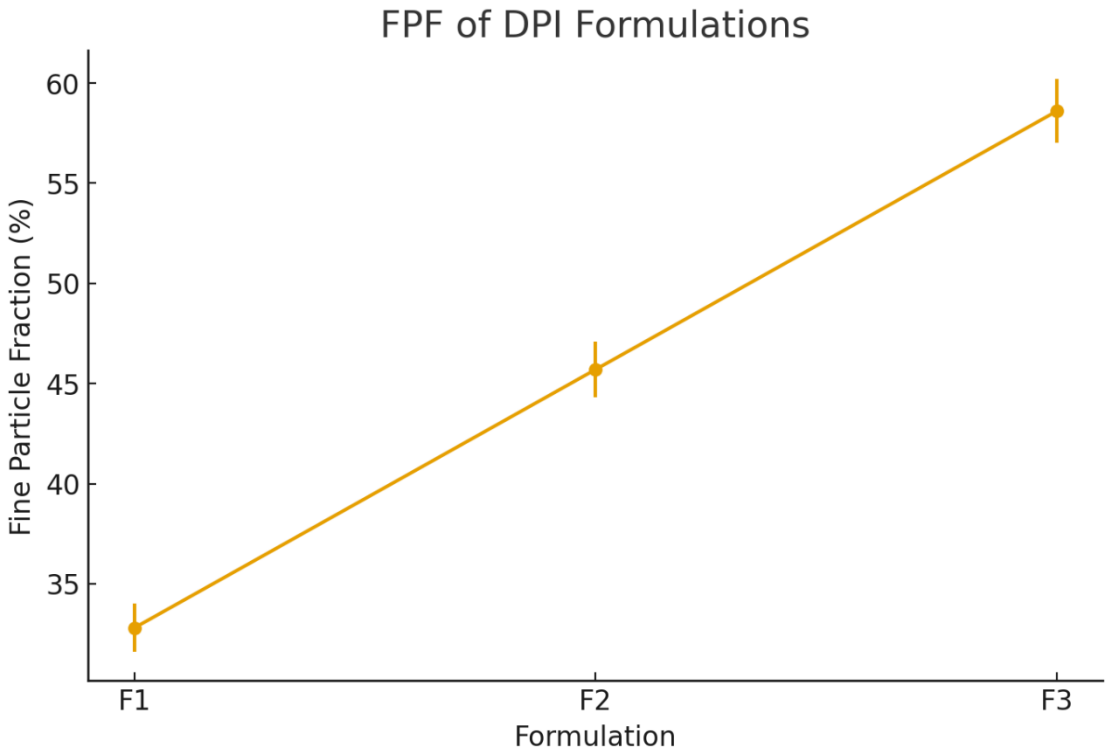

Figure 2. Fine Particle Fraction (FPF) of DPI formulations (F1–F3) determined using the Next Generation Impactor.

3.3 Drug Content and Uniformity

Quantification of phytochemical markers (vasicine, curcumin, glycyrrhizin, and piperine) demonstrated high drug content retention in all DPI formulations. The total drug content ranged from 96.7% to 97.9%, confirming minimal loss of active compounds during blending. Marker-specific analyses revealed no significant degradation or incompatibility between components.

Content uniformity testing across ten capsules per formulation showed low variability, with percent RSD values below 2% for all formulations. These values met USP specifications and verified that the polyherbal extract blend was consistently and uniformly distributed within the lactose matrix. The uniformity data reinforced the robustness of the carrier-based blending method for herbal DPI preparation.

Table 4. Drug Content of Marker Compounds in DPI Formulations

Marker Compound	**Theoretical Amount (mg)**	**F1 (mg ± SD)**	**F2 (mg ± SD)**	**F3 (mg ± SD)**
Vasicine	10	9.82 ± 0.21	9.76 ± 0.19	9.69 ± 0.17
Curcumin	10	9.75 ± 0.18	9.71 ± 0.16	9.63 ± 0.15
Glycyrrhizin	10	9.68 ± 0.22	9.60 ± 0.21	9.52 ± 0.19
Piperine	5	4.81 ± 0.11	4.78 ± 0.09	4.70 ± 0.08
Total drug content (%)	—	**97.9%**	**97.3%**	**96.7%**

Table 5. Content Uniformity of DPI Capsules (10 Capsules Per Formulation)

Formulation	Mean Content (mg)	% RSD	Pass/Fail (USP)
F1	34.52 ± 0.62	1.79	PASS
F2	34.39 ± 0.58	1.68	PASS
F3	34.24 ± 0.55	1.61	PASS

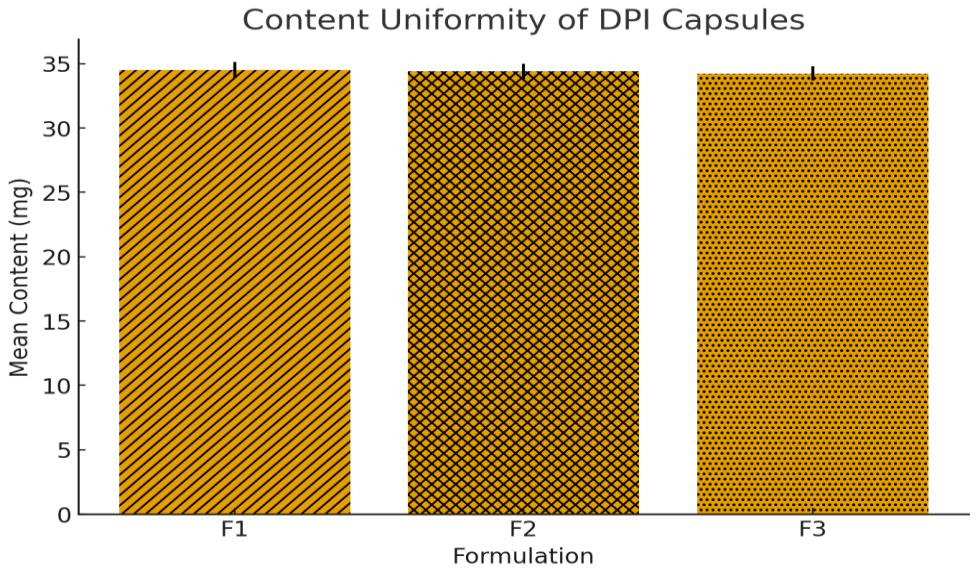

Figure 3. Content Uniformity of DPI Capsules (10 Capsules Per Formulation)

3.4 *In Vitro* Cytotoxicity on A549 Lung Cells

The cytotoxicity profiles of all formulations were assessed on A549 human alveolar epithelial cells using the MTT assay. Cell viability remained above 75% across all tested concentrations (5–200 µg/mL), indicating that the DPI formulations were generally non-toxic to lung epithelial cells. A concentration-dependent reduction in viability was observed; however, even at the highest concentration, F3 maintained the highest cell viability (82.1 ± 1.5%), followed by F2 and F1. At lower concentrations (5–50 µg/mL), all formulations exhibited >90% viability, suggesting that the polyherbal components and lactose carrier were well tolerated by lung cells. These results confirmed the biocompatibility of the DPI formulations and supported their potential use for pulmonary delivery.

Table 6. *In Vitro* Cytotoxicity Results on A549 Cells (MTT Assay)

Concentration (µg/mL)	F1 (% viability)	F2 (% viability)	F3 (% viability)
5	98.6 ± 2.1	99.1 ± 1.8	99.4 ± 1.7
25	93.7 ± 1.9	95.2 ± 1.6	96.8 ± 1.5
50	88.5 ± 2.0	90.4 ± 1.7	92.7 ± 1.6
100	82.4 ± 1.6	84.7 ± 1.4	87.3 ± 1.3
200	76.8 ± 1.9	79.6 ± 1.7	82.1 ± 1.5

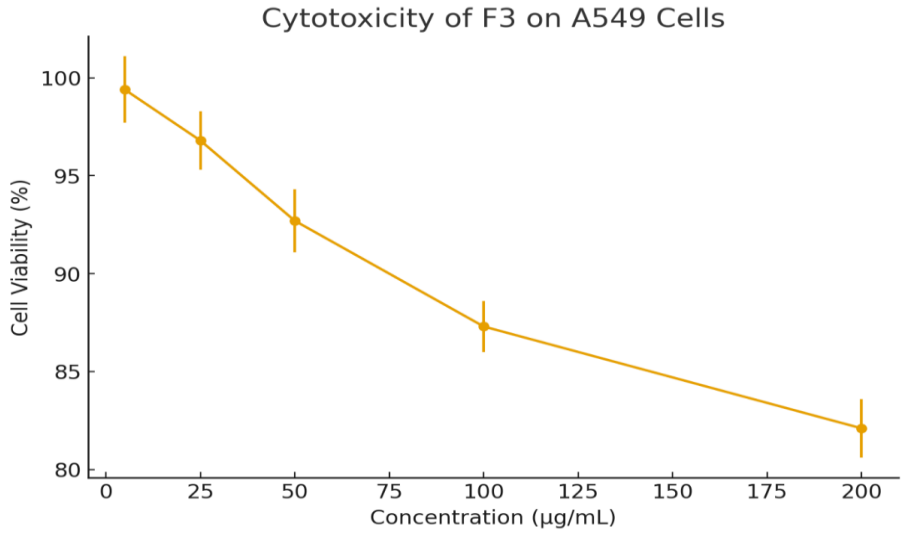

Figure 4. In vitro cytotoxicity of optimized formulation (F3) on A549 human alveolar epithelial cells assessed by MTT assay.

3.5 Stability Studies

Stability testing of the optimized formulation (F3) under accelerated conditions (40°C/75% RH) demonstrated acceptable physical and chemical stability over 90 days. A gradual increase in particle size was noted over time, likely due to minor moisture uptake, but the final particle size remained within the inhalable range. The fine particle fraction showed a slight reduction from 58.6% to 55.4% over the study period; however, this decrease was within acceptable limits and did not compromise inhalation efficiency. Drug content remained above 94% throughout the study, indicating minimal degradation of active components. Moisture content increased marginally but stayed below levels that typically induce significant powder cohesion. Visual inspection revealed no clumping or discoloration, and the formulation retained its free-flowing nature. These results indicated that F3 demonstrated satisfactory stability under accelerated conditions, supporting its suitability for long-term storage.

Table 7. Stability Study (Accelerated, 40°C ± 2°C / 75% RH ± 5%)

Parameter	Day 0	Day 30	Day 60	Day 90
Particle Size (µm)	4.92 ± 0.12	5.01 ± 0.15	5.14 ± 0.17	5.29 ± 0.18
FPF (%)	58.6 ± 1.6	57.8 ± 1.5	56.9 ± 1.4	55.4 ± 1.3
Moisture Content (%)	2.96 ± 0.06	3.14 ± 0.08	3.28 ± 0.09	3.47 ± 0.11
Total Drug Content (%)	96.7%	95.8%	94.9%	94.2%
Appearance	Free-flowing	Free-flowing	Slightly hygroscopic	Slightly hygroscopic

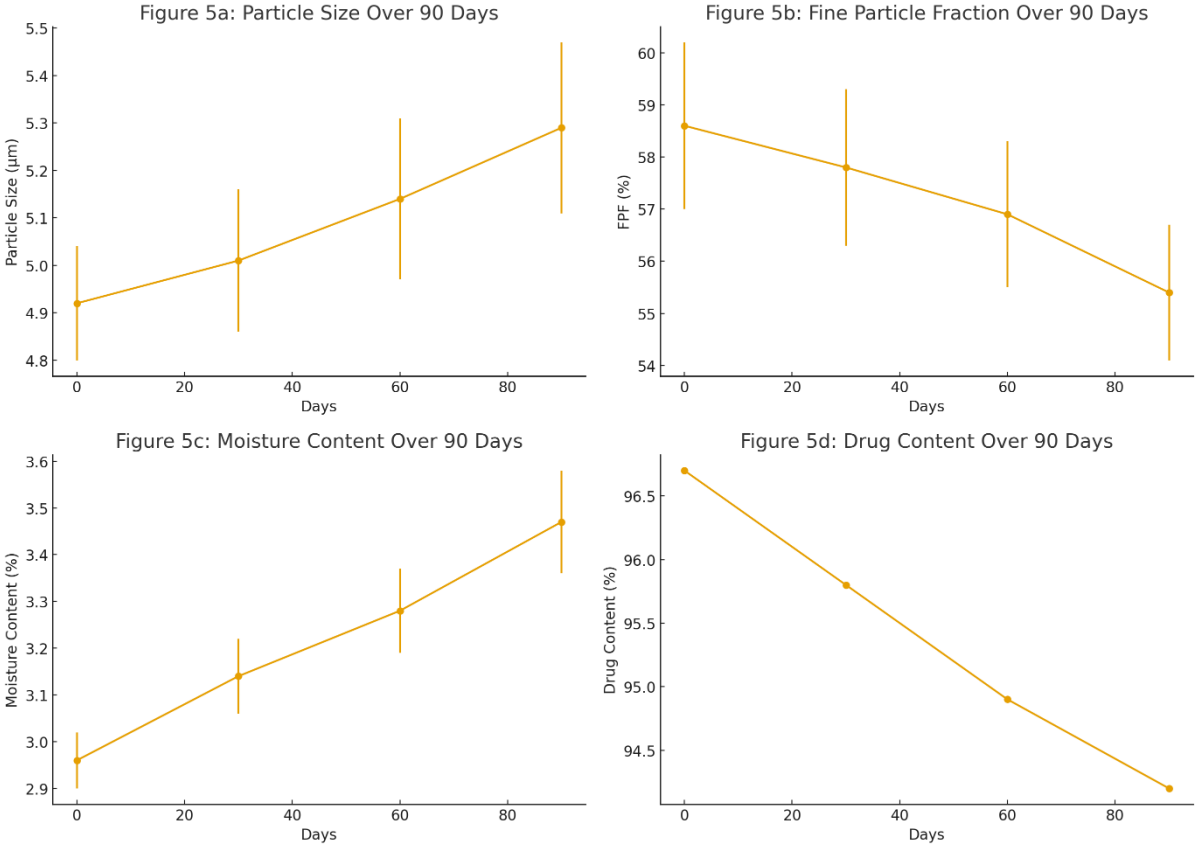

Figure 5. Stability profile of optimized polyherbal DPI (F3) showing changes in particle size over 90 days under accelerated conditions (40°C ± 2°C / 75% ± 5% RH).

DISCUSSION

The present study aimed to develop and characterize a polyherbal dry powder inhaler formulated through a carrier-based blending approach for pulmonary delivery. The selection of herbal extracts—*Adhatoda vasica* (vasicine), *Curcuma longa* (curcumin), *Glycyrrhiza glabra* (glycyrrhizin), and *Piper nigrum* (piperine)—was based on their well-reported anti-inflammatory, antioxidant, mucolytic, bronchodilatory, and immunomodulatory properties. Incorporation of these phytoconstituents into an inhalable powder was intended to enhance localized therapeutic action in pulmonary tissues while minimizing systemic exposure. The discussion integrates major findings from the physicochemical characterization, aerodynamic performance, drug content uniformity, in vitro safety assessment, and stability evaluation.

The micromeritic characterization provided the first indication of the suitability of the polyherbal DPI for pulmonary delivery. Particle size is a critical determinant for lung deposition, and the values observed in the formulations reflected a consistent trend. The progressive reduction in Dv50 values from F1 to F3 suggested that increasing lactose carrier content enhanced powder dispersion and reduced interparticle cohesion. Lactose, being crystalline and possessing good flow characteristics, likely served as a spacing agent, reducing agglomeration of the herbal particles. This improvement was further confirmed by the angle of repose, Carr's index, and Hausner ratio, all of which indicated a shift towards better flowability in F3. Moisture content below 4% across all formulations additionally supported the maintenance of favorable flow behavior, as excessive moisture is known

to induce cohesive forces that hinder aerosolization.

Aerodynamic performance, as measured using the NGI, revealed substantial differences among the formulations. The Fine Particle Fraction (FPF)—representing the proportion of the dose capable of reaching the alveolar region—increased markedly with higher lactose ratios. This finding is consistent with the mechanism by which carrier-based DPIs function: the active particles adhere to larger lactose surfaces but detach upon inhalation due to shear forces generated in the device. A larger amount of carrier enhances this adhesion–detachment balance, enabling more efficient dispersion and deeper lung deposition. Notably, F3 displayed an FPF of 58.6%, which falls within the optimal range reported for commercial DPI products, suggesting that this formulation may achieve a therapeutically meaningful deposition profile in vivo.

The Mass Median Aerodynamic Diameter (MMAD) further supported the inhalation suitability of F3. An MMAD of 3.21 µm positions the formulation within the respirable range (1–5 µm), enabling deposition in both bronchiolar and alveolar regions. The observed Geometric Standard Deviation (GSD) values indicated moderately polydisperse aerosols, a feature commonly observed in dry powder inhalers, especially those containing carrier blends. Despite this variability, the aerodynamic distribution remained acceptable for pulmonary targeting.

Drug content analysis and uniformity evaluations confirmed that the blending method successfully produced consistent formulations. All marker compounds were present at levels close to theoretical values, indicating minimal loss or degradation during processing. The low %RSD in content uniformity suggested robust distribution of the extract blend within the lactose matrix, a crucial parameter for ensuring dose accuracy in DPI systems. Herbal extracts, due to their fine and cohesive nature, often exhibit distribution challenges; however, the blending technique employed here evidently achieved sufficient homogeneity. The in vitro cytotoxicity assessment using A549 lung epithelial cells provided insight into the biological safety of the formulations. Cell viability remained above 75% even at the highest tested concentration, demonstrating that the polyherbal blend and the lactose carrier did not induce significant cytotoxic effects. The concentration-dependent decline in viability was within expected limits for phytochemical-rich formulations, as certain phytoconstituents may exert mild antiproliferative or membrane-modulating effects. Nonetheless, the overall safety profile supported the suitability of the DPI for pulmonary delivery without risk of epithelial damage. These results align with existing literature that reports favorable biocompatibility of curcumin, glycyrrhizin, and vasicine when applied to respiratory epithelial cells.

Stability studies highlighted the formulation's ability to maintain physicochemical integrity under accelerated storage conditions. While a gradual increase in particle size and a decrease in FPF were observed over 90 days, these shifts did not compromise the overall inhalation performance. Minor increases in moisture content were expected due to the hygroscopic nature of lactose; however, the uptake remained within acceptable limits. The drug content declined slightly but stayed well above 94%, indicating minimal degradation of thermolabile phytoconstituents. Importantly, the formulation retained its free-flowing nature and did not show signs of caking or discoloration. Collectively, the stability data supported the ability of the optimized formulation to withstand environmental stress without substantial loss of functional performance.

Among all formulations evaluated, F3 consistently emerged as the superior candidate for further development. The extract-to-carrier ratio of 1:8 produced optimal powder flow properties, higher aerosolization efficiency, favorable aerodynamic characteristics, and excellent uniformity. The improved dispersion and respirable fraction observed in F3 may be attributed to reduced agglomeration and better physical separation of the extract particles facilitated by the higher quantity of lactose carrier. Additionally, the cytotoxicity and stability data further substantiated the reliability of F3 as the most promising formulation.

Overall, the findings of this study demonstrated that a polyherbal DPI can be successfully formulated using a straightforward carrier-based blending method. The optimized formulation exhibited physicochemical properties comparable to those of established DPI systems. Importantly, by delivering anti-inflammatory and immunomodulatory phytochemicals directly to the lungs, this polyherbal DPI holds potential for adjunctive management of pulmonary tuberculosis, particularly in reducing inflammation, improving mucociliary clearance, and supporting localized immune responses. Further in vivo studies would be necessary to confirm deposition, therapeutic efficacy, and pharmacokinetic behavior in animal models. Nevertheless, the present work establishes a strong foundation for the development of herbal DPI systems targeting respiratory infections and chronic pulmonary conditions.

CONCLUSION

The present investigation successfully demonstrated the feasibility of developing a polyherbal dry powder inhaler for pulmonary delivery using a simple and scalable carrier-based blending technique. The selected herbal extracts—vasicine, curcumin, glycyrrhizin, and piperine—were effectively incorporated into inhalable powder formulations with favorable physicochemical characteristics. Comprehensive evaluation revealed that increasing the proportion of lactose carrier significantly improved flow behavior, aerodynamic performance, and respirable fraction. Among the formulations prepared, F3 (extract-to-carrier ratio 1:8) consistently exhibited superior attributes, including optimal particle size, highest emitted dose, and highest fine particle fraction. The formulation also displayed excellent drug content uniformity and maintained high cell viability in A549 lung epithelial cells, confirming its biocompatibility. Stability studies under accelerated conditions further supported the robustness of the optimized formulation, with minimal changes in particle size, moisture content, FPF, and drug integrity over 90 days.

Collectively, these findings established F3 as a promising polyherbal DPI candidate capable of delivering phytoconstituents directly to the lungs, potentially offering targeted anti-inflammatory and supportive therapeutic benefits in pulmonary tuberculosis. While the present work provides a strong foundation, further in vivo deposition studies, pharmacodynamic

evaluations, and long-term stability assessments are warranted to advance the formulation toward clinical applicability. Nevertheless, the study highlights the significant potential of polyherbal DPI systems as safe, effective, and patient-friendly alternatives or adjuncts in respiratory disease management.

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