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Original Article

Design and Optimization of Novel Anti-Tubercular Polyherbal Chewable Tablet Using Design Expert

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ABSTRACT

Introduction: Scientific and Ayurvedic literature provides strong evidence that plants, their phytoconstituents, and extracts have significant anti-tubercular activity. However, developing effective herbal formulations is a complex process due to the presence of multiple bioactive compounds with diverse chemical properties. This complexity requires a systematic and scientifically driven approach in formulation development. Implementing a Quality by Design (QbD) framework is therefore essential, as it allows for a deeper understanding of critical material attributes and process parameters involved in manufacturing. By adopting QbD principles, the formulation process becomes more robust, predictable, and efficient, reducing dependence on traditional trial-and-error methods and ensuring consistent quality, safety, and efficacy of the final herbal product for therapeutic use. **Objectives:** The study aims to design and develop anti-tubercular polyherbal chewable tablets using a 3^2 factorial design, with standardization achieved through HPLC. **Materials and Methods:** The formulation was developed systematically using Design-Expert Software (version 11) to ensure a scientific and optimized approach. Based on extensive evidence from scientific research and Ayurvedic literature, four medicinal plant extracts were selected for their proven anti-tubercular potential. These included *Glycyrrhiza glabra* (Yastimadhu), known for its anti-inflammatory and immunomodulatory properties; *Piper longum* (Pippali), recognized for its bio-enhancing and respiratory benefits; *Curcuma longa* (Haridra), valued for its potent anti-inflammatory and antimicrobial effects; and *Adhatoda vasica* (Vasaka), traditionally used for respiratory disorders. These extracts formed the core active components for the optimized polyherbal chewable tablet formulation. **Results:** Pre-compression, post-compression, and organoleptic evaluations indicated that formulation F5 exhibited superior taste and overall performance compared to all other formulations. The optimized batch demonstrated desirable hardness, disintegration time, and acceptable mouthfeel, making it the most suitable for patient compliance. Quantitative analysis using HPLC confirmed the presence of key active constituents, with the formulation containing 1.035% Glycyrrhin, 0.44% Piperine, 1.37% Curcumin, and 0.086% Vasicine. These results validated the consistency and standardization of the tablet. Additionally, accelerated stability studies were conducted, which showed that the formulation retained its physicochemical properties and active content throughout the study period, confirming its stability and suitability for long-term storage under recommended conditions. **Conclusion:** This factorial design approach can help to develop and optimize polyherbal chewable tablets and lead to the development of precious and cost-effective products in a minimal period of time. Quantification of active phytoconstituents in polyherbal chewable tablets by HPLC showed the product quality and through this, we can fluently control the batch- to- batch variations in the finished product which will also help to deliver consistent efficacy. Therefore, by adopting newer techniques of formulation development and manufacturing process, we can minimize the trials and improve the product quality, efficacy and shelf life. Moreover, this polyherbal formulation may offer therapeutic potential in the early stages of tuberculosis due to the anti-tubercular properties of the selected plants.

Keywords: Polyherbal Chewable Tablet, Quality by Design, Tuberculosis, HPLC, Stability study, Ayurvedic Medicines.

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INTRODUCTION

Tuberculosis is one of the international health issues, particularity in developing countries. According to the World Health Organization, tuberculosis is an infectious disease caused by a bacteria called Mycobacterium tuberculosis. It mainly affects the lungs but can also affect other parts of the body, which is known as extra-pulmonary TB. The disease spreads when people breathe in droplets from the coughs of infected individuals. People who have the bacteria in their body have a 5-15% chance of developing TB during their lifetime. Those with weak immune systems, such as people with HIV, those who are malnourished, or those with diabetes, or those who use tobacco, are more likely to get the disease. India is the second most populous country in the world. More than a quarter of all TB cases and deaths happen there each year. India has the highest number of new TB cases, drug-resistant TB cases, and TB-related deaths every year. About 2.2% of new TB cases in India are resistant to multiple drugs. The development of drug-resistant strains like MDR-TB and XDR-TB has made it harder to treat TB and has increased the need for new treatments. In this situation, plant-based medicines are getting more attention as a possible complement or alternative to standard TB treatments. For centuries, medicinal plants have been used in traditional systems such as Ayurveda, Traditional Chinese Medicine, and African traditional medicine to treat respiratory issues, including symptoms similar to TB. The rise of drug-resistant TB bacteria is a major cause of TB deaths. Certain chemicals found in plants, like Alkaloids, Flavonoids, Terpenoids, and Phenolics, have shown promise in fighting TB in early research.[1] These chemicals may work in different ways, such as stopping the growth of TB bacteria, breaking down biofilms, and supporting the immune system. Plant-based compounds often have fewer side effects and may help shorten the time needed for standard TB treatment when used alongside it. The Ayurvedic and herbal medicine industry is changing to meet modern medical standards. Research is now focusing on making traditional medicines simpler, stronger, and more effective. Scientists are also working to make sure that the active ingredients in these medicines are consistent across different batches. Efforts are being made to create easier-to-use forms like tablets, capsules, syrups, and nano-formulations to improve patient use and acceptance. These developments help connect traditional healing knowledge with modern medicine. A key part of this progress is using Quality by Design principles and advanced tools like Design of Experiments in developing new medicines. Quality by Design helps create medicines that are safe, effective, and stable by identifying important factors that affect these qualities. Even though these methods are beneficial, only a small number of Ayurvedic and herbal companies have started using them to improve and standardize their medicines.[2] Quality by Design (QbD) is progressively accepting an essential and commonly used term in the pharmaceutical industry. It is a systematic way to design quality product,[3] it will provide knowledge and scientific recognition to support pharmaceutical development.[4] Conventional pharmaceutical formulation and evolution approaches are usually limited by experiments that analyze only one-at-a-time variability. The design and development of any herbal or pharmaceutical drug is a science. Experimental Design or DoE is a structured method for determining the relationships between controlled input factors i.e., independent variables affecting one or more output responses i.e., dependent variables. The identification of input factors leads to optimized output responses and clarification of interactions between input factors. Over centuries, Ayurveda has offered a wealth of therapeutic knowledge and numerous classical formulations specifically designed for the management of tuberculosis, known traditionally as Rajayakshma, as well as its related symptoms. Ancient Ayurvedic texts such as *Charaka Samhita*, *Sushruta Samhita*, and *Ashtanga Hridaya* provide detailed descriptions of the disease, its causative factors, clinical manifestations, and its management through herbal medicines, mineral preparations, diet regulation, and rejuvenation therapies. These formulations, which include single-herb preparations, polyherbal combinations, and Rasayana (rejuvenation) therapies, aim not only to control the infection but also to restore overall vitality and strengthen immunity.[5] Despite this rich therapeutic heritage, the modern manufacturing and large-scale standardization of Ayurvedic formulations remain significant challenges. Unlike conventional pharmaceuticals, where Active Pharmaceutical Ingredients (APIs) are chemically well-defined, Ayurvedic formulations are inherently complex and often contain multiple bioactive phytoconstituents. The therapeutic effect of such preparations may rely on synergistic interactions among these components, making their standardization difficult. Consistency in raw material sourcing, seasonal variations in plant phytochemistry, differences in geographical origin, and traditional methods of preparation can all lead to batch-to-batch variability in the final product. Manufacturing challenges also include ensuring quality control at every stage, from the collection and authentication of raw herbs to the extraction, processing, and final formulation. Modern pharmaceutical expectations demand that products meet stringent quality parameters such as identity, purity, potency, and stability. Numerous ayurvedic classical formulations[5] are available since the periods for the treatment of tuberculosis and affiliated symptoms but manufacturing of ayurvedic and polyherbal phrasings and their standardization is still a challenge. According to scientific literature, a few of the Phyto molecules and extracts that are obtained from plants were found to be synergistically effective in combination with presently available tuberculosis treatments. *Glycyrrhiza glabra* (Yastimadhu), *Piper longum* (Pippali), *Curcuma longa* (Haridra) and *Adhatoda vasica* (Vasaka) plants extracts were chosen based on traditional and scientific literature for the formulation of an anti-tubercular polyherbal chewable tablet. Some of the study concluded that 100µg/mL of Glycyrrhizin[6] which is the main

active constituent of *Glycyrrhiza glabra* is effective against Avirulent M. tuberculosis H37Ra, Virulent H37Rv and INH resistant clinical isolates of M. tuberculosis. Acetone extract[7] (0.97-1.95 µg/mL) of *Glycyrrhiza glabra* have potential against H37Rv (ATCC 27294) and U937 human macrophage cell lines infected with Mycobacterium tuberculosis H37Rv. Various extracts[8-10] of *Glycyrrhiza glabra* (Yastimadhu) have antitubercular activity. Grover *et al*.,[11] reported that *Glycyrrhiza glabra* extract act as an adjuvant in conjunction with anti-TB drugs used as DOTS showed a favorable effect in patients with pulmonary TB. Piperine[12] obtained from *Piper longum* showed an MIC of 3000 µg/mL against Mycobacterium smegmatis GN/ms43 when tested using the disk diffusion assay. Against Mycobacterium tuberculosis GN/mt-75, the same assay revealed a significantly lower MIC of 39 µg/mL, indicating greater potency. The chloroform extract[13] of *Piper longum* exhibited an MIC of 8.66±0.21 mg/mL against Mycobacterium smegmatis, determined through the agar well diffusion method. Additionally, piperine[14] demonstrated inhibitory activity at 100 µg/mL against Mycobacterium tuberculosis H37Rv in the Resazurin Microplate Assay (REMA), highlighting its potential as an anti-mycobacterial agent. Piperine[15] has been studied for its immunomodulatory properties and its protective effects in mice infected with Mycobacterium tuberculosis when administered at a dose of 1 mg/kg. When combined with rifampicin[16] at a dose of 20 mg/kg along with 10 mg/kg of piperine, it acts as a bioavailability enhancer, potentially improving the therapeutic effectiveness of the antitubercular drug. A fixed-dose combination known as Risorine Capsule,[17] which contains rifampicin (200 mg), isoniazid (INH, 300 mg), and piperine (10 mg), has been reported to be highly effective and well tolerated in the treatment of drug-susceptible pulmonary tuberculosis, offering a synergistic and patient-compliant formulation. The anti-mycobacterial activity of root Dichloromethane (DCM) extract and curcuminoids[18] was evaluated against various Mycobacterium species using the broth dilution assay. The Minimum Inhibitory Concentration (MIC) values for both the DCM extract and curcuminoids were found to be 512 µg/mL against Mycobacterium smegmatis, M. simiae, and M. terrae; 128 µg/mL against M. kansasii; and 256 µg/mL against M. szulgai, indicating moderate activity across different non-tuberculous mycobacteria. Additionally, the effect of curcumin[19] on Mycobacterium tuberculosis (MTB) was studied using an *in vitro* human macrophage infection model, which helped assess its ability to inhibit MTB survival within host immune cells, suggesting its potential role in tuberculosis therapy. Kumari B *et al*.,[20] reported that *Curcuma longa* showed hepatoprotective effect against anti-tubercular drugs induced hepatotoxicity. An aqueous extract[21] of *Adhatoda vasica* (Vasaka) exhibited inhibitory activity against M. smegmatis (MTCC 6) with a Minimum Inhibitory Concentration (MIC) of 100 µg/mL using the agar diffusion cup method. The ethanol extract[22] of *Adhatoda vasica* showed activity against M. tuberculosis H37Rv with an MIC of 200 µg/mL in a broth microdilution assay. Among the isolated compounds, vasicinone[22] obtain from *Adhatoda vasica* demonstrated potent activity with an MIC of 64 µg/mL against M. tuberculosis. Additionally, a hexane extract[23] and vasicine acetate of *Adhatoda vasica* showed MIC values of 100 µg/mL against M. tuberculosis H37Rv (HR-sensitive, ATCC 27294) and HR-resistant strain Rf-10385, respectively, using the agar dilution assay. Another compound, 2-acetyl benzylamine from *Adhatoda vasica* showed inhibitory activity at 200 µg/mL. Moreover, water extracts[24] were tested on Lowenstein Jensen (L-J) medium and the BACT/ALERT 3D colorimetric system against both drug-sensitive M. tuberculosis H37Rv and multidrug-resistant isolates DKU-156 and JAL-1236, indicating the potential of aqueous formulations in managing resistant TB strains.

Literature based evidence confirmed that extracts and isolated compounds from selected plants exhibit significant *in vitro* activity against *Mtb* H37Rv and resistant *Mycobeacterium* strain. Initial symptoms of Pulmonary Tuberculosis are Breathing difficulty, Chest pain, Cough (usually with mucus), Coughing up blood, Excessive sweating, Fatigue, Fever, Weight loss, Wheezing, etc. So, apart from their anti-mycobacterial activity, these selected plants also exhibit bronchodilator, anti-inflammatory, antioxidant, hepatoprotective, immunomodulatory, antitussive, and bioavailability-enhancing properties, which may play a significant role in the treatment of primary-stage tuberculosis. Identification, Assay of each selected plant extract were done by HPTLC and HPLC and the effective dose concentration of selected plant extracts was decided based on the *in vitro* activity of selected plant extracts for formulation development.[25] The oral dosage form is the most effective route for drug administration due to its accuracy, low cost, ease of administration, and patient compliance.[26] Chewable tablets are an oral dosage form that is Immediately Released (IR) and have to be broken and chewed in between the teeth before consumption. It disintegrates into a smooth texture and has a pleasant taste.[27] The development of chewable tablets involves the careful consideration of multiple parameters, including powder flow, lubrication, disintegration, organoleptic properties, compressibility, compatibility, and stability. These parameters are crucial for both conventional swallowable tablets and chewable formulations. However, in the case of chewable tablets, the organoleptic characteristics such as taste, texture, and mouthfeel are of primary importance, as they play a major role in patient compliance and overall product acceptance. Chewable tablets offer several distinct advantages over traditional oral dosage forms. They provide a faster onset of action and improved bioavailability since the drug bypasses part of the disintegration step and can be absorbed more quickly. Additionally, chewable tablets enhance the safety profile of drugs that generate toxic metabolites through gastric or first-pass hepatic metabolism, thereby minimizing potential side effects. Their pleasant taste and ease of administration make them

particularly convenient for pediatric, geriatric, and dysphagic patients, as no water is required for swallowing. These attributes not only improve patient adherence but also offer a unique product differentiation advantage from a marketing perspective. The present study focuses on the design and development of polyherbal chewable tablets intended for anti-tubercular therapy. A 3^2 factorial design was employed to systematically study the effect of formulation variables on key quality attributes and to optimize the tablet characteristics. Furthermore, High-Performance Liquid Chromatography (HPLC) was used for the standardization of the final formulation by quantitatively determining the major bioactive markers, ensuring consistency, quality, and therapeutic efficacy of the chewable tablets.

MATERIALS AND METHODS

Procurement of materials

Glycyrrhiza glabra dry extract, *Piper longum* dry extract, *Curcuma longa* dry extract, *Adhatoda vasica* dry extract, and all other excipients used in the formulation were procured from Vasu Healthcare Pvt. Ltd., Vadodara, Gujarat, India, ensuring standardized and high-quality raw materials for the study. Additionally, all analytical-grade chemicals and reagents required for the experimental work were sourced from Merck, Germany, to maintain accuracy and reliability of the results. The use of authenticated plant extracts and high-purity chemicals ensured the reproducibility, consistency, and scientific validity of the formulation and subsequent analytical evaluations carried out during the research process.

Design of Experiments (DoE)

The poly-herbal chewable tablets formulation was intended using the 3^2 full factorial design. This design identifies and evaluates two specific components at three levels. The two independent variables chosen are Mannitol (bulk forming and binder) and Prolacrilin potassium (disintegrant). There are three levels for the selected variables: low, intermediate, and high. The responses, which are considered dependent variables, are the hardness and disintegration time of the polyherbal chewable tablets that have been designed and formulated. The formulation was designed using the Design Expert software (version 11). On the basis of the variables, ten different formulations were created with different binders and disintegrations (Table 1). Additionally, Stat-Ease-Design Expert was used to handle the acquired data, and Analysis of Variance (ANOVA) was used for statistical analysis. Ten batches were created in accordance with the design expert software, and surface responses were used to examine the relationship between the independent and dependent variables before a significant model was ultimately achieved.

Preparation of polyherbal chewable Tablet

The chewable tablets were formulated using the wet granulation technique, a widely used method for producing tablets with improved flow and compressibility. Initially, all plant extracts and excipients were carefully weighed to ensure precision and consistency in formulation. Each ingredient was passed through a #40 mesh sieve to break down agglomerates and ensure uniform particle size, which is essential for homogeneity. The granulation process began by preparing a moist mass using distilled water as the granulating fluid, ensuring that the powder blend achieved adequate wetness for granule formation. This moist mass was then passed through a #12 sieve to form uniform granules of appropriate size. The wet granules were dried in a hot air oven at a controlled temperature of 40–45°C until the desired moisture content was achieved, preventing over-drying or degradation of heat-sensitive constituents. Once drying was complete, the granules were collected and mixed with the remaining lubricants, which had been pre-sifted through a #80 mesh to eliminate lumps and ensure even distribution. This step ensured smooth tablet ejection during compression and reduced friction between granules and tooling. The lubricated blend was then subjected to pre-compression evaluation, where important flow properties such as angle of repose, bulk density, tap density, Hausner's ratio, and Carr's compressibility index were determined. These tests confirmed the blend's suitability for direct compression by assessing its flowability and compressibility characteristics. Finally, the prepared blend was compressed into chewable tablets using a 16-station single rotary tablet press (Model: PTCMD3-16, Prism Pharma Machinery). The machine was fitted with 12.6 mm round, biconvex punches to produce uniformly shaped tablets with an average weight of 800 mg each. Careful control of compression force ensured that the tablets possessed the desired hardness, uniform weight, and friability within acceptable pharmacopeial limits. The resulting chewable tablets were consistent in size, shape, and mechanical strength, making them suitable for further evaluation of quality parameters and stability studies. This method ensured optimal content uniformity, mechanical strength, and patient compliance by producing palatable, easy-to-chew dosage forms.

Physical characterization of the polyherbal chewable tablets

The compressed polyherbal chewable tablets were evaluated for various critical physical attributes in accordance with standard pharmacopoeial methods to ensure quality, consistency, and compliance with regulatory requirements. Parameters such as weight variation, friability, thickness, hardness, and disintegration time were systematically measured, as these characteristics are essential indicators of tablet uniformity, mechanical strength, and *in vivo* performance. Weight variation testing confirmed uniform distribution of the active and excipient components within each batch, while friability assessment evaluated the tablet's resistance

to mechanical stress during handling and transportation. Hardness testing ensured that the tablets possessed adequate strength to withstand compression forces and storage conditions, without compromising their ability to disintegrate appropriately upon administration. Thickness measurements helped confirm uniformity across the batch, which is critical for packaging and patient acceptability. Disintegration time was determined to assess how quickly the tablets break down in aqueous media, which is an important factor affecting drug release and bioavailability. Additionally, the loss on drying method was employed to quantify the moisture content of the tablets, as excessive moisture can lead to degradation, reduced shelf life, and compromised stability of the active constituents. Collectively, these evaluations ensured that the prepared polyherbal chewable tablets met the required quality standards for safety, efficacy, and reproducibility, thereby confirming their suitability for therapeutic use.[28]

Statistical optimization

The Analysis of Variance (ANOVA) was employed to evaluate the adequacy and reliability of the developed model. Various statistical parameters were analyzed and compared to identify the most appropriate and best-fitting model for the study. Each selected response parameter was fitted using a linear model to determine its statistical significance and correlation with the experimental variables. The relationship between the independent (formulation variables) and dependent (responses) factors was further interpreted through Response Surface Methodology (RSM) tools. Contour plots and three-dimensional surface plots were generated to visually represent the effect and interaction of different factors on the measured responses under specific conditions. These graphical tools provided a clearer understanding of factor influence and helped in predicting the optimal region for desired outcomes. Finally, the overall optimization of the formulation was achieved using a desirability function approach combined with an overlay plot to determine the most suitable factor combination.

Validation of the optimized formulation

The optimized polyherbal chewable tablet formulations were prepared based on the predicted mean values to evaluate the accuracy and reliability of the optimization process. This step was crucial to verify whether the formulation parameters identified through the statistical design would translate effectively into practical outcomes. To validate the finalized experimental design, a comparative analysis was conducted between the predicted values derived from the model and the actual experimental results obtained from the prepared batches. Key quality attributes, including disintegration time, hardness, friability, and active constituent content, were measured and statistically assessed to determine the level of agreement with the predicted outcomes. The close correlation observed between the experimental and predicted data confirmed the robustness of the optimization procedure, indicating that the selected formulation variables were appropriate and capable of producing a reproducible, high-quality polyherbal chewable tablet. This validation step reinforced confidence in the predictive model for future formulation development and scale-up processes.

Table 1: Batches of Chewable Tablet as per 3^2 Factorial Design.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Glycyrrhizaglabra DE	80	80	80	80	80	80	80	80	80	80
Piper longum DE	50	50	50	50	50	50	50	50	50	50
Curcuma longa DE	10	10	10	10	10	10	10	10	10	10
Adhatodavasica DE	30	30	30	30	30	30	30	30	30	30
Mannitol	390	450	450	390	420	390	420	420	420	450
Lactose	149.5	105.5	89.50	157.5	123.5	165.5	135.5	127.5	127.5	97.5
Aspartame	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Citric Acid	16	16	16	16	16	16	16	16	16	16
Flavor Guava	16	16	16	16	12	16	16	16	16	16
Cross Povidone	24	24	24	24	24	24	24	24	24	24
Prolacrilin potassium	24	8	24	16	24	8	8	16	16	16
Colloidal silicondi oxide	2	2	2	2	2	2	2	2	2	2
Magnesium stearate	5	5	5	5	5	5	5	5	5	5
Total	800	800	800	800	800	800	800	800	800	800

Organoleptic Characterization of a optimized polyherbal chewable tablet

Organoleptic evaluation was conducted to assess the sensory attributes of the formulated chewable tablets, which are crucial for patient compliance and acceptance.[27] The study focused on key sensory parameters, including color, odor, taste, texture (mouthfeel), and overall acceptability. A total of 36 healthy human volunteers participated in the evaluation to ensure a reliable assessment. Each parameter was rated on a five-point hedonic scale, where a higher score represented superior sensory quality and consumer preference. The data obtained provided insight into the palatability and overall user experience of the formulation. Sensory acceptability is particularly important for orally administered dosage forms, as it directly influences patient compliance and willingness to continue treatment over time. By systematically evaluating these characteristics, the study ensured that the final formulation was not only therapeutically effective but also organoleptically pleasant, thereby enhancing its potential for successful patient use and treatment adherence.

Quantitative evaluation of active constituents in polyherbal chewable tablets

The quantification of active markers in the final polyherbal chewable tablet Glycyrrhizin, Piperine, Curcumin, and Vasicine was performed using High-Performance Liquid Chromatography (HPLC). The analysis was carried out on a Shimadzu Prominent HPLC system equipped with a Shim-Pack Solar C18 column (250 mm × 4.6 mm, 5 µm particle size) and a Photodiode Array (PDA) detector for precise detection. Data acquisition, processing, and interpretation were performed using the "Lab Solutions" software to ensure accuracy and reproducibility. This analytical method allowed for reliable quantification of each active constituent, confirming the quality, consistency, and standardization of the polyherbal chewable tablet formulation.

Assay of Glycyrrhizin in optimized polyherbal chewable tablet

In order to prepare the sample,[29] 2 g of crushed polyherbal chewable tablets were placed in a 100 mL volumetric flask along with 100 mL of HPLC-grade methanol. The flask was then sonicated for 15 min and filtered using a 0.22 µ syringe filtration unit. Next, a sample of chewable tablets was run in glacial acetic acid: methanol:0.2M ammonium acetate (1:67:33) mobile phase solution and absorbance were detected at at UV/PDA (250 nm). Glycyrrhizin content was computed.

Assay of Piperine in optimized polyherbal chewable tablet

For sample preparation,[30] 2 g of crushed polyherbal chewable tablet was placed in a 100 mL volumetric flask then followed by 50 mL of HPLC grade methanol, which was sonicated for 3 min before being heated in a boiling water bath for 25 min. Cooled and diluted with 100 mL of HPLC-grade methanol, then filtered with a 0.22 µ syringe filtration unit. The sample was run in a mobile phase solution containing 0.136 g of potassium dihydrogen orthophosphate in 900 mL of DM water, which was adjusted to pH 2.5 with orthophosphoric acid and diluted to 1000 mL with water. B: Acetonitrile absorbance was measured at UV/PDA (345 nm). The content of piperine was calculated.

Assay of Curcumin in optimized polyherbal chewable tablet

To prepare the sample,[31] 2 g of crushed polyherbal chewable tablet was dissolved in 50 mL of HPLC grade acetonitrile, sonicated for 10 min, and then added to 100 mL of HPLC grade water. Then, filter it through a 0.22 µ syringe filtration unit. The sample was run in a mobile phase solution of A: Acetonitrile B: 2% Acetic acid (40:60), and the absorbance was measured at UV/PDA (425 nm). The curcumin content was calculated.

Assay of Vasicine in optimized polyherbal chewable tablet

To prepare the samples,[32] 2 g of crushed polyherbal chewable tablet was dissolved in 100 mL of HPLC grade methanol and refluxed for 30 min. The solution was then filtered through a 0.22 µ syringe filtration unit, and 5 mL was diluted with 10 mL of HPLC grade methanol. A filtered and gas-free mixture of 92 volumes of phosphate buffer (prepared by dissolving 0.14 g of potassium dihydrogen orthophosphate in 500 mL of water and adjusting the pH to 2.8 with orthophosphoric acid), 5 volumes of acetonitrile, and 3 volumes of tetrahydrofuran (92:5:3) was used as a mobile phase, and the results were detected at UV/PDA (280 nm). The vasicine content was determined through quantitative analysis to ensure accurate formulation standardization.

Accelerated stability study of finished goods

Stability testing is an essential part of pharmaceutical product development, conducted to ensure that a drug formulation remains safe, effective, and of consistent quality throughout its intended shelf life.[33] The primary objective of these studies is to determine the impact of various environmental conditions such as temperature, humidity, and light on the physicochemical, microbiological, and functional properties of the drug over time. Accelerated stability studies are designed to predict long-term stability within a shorter period by subjecting the product to stressed conditions. Typically, these conditions are maintained at 40 ± 2°C temperature and 75 ± 5% relative humidity. The formulation is evaluated at predefined intervals, generally at 0, 3, and 6 months, to monitor parameters such as appearance, hardness, disintegration time, drug content, and other quality attributes.

RESULTS

Pre- and post-compression studies of trial batches were conducted using a 3^2 factorial design, with concentrations of polacrilin potassium (A-mg) and mannitol (B-mg) serving as the primary formulation parameters for experiment design. Figure 1 depicts the experimental trials, which included independent variables and considered responses for the developed polyherbal chewable tablet. Table 2 displays the pre-compression trial results. Granules from formulation batches F1 to F10 had an angle of repose ranging from 31° to 37° and a compressibility index of 11.67% to 16.67%. All formulations demonstrated good to fair properties. The Hausner's ratio was found to be between 1.13 and 1.20, indicating that the flow properties of all formulations were good to fair. All batches experienced a drying loss ranging from 1.92 to 2.20% because compression requires an optimal amount of moisture. Post-compression parameters are quality control parameters for compressed tablets. Physical characterization of the formulation trials F1-F10 revealed that all physical parameters were ssufficiently good. Different physical parameters of prepared tablets are shown in Table 3. The tablets hardness was found to be between 3.5 and 12 kg/cm2. The average weight of the prepared tablets was 800±2%. The tablets' friability was found to be 0.33-0.81%, and the disintegration time ranged from 2 to 15 min. Among all formulations, F5 performed well, with a disintegration time of 3.5 min, a hardness of 6 kg/cm^2, and a pleasant taste.

Surface Responses of DoE

Statistical parameters were represented graphically. To assess the impact of each parameter on responses, response surface plots and contour plots were generated. The response surface was shown in Figure 2a and 2b, indicating that as the concentration of polacrilin potassium increased, the disintegration time decreased and as the concentration of mannitol increased, the disintegration time increased. Figure 2c and 2d show that as the concentration of mannitol increased, so did the hardness, and the concentration of polacrilin potassium had little effect on the hardness. Thus, it can be concluded that the variables polacrilin potassium and mannitol have a significant impact on responses such as disintegration and hardness. Figure 3 depicts the design space (overlay plot), with yellow indicating the successful working area and grey indicating the unsatisfied region. According to Design Space, formulation trial F5 falls under the category of successful working area. As a result, formulation trial F5 (Polacrilin Potassium-24 mg/tablet and Mannitol-420 mg/tablet) was chosen as the optimal formulation. The optimized formulations (F5) of polyherbal chewable tablets with the predicted levels of independent variables were generated to ensure the validity of the optimization procedure. The predicted and experimental values for the optimized formulation are nearly same, indicating statistical equivalence between the two. This also display the validity of the formulation variables chosen.

Optimized Concentration of the Formulation

The formulation trials were conducted based on the optimal solution suggested by the Design of Experiments (DOE) software. Experimental results obtained from these trials closely aligned with the predicted responses, confirming the reliability and accuracy of the optimization model. Among the various trial batches, F5 emerged as the most promising, exhibiting a balanced combination of desirable characteristics, including appropriate hardness, rapid disintegration, acceptable friability, and favorable organoleptic properties. Given its superior performance in both pre- and post-compression evaluations, F5 was selected as the optimized formulation for the polyherbal chewable tablet. This batch was subsequently subjected to detailed characterization, including assessment of mechanical strength, disintegration, weight uniformity, moisture content, and sensory attributes, to confirm its suitability for further development. The close correlation between predicted and observed outcomes underscores the effectiveness of the DOE-guided approach in streamlining formulation development, minimizing experimental iterations, and ensuring reproducibility and quality in herbal tablet manufacturing.

Characterization of final polyherbal chewable tablet

The pre-compression parameters of formulation F5 were evaluated to assess its flow properties and powder characteristics. The angle of repose was found to be 32°, indicating fair flowability. The bulk density and tapped density were recorded as 0.48 g/cm^3 and 0.55 g/cm^3, respectively. The calculated Hausner's ratio was 1.14, suggesting good flow properties, while the compressibility

Table 2: Pre-Compression evaluation parameters results as per 3^2 Factorial Design Trials.

Pre-Compression parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Bulk Density (g/cm^3)	0.45	0.52	0.48	0.46	0.48	0.52	0.45	0.53	0.45	0.45
Tapped density (g/cm^3)	0.54	0.59	0.55	0.54	0.55	0.59	0.53	0.60	0.53	0.53
Angle of Repose (°)	37	32	32	33	32	32	37	31	37	37
Compressibility(%)	16.67	11.86	12.73	14.81	12.73	11.86	15.09	11.67	15.09	15.09
Hausner's ratio	1.20	1.13	1.14	1.17	1.14	1.13	1.17	1.13	1.18	1.18
Loss on drying (%)	1.93	1.99	1.92	1.99	2.10	1.94	2.20	2.10	1.90	2.10

Table 3: Post Compression evaluation parameters results as per 3^2 Factorial Design Trials.

Post Compression parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Disintegration time (min.)	2.00	15.00	7.34	6.00	3.50	11	10.22	12	10	11
Hardness (kg/cm^2)	3.50	12	11	5	6	9	8	8	7	12
Friability (%)	0.4	0.81	0.46	0.46	0.36	0.81	0.4	0.81	0.42	0.33
Palatability	Slight Bitter	Slight Bitter	Slight Bitter	Slight Bitter	Good	Slight Bitter	Good	Good	Good	Slight Bitter
Hardness	6.4333	6.4333	6.00	1.2237	0.6319	4.939	7.9276	0.4289	13.295	6.4333

Std	Run	Factor 1 A:Polacrilin Pota... mg	Factor 2 B:Mannitol mg	Response 1 Disintegration ti... min.	Response 2 Hardness kg/cm2
3	1	24	390	1	3
7	2	8	450	14	12
9	3	24	450	6	11
2	4	16	390	5.3	4.5
6	5	24	420	3	6
1	6	8	390	10	8.5
4	7	8	420	12	8
10	8	16	420	10	7.5
5	9	16	420	11	7
8	10	16	450	9.3	11

Figure 1: Obtained Formulation Design Ratio by Design Expert Software.

index was 32%, indicating a relatively poor compressibility. The loss on drying was observed to be 2.10%, which falls within acceptable limits, suggesting minimal moisture content in the formulation. The optimized formulation exhibited satisfactory post-compression characteristics suitable for oral administration. The tablets had a light yellowish-brown color with a biconvex round shape and a characteristic odour. The texture was smooth, and the formulation was found to be palatable. Weight variation was within the acceptable limit of ±3%, indicating consistency in tablet mass. The hardness was measured at 6.0 kg/cm^2, suggesting good mechanical strength, while friability was low at 0.32%, confirming minimal loss due to handling. The disintegration time was found to be 3.5 min, indicating rapid breakdown of the tablet in the digestive tract. Moisture content was 3.2%, within the permissible range. The microbial analysis of the formulation revealed a total aerobic count of 8 cfu/g and a total fungal count of 5 cfu/g, both well within the pharmacopeial acceptable limits, indicating good microbiological quality. Furthermore, pathogenic microorganisms, including *Escherichia coli,* *Salmonella* spp., and *Shigella*, were completely absent in the tested samples. The absence of these harmful pathogens confirms that the formulation is microbiologically safe for human use. These results demonstrate that the manufacturing process was carried out under controlled and hygienic conditions, ensuring product safety, compliance with regulatory standards, and suitability for consumption without posing any microbiological health risks.

Organoleptic evalvation of polyherbal chewable tablet

Figure 4 presents a bar chart illustrating the organoleptic evaluation of the optimized polyherbal chewable tablet, conducted with feedback from 36 healthy volunteers. The formulation was assessed across five sensory attributes: Color (mean score 4.5/5), Odor (4.5/5), Taste (4.9/5), Texture (3.9/5), and Overall Acceptability (4.5/5). The results indicate that the chewable tablet was well-accepted by participants, with particularly high scores for taste, color, and odor. Texture received a slightly lower score but still remained within the acceptable range, confirming that the

optimized formulation successfully achieved desirable sensory characteristics suitable for patient compliance and commercial viability.

Quantification of Glycyrrhizin in polyherbal chewable tablet by HPLC

The glycyrrhizin content in the optimized polyherbal chewable tablets was determined to be 1.03% w/w, with a Retention Time (RT) of 10.118 min as illustrated in Figure 5. This quantification confirms the presence of the active phytoconstituent at a consistent and measurable level, ensuring batch-to-batch uniformity.

Quantification of Piperine in polyherbal chewable tablet by HPLC

The Piperine content in the optimized polyherbal chewable tablets was determined to be 0.44% w/w , with a Retention Time (RT) of 25.768 min , as illustrated in Figure 6. This quantification confirms the presence of the active phytoconstituent at a consistent and measurable level, ensuring batch-to-batch uniformity.

Quantification of Curcumin in polyherbal chewable tablet by HPLC

The Curcumin content in the optimized polyherbal chewable tablets was determined to be 1.37% w/w , with a Retention Time (RT) of 38.562 min , as illustrated in Figure 7. This quantification confirms the presence of the active phytoconstituent at a consistent and measurable level, ensuring batch-to-batch uniformity.

Quantification of Vasicine in polyherbal chewable tablet by HPLC

The Vasicine content in the optimized polyherbal chewable tablets was determined to be 0.086% w/w , with a Retention Time (RT) of 5.948 min , as illustrated in Figure 8.

Quantification of active phytoconstituents in the final polyherbal chewable tablet showed the consistent quality of the finished good and through this, we can maintain the efficacy of the product.

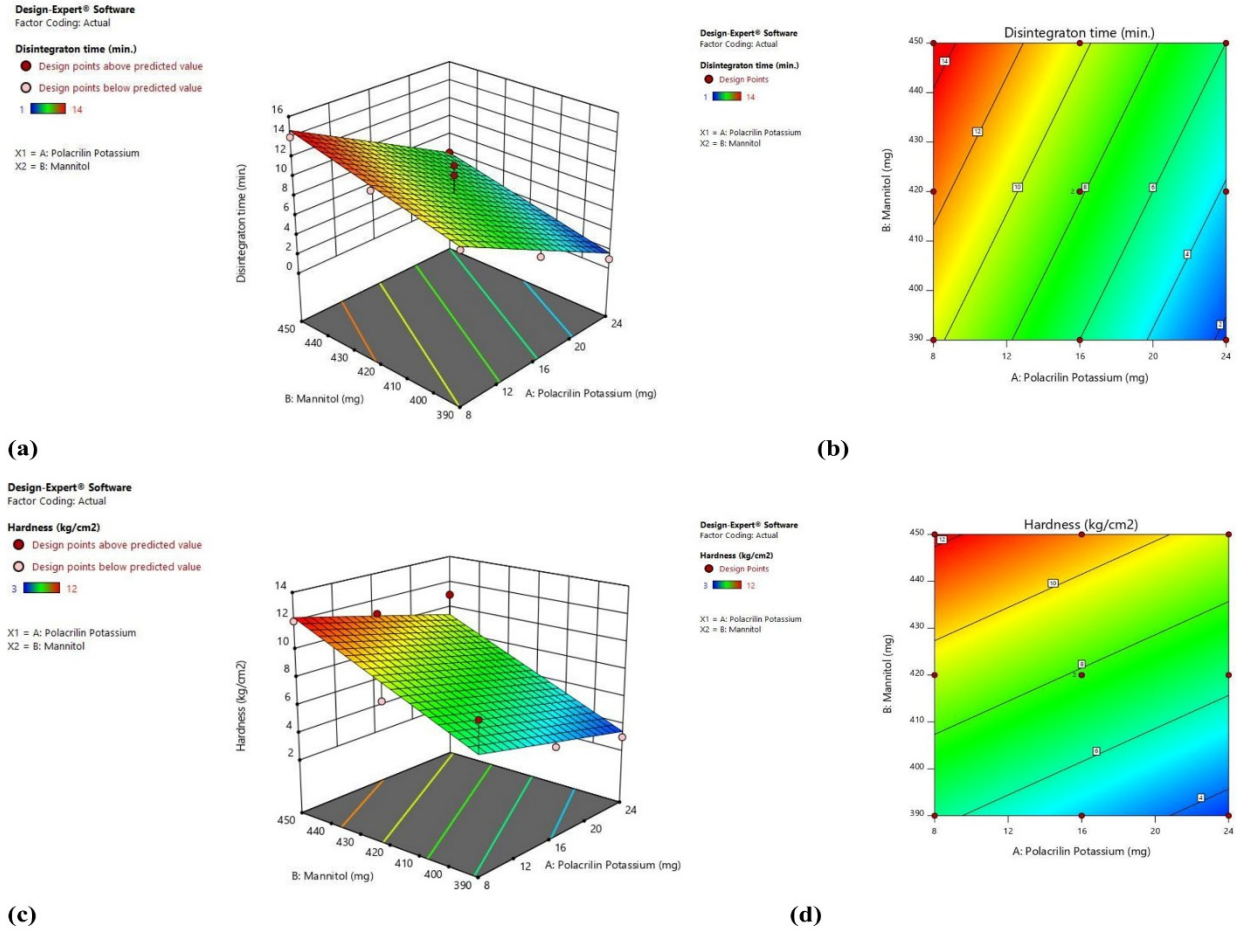

Figure 2: The effect of the factors on the responses (a) Effect on Disintegration Time- Response Surface Plot; (b) Desirability Plot (Contour Plot) for Disintegration; (c) Effect on Hardness - Response urface Plot; (d) Desirability Plot (Contour Plot) For Hardness.

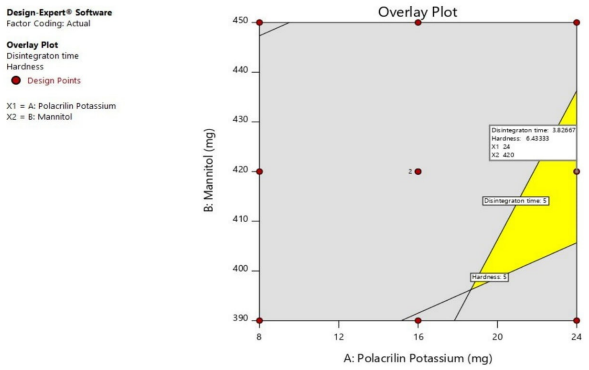

Figure 3: Overlay plot of graphical optimization indicating the design space (Yellow color region).

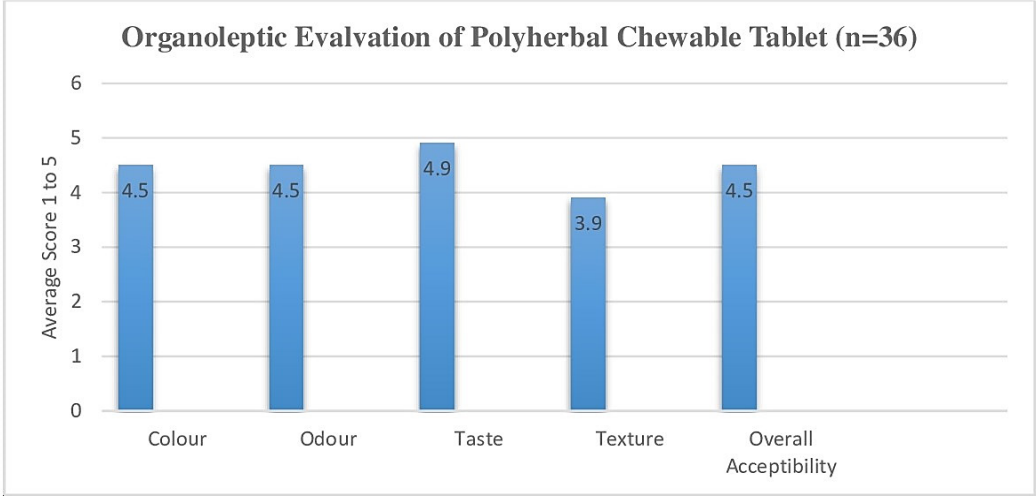

Figure 4: Results of Organoleptic Evaluation.

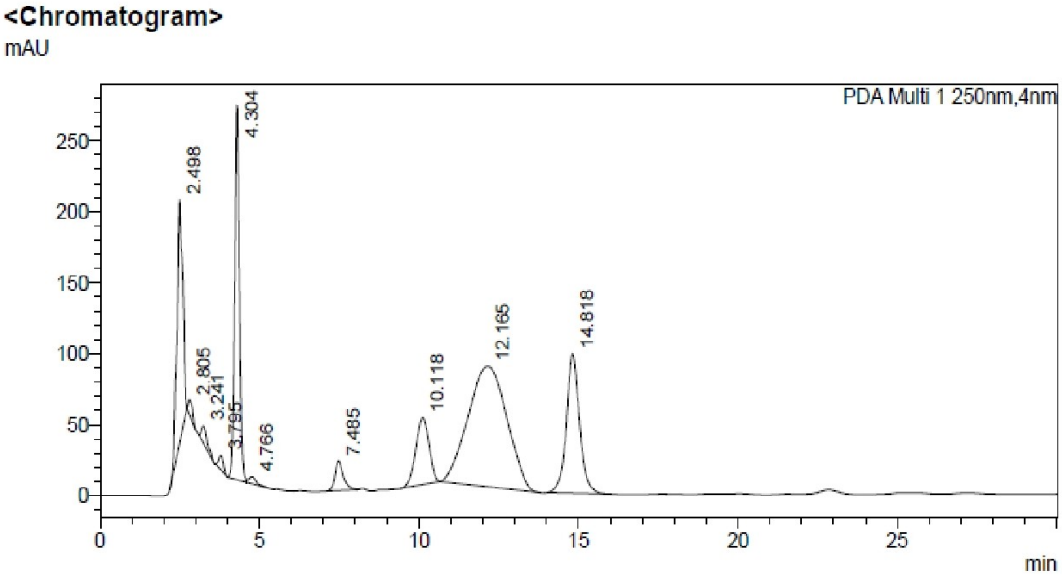

Figure 5: HPLC Chromatogram of Glycyrrhizin in Polyherbal Chewable Tablet at 250 nm.

Accelerated stability study of final optimized polyherbal formulation

A comprehensive stability study was conducted on the optimized formulation batch (F5) in accordance with the International Council for Harmonisation (ICH) guidelines for stability testing of pharmaceutical products. The primary objective of this study was to determine whether the formulation maintained its physical integrity, chemical potency, and microbiological safety over a specified storage period under accelerated conditions. The optimized polyherbal chewable tablet batch was subjected to accelerated stability conditions of 40°C ± 2°C temperature and 75% RH ± 5% relative humidity, as recommended by ICH guidelines, for a period of six months. During the study, the tablets were periodically evaluated for critical quality attributes, including appearance, weight variation, hardness, friability, disintegration time, moisture content, active constituent assay, organoleptic characteristics, and microbial contamination. The data generated during the study were compiled and tabulated (as presented in Table 4) to enable statistical comparison between initial and post-storage results. The results of the study indicated that the physical parameters of formulation F5 such as tablet hardness, friability, and weight uniformity remained largely unchanged throughout the duration of the study. This finding confirms that the optimized formulation possesses adequate mechanical strength and resilience to withstand storage stress conditions without compromising structural integrity.

There was a slight variation in disintegration time and moisture content, which are critical factors for chewable tablets. However, both parameters remained within pharmacopeial specifications and were not significant enough to affect product performance or patient acceptability. The minor fluctuation in disintegration time may be attributed to normal environmental effects during storage

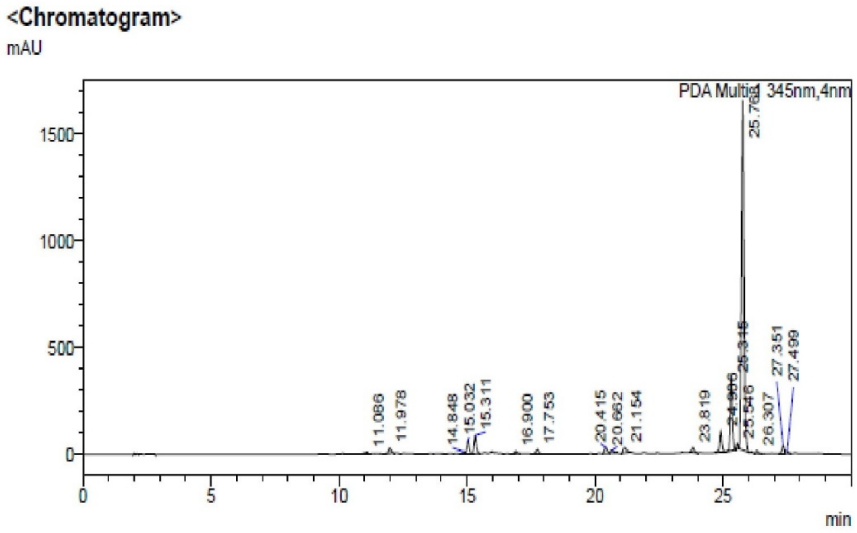

Figure 6: HPLC Chromatogram of Piperine in Polyherbal Chewable Tablet at 345 nm.

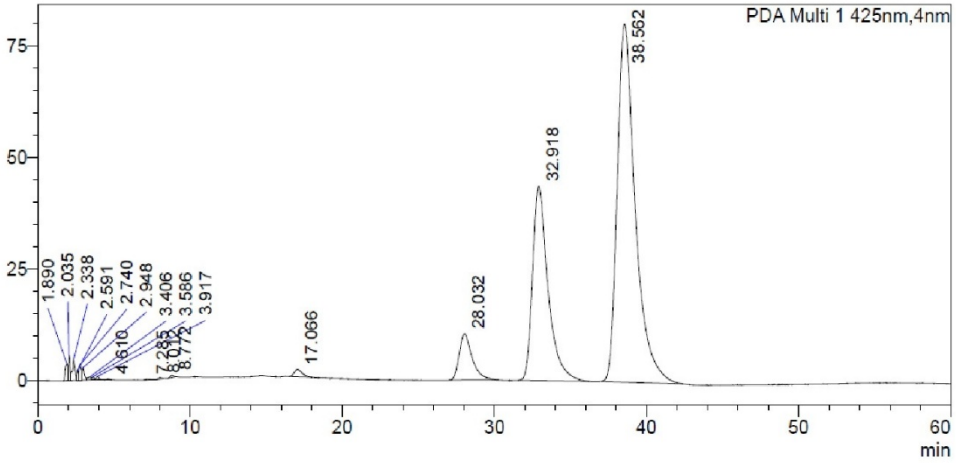

Figure 7: HPLC Chromatogram of Curcumin in Polyherbal Chewable Tablet at 425 nm.

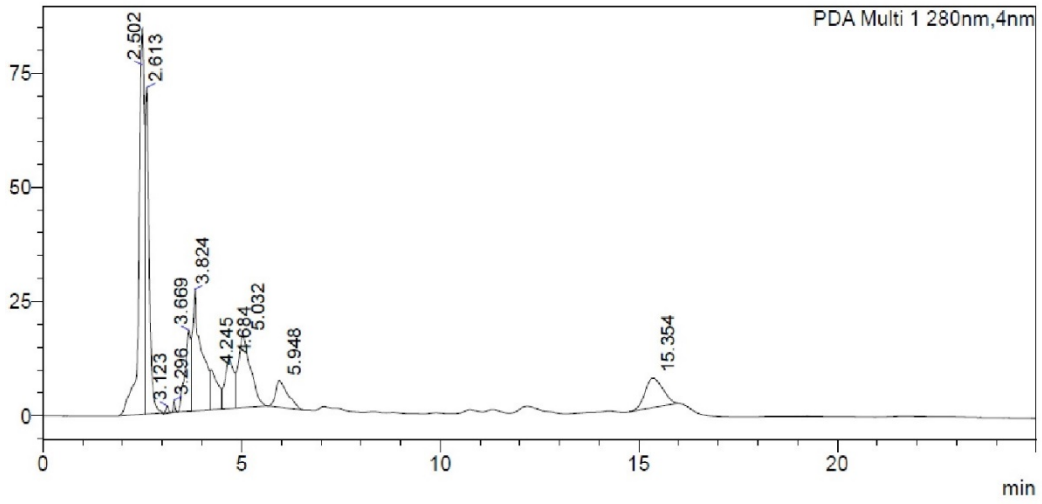

Figure 8: HPLC Chromatogram of Vasicine in Polyherbal Chewable Tablet at 280 nm.

Table 4: Results of Accelerated Stability Studies.

Storage Condition	40°C2°C and 75% 5% RH				
Pack Details	Three layers laminated (PET+MPET+POLYETHYLENE) pack				
Trial No.	F5				
Tests	Releasing Limits	Initial	1M	3M	6M
Description	Light yellowish brown color biconvex round chewable tablet	Light yellowish brown color biconvex round chewable tablet	Light yellowish brown color biconvex round chewable tablet	Light yellowish brown color biconvex round chewable tablet	Light yellowish brown color biconvex round chewable tablet
Moisture	NMT 4.0%	3.2%	3.42%	3.51%	3.56%
Disintegration time	NMT 5 min	3.5 min.	4 min.	4 min.	4 min.
Hardness	NLT 6.0 kg/cm^2	6.0 kg/cm^2	6.0 kg/cm^2	5.0 kg/cm^2	5.0 kg/cm^2
Assay of Glycyrrizin by HPLC	-	1.03%	0.991%	0.96%	0.929%
Assay of Piperine by HPLC	-	0.44%	0.45%	0.47%	0.47%
Assay of Curcumin by HPLC	-	1.37%	1.33%	1.30%	1.26%
Assay of Vasicine by HPLC	-	0.086%	0.08%	0.077%	0.076%
Total Aerobic Count	NMT 10,000 cfu/g	8 cfu/g	8 cfu/g	1°C fu/g	12 cfu/g
Total Fungal Count	NMT 1000 cfu/g	5 cfu/g	5 cfu/g	12cfu/g	1°C fu/g
Pathogens	Absent	Absent	Absent	Absent	Absent

but did not impact the tablet's ability to break down effectively in the oral cavity. A degradation rate of approximately 10% was observed in the quantification of active phytoconstituents at the end of six months. This level of degradation falls within acceptable limits and is considered normal for herbal formulations under accelerated conditions. Importantly, no qualitative change was detected in the chemical profile of the formulation, indicating that no new degradants or toxic by-products were formed during the study period. Organoleptic characteristics, which include color, odor, taste, texture, and overall appearance, were carefully monitored. No noticeable changes were observed, suggesting that the formulation remained visually and sensorially acceptable to patients. Additionally, microbial load analysis revealed no significant growth of bacteria, yeast, or molds, confirming that the formulation maintained microbiological stability throughout storage. Taken together, the findings of this accelerated stability study indicate that the optimized polyherbal chewable tablet formulation (F5) is physically stable, chemically potent, and

microbiologically safe over a minimum of six months under accelerated conditions. By applying standard shelf-life calculation principles, it can be reasonably extrapolated that the formulation will remain within acceptable limits for its entire proposed shelf life under normal storage conditions. Thus, batch F5 demonstrates robust stability and is suitable for commercial manufacturing and long-term distribution.

DISCUSSION

Tuberculosis (TB) has remained one of the world's most pressing infectious diseases for centuries, causing significant morbidity and mortality across populations. Even with the discovery of antibiotics and the development of effective multi-drug regimens, TB continues to pose a serious health burden due to the emergence of drug-resistant strains and the long duration of therapy. In this context, there has been a renewed interest in exploring traditional systems of medicine, particularly Ayurveda, which has documented evidence of numerous herbal and mineral formulations for the management of tuberculosis and its associated symptoms. Ayurvedic texts such as *Charaka Samhita*, *Sushruta Samhita*, and *Ashtanga Hridaya* describe tuberculosis under the term Rajayakshma, a wasting disease characterized by weight loss, chronic cough, weakness, fever, and progressive debility. Over centuries, Ayurvedic scholars have developed formulations that not only aim to alleviate symptoms but also restore vitality, strengthen immunity, and improve overall well-being. These formulations, ranging from single herbs like *Pippali* (Piper longum) and Vasaka (*Adhatoda vasica*) to complex polyherbal preparations like Chyawanprash, have been prescribed for both preventive and curative purposes. Clinical and preclinical studies in India have evaluated the role of Ayurvedic therapeutics as an adjunct to conventional anti-TB treatment.[34,35] Findings from these investigations suggest that several herbal drugs can significantly reduce symptom severity, mitigate drug-induced adverse effects, and enhance patient recovery. Some Ayurvedic preparations possess hepatoprotective properties,[36,37] making them particularly valuable in patients receiving prolonged anti-TB therapy, which is often associated with liver toxicity. In addition, certain plant-derived molecules act as mucoactive agents,[38-46] helping to clear airway secretions and improving respiratory function, which is a crucial aspect of symptomatic management in pulmonary TB. While antibiotics remain the cornerstone of TB treatment, their effectiveness is compromised when Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) strains emerge. This growing challenge underscores the urgent need for supportive and complementary therapies. The scientific literature increasingly highlights that Ayurvedic treatments, with their immunomodulatory, anti-inflammatory, and tissue-regenerative potential, may play a significant role in combating this problem. Unlike antibiotics, which directly target the pathogen, Ayurvedic formulations often act on the host system by strengthening immunity and restoring physiological balance, thereby providing a dual line of defense. One of the key innovations in modern Ayurvedic pharmaceutical development is the design of polyherbal chewable tablets. Classical Ayurvedic formulations are traditionally prepared as decoctions, powders, or semi-solid pastes, which often present challenges in terms of palatability, stability, and patient compliance. Chewable tablets, in contrast, offer a convenient dosage form with several distinct advantages like by chewing, the drug mixes with saliva, allowing for partial absorption in the oral cavity. This helps in bypassing first-pass metabolism, leading to better bioavailability of active phytoconstituents. As the drug disperses in the oral cavity, it can help reduce bacterial load locally and provide faster relief of primary symptoms such as cough and throat irritation. Patients, especially children and elderly individuals, are more likely to adhere to a treatment regimen when the formulation is palatable and easy to consume. Thus, the choice of polyherbal chewable tablets reflects an attempt to modernize Ayurvedic dosage forms while preserving the therapeutic essence of traditional medicine. The development of a robust polyherbal chewable tablet requires careful optimization of formulation and process variables. Statistical design tools such as Design of Experiments (DoE) and Response Surface Methodology (RSM) are valuable in this context. These tools enable systematic variation of parameters and the study of their individual and interactive effects on critical quality attributes such as hardness, disintegration time, and taste profile.

In this study, statistical modeling revealed that the coefficient of determination (R^2) for disintegration time was close to 1, indicating a strong predictive relationship and confirming that a linear model was appropriate for data analysis. Analysis of Variance (ANOVA) further supported the validity of the model, with predicted R^2 values (0.8603 for disintegration time, 0.6259 for hardness) closely matching adjusted R^2 values (0.8771 and 0.8239, respectively). This demonstrated good correlation and reproducibility of the results. The overlay plot for design space helped identify the optimal formulation, with trial batch F5 selected as the optimize formulation. The experimental data closely matched the predicted values, confirming statistical equivalence and the reliability of the chosen formulation variables. Comprehensive pre and post-compression evaluations are essential for ensuring the quality of herbal tablets. Parameters such as angle of repose, bulk density, tapped density, and compressibility index provide information on powder flowability and compressibility, which directly influence tablet uniformity and production efficiency. Post-compression tests such as hardness, friability, weight variation, disintegration time, and dissolution profile ensure that the final dosage form meets pharmacopeial standards.

Organoleptic evaluation is equally important in chewable formulations. Sensory analysis conducted on human participants indicated favorable outcomes in terms of taste, color, texture,

odor, and overall acceptability. These results reflect successful selection of excipients, sweeteners, and flavoring agents aimed at enhancing palatability. Furthermore, quantification of bioactive constituents within the tablets ensures that each batch delivers consistent therapeutic potential. This step is crucial in overcoming one of the long-standing challenges in the Ayurvedic and herbal drug industry: batch-to-batch variability. By standardizing active ingredients, manufacturers can ensure therapeutic efficacy and build global confidence in herbal products. Shelf-life determination is a mandatory requirement for licensed Ayurvedic medicines. Accelerated stability studies conducted at 40°C ± 2°C / 75% RH ± 5% for several months showed no significant changes in organoleptic properties, assay values of active constituents, or microbial contamination levels. These findings confirm that the optimized polyherbal chewable tablet is physically and chemically stable under accelerated conditions, suggesting a satisfactory shelf life under normal storage.

To achieve global acceptance, herbal medicines must adhere to the highest standards of safety, efficacy, and quality. The Quality by Design (QbD) approach, recommended by regulatory authorities, provides a systematic framework for formulation development. QbD involves identifying Critical Quality Attributes (CQAs), Critical Material Attributes (CMAs), and Critical Process Parameters (CPPs) early in the development process, and using risk assessment tools to control variability. By applying QbD principles, manufacturers can consistently produce high-quality herbal products that meet pharmacopeial requirements and patient expectations.

CONCLUSION

This investigation provides a comprehensive framework for the development and optimization of a scientifically validated polyherbal chewable tablet designed as an adjunct therapy for pulmonary Tuberculosis (TB). By systematically integrating Quality by Design (QbD) principles with traditional Ayurvedic knowledge, the study successfully produced a reproducible, pharmacopeially compliant, and patient-friendly formulation that meets modern quality expectations. The outcomes highlight how a rational, data-driven methodology can significantly reduce development timelines and enhance both product consistency and therapeutic potential. The application of statistical design of experiments enabled the precise identification and control of critical formulation variables. Optimization of disintegration time, hardness, and friability was achieved with high predictive accuracy ($R^2 > 0.8$), confirming the robustness of the chosen model and its suitability for scale-up in industrial settings. This marks a departure from conventional empirical, trial-and-error approaches to herbal formulation development, offering a pathway that is more efficient, reproducible, and scientifically defensible. One of the most significant contributions of this work is the analytical standardization of the polyherbal formulation. High-Performance Liquid Chromatography (HPLC) profiling was employed to quantify bioactive constituents, providing a reproducible chemical fingerprint for quality assurance and facilitating the monitoring of batch-to-batch consistency. This is critical for overcoming one of the key challenges facing the herbal drug industry variability in active constituents that can lead to inconsistent therapeutic outcomes. Aligning with ICH and AYUSH guidelines, this standardization step enhances the credibility and regulatory acceptability of the formulation in domestic and international markets. The choice of a chewable tablet dosage form was strategically guided by clinical and patient compliance considerations. Chewing facilitates buccal absorption, bypassing first-pass metabolism and enhancing bioavailability, while simultaneously activating salivary lysozymes, which possess natural antimicrobial activity. This may help reduce mycobacterial load in the oral cavity and throat, thereby supporting local symptom relief in TB patients. The selected plant extracts also exhibit mucoactive, hepatoprotective, and immunomodulatory properties, which can help clear dense mucus from infected lungs, mitigate drug-induced hepatotoxicity, and strengthen immune function thereby complementing conventional anti-tubercular therapy. Accelerated stability studies conducted under ICH Q1A (R2) conditions further confirmed the robustness of the formulation. Over six months, the chewable tablet retained its organoleptic characteristics, exhibited minimal changes in disintegration time and moisture content (both within pharmacopeial limits), and showed less than 10% degradation in active phytoconstituents. Microbial load remained compliant with pharmacopeial requirements, confirming the microbiological safety of the product. These results collectively indicate an acceptable shelf life and commercial feasibility of the formulation. From a broader perspective, this research emphasizes the untapped potential of QbD in the herbal pharmaceutical industry. Although QbD principles are widely applied in synthetic drug development, their use in Ayurvedic and herbal formulations has been limited, often confined to academic research. The methodology presented here demonstrates that QbD can ensure consistent quality, reproducibility, and scalability of herbal dosage forms crucial steps toward building trust among prescribers and patients and meeting global regulatory expectations. However, pharmacological activity of a finished formulation is a critical parameter for confirming its therapeutic efficacy. To evaluate this, the present study has been extended to investigate the in vitro antibacterial activity of the optimized polyherbal chewable tablet against Mycobacterium smegmatis, Mycobacterium tuberculosis, and multidrug-resistant M. tuberculosis strains. These assessments were conducted using the Agar well diffusion method in conjunction with the MGIT BACTEC system to ensure accurate and reproducible results. The detailed findings of these pharmacological evaluations, which will provide insight into the tablet's potential as an adjunct anti-tuberculosis therapy, will be reported in a forthcoming publication.

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ABBREVIATIONS

MDR: Multidrug-resistant; **XDR:** Extensively drug-resistant; **TB:** Tuberculosis; **QbD:** Quality by Design; **HPLC:** High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **IR:** Immediately Release; **DCM:** Dicholoromethene; **DoE:** Design of experiments; **mM:** Millimeter; **DM:** Demineralize; **ANOVA:** Analysis of Variance; **mL:** Milliliter; **µ:** Micron; **nm:** Nanometre; **mg:** Milligram; **RT:** Retention Time; **ICH:** International Council for Harmonization; **RH:** Relative Humidity; **°C:** Degree Centigrade; **W/W:** Weight by Weight; **CFU:** Cell Forming Unit; **gm:** Gram; **Kg:** Kilogram; **cm:** Centimeter; **%:** Percentage.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research was supported by Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.,) Vadodara, Gujarat.

ETHICAL APPROVAL

This study did not require ethical approval as it involved only formulation and optimization and quantification of polyherbal chewable tablet.

SUMMARY

The objective of the present study is to design, development and quantification of anti-tubercular polyherbal chewable tablets by using a 3^2 factorial design and standardization by HPLC. Four standardized herbal extracts *Glycyrrhiza glabra, Piper longum, Curcuma longa*, and *Adhatoda vasica* were included in the polyherbal formulation based on their antitubercular potential. A 3^2 factorial design was employed to investigate the influence of two independent variables, Mannitol (bulk forming and binder) and Prolacrilin potassium (disintegrant), on critical quality attributes such as hardness, friability, and disintegration time. Design Expert software was utilized to optimize the formulation based on pre-compression and post-compression parameters. The optimized formulation (F5) exhibited desirable characteristics in terms of taste and performance. HPLC analysis of optimized formulation quantified the active constituents in the final tablet. Stability studies demonstrated the robustness of the formulation. The study concludes that this approach, utilizing Design Expert software, can effectively optimize polyherbal tablet formulations, leading to improved product quality, reduced development time, and enhanced batch-to-batch consistency.

REFERENCES

1. Martolia J, Soni H, Tandel F. Natural perspective for management of drug-resistant tuberculosis: a review. Res J Pharm Technol. 2020;13(8):4002-8. doi: 10.5958/0974-360X.2020.00708.8.
2. Zhang L, Yan B, Gong X, Yu LX, Qu H. Application of quality by design to the process development of botanical drug products: a case study. AAPS PharmSciTech. 2013;14(1):277-86. doi: 10.1208/s12249-012-9919-8, PMID 23297167.
3. Food and Drug Administration. CDER, guidance for industry Q8 pharmaceutical development. Rockville; 2006.
4. Sangshetti JN, Deshpande M, Zaheer Z, Shinde DB, Arote R. Quality by design approach: regulatory need. Arab J Chem. 2017; 10 Suppl 2:S3412-25. doi: 10.1016/j.arabjc.2014.01.025.
5. Ayurvedic formulary of India (AFI). Part-I, Part II and Part-III. Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy.
6. Chidambaram S, Swaminathan R. *In vitro* antimycobacterial activity of selected Indian medicinal plants to resistant strains of *Mycobacterium tuberculosis*. Int J Pharm Sci Res. 2016;7(10):4130-33.
7. Nair SS, Pharande RR, Bannalikar AS. *In vitro* antimycobacterial activity of G. glabra. J Pharm Pharmacogn Res. 2015;3(4).
8. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, *et al.* Antimicrobial potential of *Glycyrrhiza glabra* roots. J Ethnopharmacol. 2008;116(2):377-80. doi: 10.1016/j.jep.2007.11.037, PMID 18182260.
9. Gaur R, Thakur JP, Yadav DK, Kapkoti DS, Verma RK, Gupta N *et al.* Synthesis, antitubercular activity, and molecular modeling studies of analogues of isoliquiritigenin and liquiritigenin, bioactive components from *Glycyrrhiza glabra*. Med Chem Res. 2015;24(9):3494-503. doi: 10.1007/s00044-015-1401-1.
10. Kaur R, Kaur H. Anti-tubercular Activity and Phytochemical Screening of Selected Medicinal Plants. Orient J Chem. 2015;31(1):597-600. doi: 10.13005/ojc/310176.
11. Grover IS, Rai J, Kajal NC, Bhushan B. Effect of liquorice [*Glycyrrhiza glabra* Linn.] as an adjuvant in newly diagnosed sputum smear-positive patients of pulmonary tuberculosis on directly observed treatment short course (dots) therapy. Chest. October 2006;130(4):95S. doi: 10.1378/chest.130.4_MeetingAbstracts.95S-c.
12. Singh C, Singh KS, Nath G, Rai PN. Anti-mycobacterial activity of *Piper longum* L. fruit extracts against multi drug resistant Mycobacterium spp. Int J Phytomed. 2011:353-61.
13. Barua CC, Singh SS, A, Barua AG. *In vitro* Antioxidant and antimycobacterial Activity of Seeds of *Piper longum* Linn: A comparative Study. SAJ Pharm Pharmacol. 2014;262:2375.
14. Deepthi PR, Janise V, Shibin P, Senthila S, Rajesh RS. Isolation, identification and antimycobacterial evaluation of piperine from *Piper longum*. Pharm Lett. 2012;4(3):863-68.
15. Sharma S. Piperine as an inhibitor of Rv1258c of *Mycobacterium tuberculosis*'. J Antimicrob Chemother. 2010;65:1694-701.
16. Prasad R, Singh A, Gupta N, Tarke C. Role of Bioemhncers in tuberculosis. Int J Health Sci Res. June 2016; 309; 6(6).
17. Vora A, Patel S, Patel K. Role of Risorine in the treatment of drug - susceptible pulmonary tuberculosis: a pilot study. J Assoc Physicians India. 2016;64(11):20-4. PMID 27805329.
18. Cikrikci S, Mozioglu E, Yilmaz H. Biological Activity of curcuminoids isolated from *Curcuma longa*. Rec Nat Prod. 2008;2(1):19-24.
19. Bai X, Oberley-Deegan RE, Bai A, Ovrutsky AR, Kinney WH, Weaver M, *et al.* Curcumin enhances human macrophage control of Mycobacterium tuberculosis infection. Respirology. 2016;21(5):951-7. doi: 10.1111/resp.12762, PMID 27012592.
20. Kumari B, Kumar T, Kaur V. Hepatoprotective effect of ethanolic extract of *Curcuma longa* Linn. on antitubercular drugs induced hepatotoxicity in albino rats. Int Res J Pharm. 2018;9(10):106-10. doi: 10.7897/2230-8407.0910235.
21. Jethva K, Bhatt D, Zaveri M. Phytochemical evaluation and anti- tuberculosis activity of selected Nine ethnomedicinal plants. Int J Herb Med. 2017;5(1):25-30.
22. Balachandran C. Antimycobacterial and cytotoxic (A549) Properties of Vasicinone isolated from *Adhatoda vasica* (L.) Nees. Asian Pac J Trop Biomed. 2012;1:1-4.
23. Ignacimuthu S, Shanmugam N. Antimycobacterial activity of two natural alkaloids, vasicine acetate and 2-acetyl benzylamine, isolated from Indian shrub *Adhatoda vasica* Ness. leaves. J Biosci. December 2010;35(4):565-70. doi: 10.1007/s12038-010-0065-8, PMID 21289439.
24. Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM, *et al.* Anti- tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates. Indian J Med Res. 2010;131:809-13. PMID 20571171.
25. Jaya M, Hardik S, Falguni T. *In vitro* Anti-tubercular and physicochemical standardization of selected medicinal plant extracts. Indian J Pharm Sci. 2021;83(2):221-8.
26. Konapure AS, Chaudhari PS, Pswal RJ, Kshirsagar SS, Antre RV, Chorage TV. Mouth dissolving tablets - an innovative technology. Int J App Biol Pharm Tech. 2011;2(1).

27. Quality attribute considerations for chewable tablets guidance for industry, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Quality: Pharmaceutical Press/CMC; 2018.
28. World Health Organization. Quality control methods for medicinal plant materials, Geneva. 1998.
29. Indian pharmacopoeia (IP).Vol. 3 India. Ghaziabad: The Indian Pharmacopoeia Commission; 2014; 3:3283.
30. Indian pharmacopoeia (IP).Vol. 3 India. Ghaziabad: The Indian Pharmacopoeia Commission; 2014; 3:3255.
31. Wichitnithad WJ, JONGAROONNGAMSANG N, Pummangura S, Rojsitthisak P. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric Extracts. Phytochem Anal. 2009;20(4):314-9. doi: 10.1002/pca.1129, PMID 19402187.
32. The ayurvedic pharmacopoeia of India. Part j. 2011;8:164.
33. The ayurvedic pharmacopoeia of India. Part j. 1st ed. 2016;9:136.
34. Ayurvedic formulary of India (AFI). Part-I, Part II and Part-III. Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy.
35. Ayurvedic formulary of India (AFI). Part-I, Part II and Part-III. Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy.
36. Vyas P, Chandola HM, Ghanchi F, Ranthem S. Clinical evaluation of Rasayana compound as an adjuvant in the management of tuberculosis with anti-Koch's treatment. Ayu. 2012;33(1):38-43. doi: 10.4103/0974-8520.100307, PMID 23049182.
37. Dornala SN, Dornala SS. Clinical efficacy of Bhringarajasava as Naimittika Rasayana in Rajayakshma with special reference to pulmonary tuberculosis. Ayu. 2012;33(4):523-9. doi: 10.4103/0974-8520.110536, PMID 23723670.
38. Debnath PK, Chattopadhyay J, Mitra A, Adhikari A, Alam MS, Bandopadhyay SK, *et al.* Adjunct therapy of Ayurvedic medicine with anti-tubercular drugs on the therapeutic management of pulmonary tuberculosis. J Ayurveda Integr Med. 2012;3(3):141-9. doi: 10.4103/0975-9476.100180, PMID 23125511.
39. Kokate CK, Gokhale AS, Gokhale SB. Cultivation of medicinal plant. 5th ed; 2006; 11-79.
40. Nadkarni AK. Materia medica. 3rd ed. Vol. I., Popular Prakashan Pvt. Ltd., Bombay; 1976; 561-611.
41. Gomaa AA, Abdel-Wadood YA. The potential of glycyrrhizin and licorice extract in combating COVID-19 and associated conditions. Phytomed Plus. 2021;1(3):100043. doi: 10.1016/j.phyplu.2021.100043, PMID 35399823.
42. Tiwari A, Mahadik KR, Gabhe SY. Piperine: A comprehensive review of methods of isolation, purification, and biological properties. Med Drug Discov. 2020;7:100027. doi: 10.1016/j.medidd.2020.100027.
43. Dada Khalandar S, Naga Adithya T, Jilani Basha S, *et al.* A current review on *Curcuma longa* Linn. plant, IJPCBS. 2018;8(1):68-73, ISSN: 2249-9504.
44. Dhankhar S, Kaur R, Ruhil S, *et al.* A review on *Adhatoda vasica* Nees-An important and high demanded medicinal plant. Indo Am J Pharm Res. 2013; ISSN No. 2231-6876.
45. Thimmulappa RK, Mudnakudu-Nagaraju KK, Shivamallu C, Subramaniam KJ, Radhakrishnan A, Bhojraj S *et al.* Antiviral and immunomodulatory activity of curcumin: A case for prophylactic therapy for COVID-19. Heliyon. 2021;7(2):e06350. doi: 10.1016/j.heliyon.2021.e06350, PMID 33655086.
46. Khanal H, Khanal U, Koirala J. Medicinal plant vasaka could be a therapeutic option for the management of COVID-19 symptoms. J Med Plants Stud. 2020;8(5):44-8.
47. Ghosh R, Chakraborty A, Biswas A, Chowdhuri S. Identification of alkaloids from *Justicia Adhatoda* as potent SARS CoV-2 main protease inhibitors: An *in silico* perspective. J Mol Struct. 2021; 1229:129489. doi: 10.1016/j.molstruc.2020.129489, PMID 33100380.

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