FORMULATION AND EVALUATION OF A POLYHERBAL IMMUNITY BOOSTER TABLET

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ABSTRACT

The objective of the current study was to standardize and create a polyherbal pill that would increase immunity by containing Withania somnifera, Tinospora cordifolia, Curcuma longa, Zingiber officinale, and Phyllanthus emblica L. The Drugs were found to have acceptable quality characteristics, including foreign matter ranging from 0.36 to 0.55%, drying loss ranging from 5.46 to 7.12%, and total ash values less than 6.24%. The polyherbal mixture was identified by quantitative phytochemical analysis as having 88.5 ± 2.4 mg GAE/g of total phenolics and 55.6 ± 1.8 mg QE/g of total flavonoids, along with half-maximal inhibitory concentrations (IC50) of 15.2 ± 0.5 µg/mL that demonstrated 95.4 ± 1.3% of free radical scavenging activity as determined by the DPPH assay. The lack of notable drug-excipient interactions was confirmed by FTIR and DSC analyses. Out of the five wet granulation formulations (F1-F5), F3 was chosen as an optimized batch due to its favorable pre-compression and post-compression properties, such as drug content (98.8±1.1%), hardness (6.3±0.2 kg/cm2), friability (0.54±0.02%), and disintegration time (11.4±0.4 min). The release kinetics of the most optimal formulation, which released 96.4±1.6% drug in cumulative drug release data after 45 minutes, were fitted by the Korsmeyer-Peppas model (R2=0.993). According to the accelerated stability studies, there were no significant changes in the drug content (98.8% to 97.3%) and dissolution (96.4% to 95.1%) under ICH Q1A (R2) conditions (40 ± 2°C/75 ±5% RH), and all spoilage-related quality standards remained well within the acceptable limits specified by pharmacopoeia. Our study's findings show that the optimized polyherbal tablet is pharmaceutically stable and that it may be a viable alternative for a novel oral herbal formulation with immunomodulatory and antioxidant properties.

Keywords: Polyherbal tablet; Immunity booster; Withania somnifera; Tinospora cordifolia; Curcuma longa; Antioxidant activity; Wet granulation; Drug release kinetics; Stability study.

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INTRODUCTION

The whole body is protected from pathogenic microbes by the immune system, an intricate network of cells, tissues, and signaling chemicals. Toxins and aberrant cellular alterations. Timely regulation of immune response is crucial for homeostatic immunity and to restrain the disease processes driving infectious diseases and chronic conditions [1]. Nonetheless, several factors like aging, malnutrition, psychological factors, environmental pollutants, sedentary lifestyle, and metabolic disorders impairing adaptation lead to immune function defects, thus increasing vulnerability to recurrent infections and inflammatory diseases. Globally, the outbreaks of viral infections, as well as some viral recurrences, have created an urgent need for preventive healthcare that could augment host immunity [2].

Natural products have provided a wellspring of therapeutic agents through their diverse phytochemical composition and a better safety profile across medical

archetypes. According to WHO estimates, up to 80% of people use traditional herbal remedies as a primary and essential form of therapy, highlighting the ongoing significance of herbal medicines in international healthcare systems [3]. Combination products such as traditional phytomedicine from botanicals are especially appealing because they have the potential for a synergistic relationship among bioactive phytoconstituents, explaining the broader pharmacological activity, greater therapeutic efficacy, and decreased side effects compared to single-herb preparations [4].

Although medicinal plants have been shown to modulate the immune system's immunomodulatory characteristics, withanolides are largely responsible for the adaptogenic, antioxidant, anti-inflammatory, and immune-boosting effects of Withania somnifera (Ashwagandha) [5]. Molecules known as antioxidants can stop harmful bacteria from growing. Immunostimulatory, hepatoprotective, antioxidant, and antibacterial properties have been demonstrated for the plant material's bioactive substances. The Moonseed family (Menispermaceae) includes the climbing shrub Tinospora cordifolia (Giloy), which has acicular stems [6]. The bioactive substances that have been separated from T. cordifolia are categorized into several substances, including polysaccharides, alkaloids, and diterpenoid lactones. Curcuma longa, the scientific name for turmeric, is a member of the ginger family of spices. Curcuminoids, one of curcumin's many bioactive components, have strong antioxidant, anti-inflammatory, antibacterial, and immunomodulatory qualities by modifying inflammatory signaling pathways and oxidative stress [7]. Ginger's antibacterial, anti-inflammatory, and antioxidant properties are attributed to gingerols and shogaols [8]. Similar to this, Phyllanthus emblica (Amla), which is rich in vitamin C, hydrolyzable tannins, gallic acid, ellagic acid, and flavonoids, is well-known for having strong immunomodulatory and antioxidant properties [9].

There are a lot of commercially available herbal immunity boosters, but many of these products are not fully characterized for pharmaceutical standards or any assessments of quality, optimization, and stability of formulation variables. Changes in raw material quality, phytochemical content, manufacturing conditions, and the attributes of the dosaged form can seriously impact product efficacy and reproducibility [10]. As a result, it has become critically important to accomplish a standardization of herbal formulation by scientific pharmaceutical approaches to obtain reproducible, stringent quality, safety, and therapeutic efficiency [11].

Tablet dosage forms are among the most widely used oral drug delivery systems because they are more convenient, require less equipment to administer, are easier to handle, ensure an accurate dosage, increase patient compliance, are more affordable, and provide better stability than liquid herbal formulations [12]. However, choosing raw materials, optimizing excipients, testing compatibility, flow characteristics, compressibility, mechanical strength, disintegration and dissolution capabilities, and long-term stability assessment are all necessary for creating an appropriate herbal tablet [13].

These days, many profiles of pharmacognostic and physicochemical parameters such as organoleptic characteristics, foreign matter, moisture content, ash values, extractive values, powder flow qualities, etc. are used to identify and standardize each medicinal herb [14]. The quantitative assessment of total phenolic and flavonoid levels was supplemented by qualitative phytochemical screening. Finding each herb's and the polyherbal mixture's phytochemical contents was the goal. The DPPH free radical scavenging assay was used to assess the formulation's biological activity and ascertain its antioxidant capability [15].

The physicochemical interactions between the powdered herbal blend and formulation excipients were assessed using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) as drug-excipient compatibility assays [16]. The wet granulation procedure was used to create the following five formulations, which were then methodically examined for both pre-compression and post-compression parameters. The improved formulation was evaluated using drug content, dissolution studies, release kinetic modeling, scanning electron microscopy (SEM), and accelerated stability experiments in accordance with ICH Q1A(R2) standards [17].

Thus, the current study's goals were to develop, refine, and assess a polyherbal tablet with expected immunomodulatory and antioxidant properties that would be physiologically stable, approved by pharmaceutical markings, and scientifically standardized. The results will yield a quality-based approach for the formulation of evidence-driven herbal formulations with better therapeutic reproducibility and patient acceptability.

MATERIALS AND METHODS

Selection of Medicinal Herbs

Based on their documented immunomodulatory, antioxidant, anti-inflammatory, and adaptogenic properties, the chosen medicinal plant elements were examined. Numerous journal articles from various scientific journals as well as traditional Ayurvedic

literature were screened. Withania somnifera, Tinospora cordifolia, Curcuma longa, Zingiber officinale, and Phyllanthus emblica L. are the five medicinal herbs that were chosen because of their well-known synergistic effect in boosting the immune system and lowering oxidative stress. Withanolides, curcuminoids, gingerols, polyphenols, alkaloids, and other phytochemical components that contribute to these plants' diverse pharmacological properties have all been thoroughly investigated. Table 1 summarizes the botanical traits of a few chosen medicinal plants.

Table 1. Botanical details of selected medicinal plants

Herb	Botanical Name	Family	Plant Part Used	Source of Procurement	Reference
Ashwagandha	*Withania somnifera* (L.) Dunal	Solanaceae	Root	Botany Department, Government V.Y.T. PG Autonomous College, Durg	[5]
Giloy	*Tinospora cordifolia* (Willd.) Hook.f. & Thomson	Menispermaceae	Stem	Botany Department, Government V.Y.T. PG Autonomous College, Durg	[6]
Turmeric	*Curcuma longa* L.	Zingiberaceae	Rhizome	Botany Department, Government V.Y.T. PG Autonomous College, Durg	[7]
Ginger	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome	Botany Department, Government V.Y.T. PG Autonomous College, Durg	[8]
Amla	*Phyllanthus emblica L.*	Phyllanthaceae	Fruit	Botany Department, Government V.Y.T. PG Autonomous College, Durg	[9]

Authentication of Plant Materials

All medicinal plant materials were authenticated by a qualified taxonomist at the Botany Department, Government V.Y.T. PG Autonomous College, Durg, Chhattisgarh, India. Voucher specimens of all selected herbs were prepared and authenticated. Voucher specimens were deposited as VYT/BOT/2026/WS-001 (Withania somnifera), VYT/BOT/2026/TC-002 (Tinospora cordifolia), VYT/BOT/2026/CL-003 (Curcuma longa), VYT/BOT/2026/ZO-004 (Zingiber officinale), and VYT/BOT/2026/PE-005 (Phyllanthus emblica), respectively.

Extraction of Medicinal Herbs

Plant materials were cleaned using distilled water after being filtered to get rid of impurities. For ten to fourteen days, the samples were shade-dried at room temperature (25 to 30°C) until their weight remained constant. To create a consistent powder, dried plant materials were separately processed in a mechanical grinder equipped with a 60-mesh sieve device. Before extraction, ground powder was kept in airtight, dark containers. The Soxhlet extraction method was used to extract each powdered plant independently, and the hydroalcoholic method was then devised. A cellulose extraction thimble was filled with 200 g of powdered plant material. After that, the thimble was extracted using 70% ether (ether: water, 70:30 v/v) for six to eight hours, or until the siphon shade faded. To create semisolid masses, the extracts were concentrated at lower pressure, filtered through Whatman No. 1 filter paper, and dried in a vacuum oven at an equilibration temperature of 40–45 °C using a rotating vacuum evaporator. Next, each extract's yield percentage was computed. Until more phytochemical and formulation research was completed, the dried

extracts were kept in an airtight amber glass container at 4°C in the dark [18].
The extractive yield % was estimated according to the subsequent formula:

"Percentage Yield (% w/w)"="Weight of dried extract" /"Weight of powdered crude drug" ×100

Raw Drug Standardization
Each crude herbal material utilized in formulation (Withania somnifera, Tinospora cordifolia, Curcuma longa, Zingiber officinale, and Phyllanthus emblica L.) was standardized individually before formulation according to WHO guidelines for quality control of medicines and the Ayurvedic Pharmacopoeia of India [19].

Organoleptic Evaluation
Physicochemical properties of every herbal powder material were assessed visually and by sensory evaluation with the aid of physical/chemical parameters such as Colour, Odour, Taste, Texture, and Appearance. The observations were recorded to confirm the identity and quality of the starting materials [20].

Determination of Foreign Matter
Foreign organic matter (stones, soil, insects, and other plant detritus) was manually extracted and weighed for each visually inspected sample of approximately 100 g of each herbal material. According to the WHO standard, the foreign matter content was determined [20].

Loss on Drying (LOD)
Two grams of each powdered herb were weighed at 105 °C until their weight remained constant in order to calculate the moisture content [20].

Determination of Total Ash
Total ash was determined by scorching the powder in a silica crucible to obtain a char-free ash at 550–600 °C [21].

Acid Insoluble Ash
After filtering and washing the entire ash with diluted hydrochloric acid, the acid-insoluble ash fraction that remained was burned to produce acid-insoluble ash [21].

Water Soluble Ash
The difference between total ash and insoluble residual after boiling the total ash with distilled water was defined as water-soluble ash [21].

Extractive Value

Alcohol Soluble Extractive Value
The powdered drug (5g) was macerated with 95% ethanol (1:10 w/v Drug-Ethanol) for a full day. After the filtrate was evaporated, the percentage extractive value was determined [22].

Water Soluble Extractive Value
Using distilled water as the solvent, the same technique was also used to calculate the water-soluble extractive value [22].

Particle Size Analysis
Standard sieves (No. 40–100) were then used to sieve each powdered herb. Powder that crossed a 60-mesh screen was chosen for formulation to provide a well-mixed powder [20].

Phytochemical Screening of Individual Herbs
Alkaloids
Dragendorff's reagent was used to analyze alkaloids; in the absence of blue color, Mayer's reagent produced a cream yellow precipitate; and Wagner's reagent produced a reddish-brown precipitate [23].

Flavonoids
Detection Tests for flavonoids were performed by the Shinoda test and the alkaline reagent test. The pink-red colour developed after the addition of magnesium turnings and concentrated hydrochloric acid indicated the presence of flavonoids [24].

Tannins
Lead acetate and ferric chloride were used for testing after the tablet's powdered form was removed, screened, and separated. According to the ferric chloride test, which shows a blue-black or greenish-black coloration that indicates the presence of tannins, and the lead acetate test, which produces a bulky precipitate [24].

Saponins
The Foam Test and Hemolysis Test were used to evaluate the saponins in the tablet powder extract. As seen in Table 4, saponins demonstrated hemolytic activity in the hemolysis test and stable, persistent froth production in the foam test, indicating the presence of saponins in the extract [24].

Glycosides
The Keller-Kiliani test and Legal's test were used to detect glycosides in the methanolic extract of the tablet powder. The presence of glycosides in the extract was indicated by a brown ring at the interface in the Keller-Kiliani test and a pink to red color in Legal's test [24].

Terpenoids

Terpenoids were found in the tablet powder extract according to the Salkowski test. The emergence of a reddish-brown color at the mixture's interface following the addition of strong sulfuric acid was the first indication that terpenoids were present in the extract [24].

Phenolic Compounds

The Folin-Ciocalteu reagent was used to identify the phenolic components of the tablet powder extract. By combining extracts with distilled water and FeCl3 in a test tube that produced a blue color during the reaction, the total phenolic content was determined [24].

Final Polyherbal Blend Phytochemical Screening

The qualitative phytochemical analysis of the final polyherbal blend prepared by mixing standardized powders of all five medicinal plants was also performed using the same standard procedure. It was found that all the major classes of bioactive phytoconstituents contributed by the individual herbs were present in higher quantities in the study [25].

Quantitative Phytochemical and Biological Evaluation

Determination of Total Phenolic Content (TPC)

Generally, 0.5 mL of an appropriately diluted extract was incubated for five minutes with a 1:10 dilution of Folin-Ciocalteu reagent (2.5 mL). After adding 2.0 mL of sodium carbonate (7.5% solution), the mixture was allowed to stand at room temperature for half an hour in the dark. The absorbance at 765 nm was measured using a UV-visible spectrophotometer. Based on a gallic acid calibration curve, these findings were reported as milligrams of gallic acid equivalent (GAE) per gram dry extraction (mg GAE/g) [26].

Determination of Total Flavonoid Content (TFC)

The total flavonoid content was measured using a colorimetric technique (aluminum chloride). Specifically, distilled water, potassium acetate, and aluminum chloride reagent were mixed with 1 mL of the sample solution. After 30 minutes of room temperature incubation, absorbance was measured at 415 nm. Using quercetin as a standard, the results were reported as mg QE/g (mg quercetin equivalent/g of dry extract) [27].

Antioxidant Activity

The DPPH free radical scavenging assay was used to assess the antioxidant capacity of both the final polyherbal blend and the herbal extracts. For thirty minutes, the freshly made DPPH solution was combined with various extract concentrations in the dark. In particular, the absorbance at 517 nm was measured to assess radical scavenging activity per se and calculate the pertinent percentage of radical scavenging activity. Ascorbic acid served as the test's positive control. Concentration-response curves could be used to calculate $IC5_0$ values [28].

Drug–Excipient Compatibility Studies

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was used to assess the polyherbal blend's compatibility with the chosen excipients. An FTIR spectrophotometer (IRAffinity-1S, Shimadzu Corporation, Kyoto, Japan) was used to record the spectra using the potassium bromide (KBr) pellet method. Each sample was crushed into thin, translucent pellets (about 2 mg) after being mixed with spectroscopic-grade KBr in a ratio of 1:100 (sample: KBr). Spectra were recorded at 298 K in the 4000–400 cm−1 range (spectral resolution of 4 cm−1; 32 scans per sample). Drug-excipient interaction is shown by analysis of the prominent peak shift, peak disappearance, and the existence of other absorption bands [29].

Differential Scanning Calorimetry (DSC)

The thermal compliance of the polyherbal blend with the chosen excipients in the generated optimized tablet formulation was assessed using Differential Scanning Calorimetry (DSC) technique. Differential thermal analysis and thermogravimetric measurement. A DSC 60 Plus DSC (Shimadzu Corporation, Kyoto, Japan) was used to obtain thermograms. Five to eight milligrams of the meticulously weighed sample were sealed in a standard sealed aluminum pan, while the reference sample was an empty sealed aluminum pan. To reverse oxidative degradation, the samples were heated (10°C min^{-1}, 30–350°C) in a nitrogen atmosphere (50 mL min^{-1}). To evaluate any notable divergence from endothermic or exothermic transitions suggestive of physicochemical incompatibility, the data supplied for all the thermograms of various herbal blends, excipients, and ideal formulation were compared [30].

Scanning Electron Microscopy (SEM)

A scanning electron microscope (JSM-IT200, JEOL Ltd., Tokyo, Japan) was used to examine the nature and surface morphology of the optimized polyherbal tablet. Before imaging, the tablets were attached to aluminum stubs using double-sided conductive carbon tape and sputter-coated with 5 nm of gold using a vacuum sputter coater. Using high vacuum micrographs (all crude data were taken at an accelerating voltage of 15 kV at

magnifications of 100 ×, 500 ×, and 1000 ×), this method was used to evaluate the particle distribution, permeability, and strength of the crushed tablet [31].

Preparation of Polyherbal Tablet Formulations

The active ingredients of a few medicinal plants are used to create polyherbal formulations, which must operate in concert to boost immunity. Using the wet granulation method, five polyherbal immune booster tablet formulations (F1–F5) were created. While keeping the total tablet weight at 500 mg, we changed the formulation design specifications (acacia, binder; starch, disintegrant; and microcrystalline cellulose, diluent) (Table 2). To investigate the impact of excipient content on the primary powder characteristics of flow and tablet quality, all formulations included the same quantities of the herbal elements. An analytical balance was used to weigh the excipients and herbal powders. A single-punch tablet compression machine was used to compress the powders into tablets after they had been uniformly blended, granulated with prepared acacia mucilage, filtered through mesh size 16, dried in an oven at 45 ± 2 °C, and blended with talc and magnesium stearate in a 9:4 ratio [32].

Table 2. Trial polyherbal tablet formulations' composition (per 500 mg tablet)

Ingredients (mg/tablet)	**F1**	**F2**	**F3**	**F4**	**F5**
Ashwagandha	60	60	60	60	60
Giloy	60	60	60	60	60
Turmeric	60	60	60	60	60
Ginger	60	60	60	60	60
Amla	60	60	60	60	60
Microcrystalline cellulose	180	170	165	175	160
Acacia	10	15	20	25	15
Starch	50	55	60	45	65
Talc	10	10	10	10	10
Magnesium stearate	10	10	10	10	10
Total weight (mg)	500	500	500	500	500

Wet Granulation Method

The weight of standardized herbal powder was determined by the formulation's composition. Ten minutes was taken into consideration when combining the powders to obtain a homogeneous slurry. This formulation's granulating agent was acacia mucilage made with pure water. A homogeneous wet mass was created by gradually adding the binder solution while stirring. Granules were produced with a sieve. The majority of the wet mass was not put through a sieve. Granules can be dried using a tray dryer (≤ 5%, 45 ± 2°C). After that, granules were processed using sieve No. 20 to obtain a consistent granule size. After that, talc and magnesium stearate were combined with the dry granules for five minutes [32]. A single-punch compression tablet machine was then used to compact the lubricated granules into tablets.

Figure 1. Polyherbal Immunity Booster Tablet

Evaluation of Trial Formulations

Pre-compression Evaluation of Granules (F1–F5)

Bulk Density

A specific mass of granules was transferred, without tapping, to a graduated measuring cylinder (100 ml) in order to calculate the bulk density [33]. The following formula was used to determine bulk density based on the initial volume occupied by granules:

"Bulk Density"="Mass of Granules (g)" /"Bulk Volume (mL)"

Tapped Density

The graduated cylinder holding the grains was mechanically tapped until a consistent volume was reached in order to compute the tapped density [33]. The following formula was used to determine the tapped density:

"Tapped Density"="Mass of Granules (g)" /"Tapped Volume (mL)"

Carr's Index of Compressibility

The compressibility of the granules was evaluated using Carr's compressibility index, which was computed using the formula shown below from bulk and tapped density values [34].

"Carr’s Index (%)"=("Tapped Density" -"Bulk Density")/"Tapped Density" ×100

Hausner Ratio

The Hausner ratio was calculated using a simple formula to describe the granules' flowability.
"Hausner Ratio"="Tapped Density" /"Bulk Density"

Angle of Repose

The angle of repose was measured using the fixed funnel method. As the grains emerged from a funnel onto a level surface, they gathered into a conical stack. The height (h) and radius (r) of the heap are used to compute the angle of repose [34].

$\theta = [\![\tan \;]\!]^{(-1)} (h/r)$

where θ is the angle of repose.

Post-compression Evaluation of Tablets (F1–F5)

Appearance

The visual assessment of colour, shape, surface smoothness, cracks, capping, chipping, lamination, and uniformity of all compressed tablets was done [35].

Thickness

A digital Vernier caliper was used to measure the thickness of ten tablets from each formulation. The average of the four thicknesses, stated in millimeters, is known as the mean thickness [35].

Diameter

To ensure consistency, ten randomly chosen tablets from each formulation were weighed, and the average value was determined [35].

Weight Variation

Using an analytical balance, the net weight of twenty pills for each formulation. The Indian Pharmacopoeia [35] was used to calculate the tablets' mean weight and percentage variance from mean weight.

Hardness

A Monsanto hardness test (Pfizer hardness tester gadget) was used to determine the hardness of tablets. The crushing power for each formulation was calculated using thirty pills and represented as kg/cm [35].

Friability

A Roche Friabilator was used to measure the friability. Twenty already weighed tablets were rotated at a speed of 25 rotations × min−1 for 4 minutes, totaling 100 revolutions. Monolayer tablet weight loss was computed as an indicator of friability [35].

$\text{Friability (\%)} = (W_1 - W_2)/W_1 \times 100$

where:

W_1 = Initial weight

W_2 = Final weight

Disintegration Time

Using distilled water at 37±0.5 °C, the USP disintegration test apparatus was used to measure the disintegration time. Each formulation's six tablets were weighed, and the time was noted.

Selection of optimized formulation

The best formulation was chosen by comparing the pre and post-compression parameters of five trial formulations (F1–F5). Selection criteria include pharmaceutical examination (granule flow characteristics, tablet hardness, friability, weight fluctuation, and disintegration time). The formulation with the best compromise was selected for additional characterisation and in vitro performance testing in order to attain the intended balance of flowability, compressible mechanical strength, quick disintegration, and pharmacopoeial conformance [36].

Evaluation of the Optimized Formulation

Assay of marker phytoconstituent(s)

Twenty of the optimized polyherbal tablets were weighed and ground into a powder to determine the overall drug content. A UV-visible spectrophotometer was used to evaluate one tablet that had been extracted using the proper solvent, filtered, and diluted to the required amounts. The percentage of active phytoconstituents present was recorded for each test, which was conducted in triplicate [37].

In Vitro Dissolution Study

A USP Type II (Paddle) dissolution device (Electrolab TDT-08L, Electrolab India Pvt. Vibro Shaking Dissolution Tester, Pioneer Instruments Pvt. 900 mL of phosphate buffer (pH 6.8) kept at 37 ± 0.5 °C with a paddle spinning at 50 rpm made up the dissolving medium. At 5, 10, 15, 20, 30, and 45 minutes, the samples (5 mL) were taken out and replaced with an equivalent volume of brand-new dissolving medium maintained at the same temperature. Because curcumin (from Curcuma longa) has been established as a general standardized phytochemical marker for quality control of the structure and composition of most polyherbal formulations, it was chosen for analysis as a dissolution marker compound in light of the polyherbal formulation's multiple herbal ingredients. A UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) was used to measure the samples at 425 nm after they had been filtered through a 0.45 µm membrane filter and diluted to the proper quantities. Using the previously acquired calibration curve, the % release of curcumin value at each time point was determined [38].

Drug Release Kinetics

The improved formulation's in vitro dissolution data were fitted to zero-order, first-order, Higuchi, Hixson-

Crowell, and Korsmeyer-Peppas kinetic models. The model with the highest R2 value was the one that predicted the release mechanism [39].

Study of the Optimized Formulation Stability

The ICH Q1A(R2) standards for physical and chemical stability at 0, 1, 2, 3, 6, and 12 months of storage were followed in the accelerated stability testing for the optimised polyherbal tablet formulation. The pills were kept in HDPE bottles in a stability room at 40 ± 2°C and 75 ± 5% humidity for six months. Samples were taken out and examined for hardness, friability, drug content, drug release profile, and visual appearance at predetermined intervals (0, 1, 2, 3, and 6 months) [40].

RESULT

Raw Drug Standardization

Organoleptic characteristics

The organoleptic investigation showed that each of the selected medicinal herbs had unique colors, tastes, smells, and textures that matched their pharmacopoeial descriptions (Table 3). The powdered herbal components were found to be free of visible impurities and appropriate for further investigation.

Table 3. Organoleptic characteristics of individual herbs

Herb	Colour	Odour	Taste	Texture
Ashwagandha	Light brown	Characteristic	Slightly bitter	Fine powder
Giloy	Greenish brown	Characteristic	Bitter	Fine powder
Turmeric	Bright yellow	Aromatic	Bitter	Fine powder
Ginger	Light yellow	Aromatic	Pungent	Fine powder
Amla	Brownish green	Characteristic	Sour-astringent	Fine powder

Foreign matter in herbal materials

All of the herbal samples (Table 4) had a foreign matter level between 0.36 and 0.55%, which was within pharmacopoeias' allowed bounds. The lowest amount of foreign matter (0.36%) was found in turmeric, which indicates the raw material's quality.

Table 4. Herbal materials containing foreign substances

Herb	Foreign matter (%)
Ashwagandha	0.42
Giloy	0.55
Turmeric	0.36
Ginger	0.48

Loss on drying

For all therapeutic herbs, the moisture content ranged from 5.46% to 7.12% after drying (Table 5). These numbers imply that the plant materials were thoroughly dried before extraction.

Table 5. Loss on drying

Herb	LOD (%)
Ashwagandha	6.18
Giloy	7.12
Turmeric	5.46
Ginger	6.05
Amla	5.72

Ash values

The findings showed that the medicinal plants' total, acid-insoluble, and water-soluble ash levels were all within permissible bounds (Table 6). Compared to Ashwagandha and Giloy, Amla's total ash yield was much lower.

Table 6. Ash values

Herb	Total Ash (%)	Acid Insoluble Ash (%)	Water Soluble Ash (%)
Ashwagandha	5.38	0.44	2.83
Giloy	6.24	0.61	3.12
Turmeric	5.02	0.36	2.65
Ginger	4.76	0.29	2.48
Amla	3.95	0.24	2.11

Extractive values

Amla had the highest water-soluble extractive levels (39.5%) and total alcohol (28.6%) (Table 7). These findings all point to the phytoconstituents' high solubility in amla.

Table 7. Extractive values

Herb	Alcohol Soluble (%)	Water Soluble (%)
Ashwagandha	17.5	24.8
Giloy	12.4	21.6
Turmeric	14.8	18.3
Ginger	11.6	15.2
Amla	28.6	39.5

Powder flow properties of individual herbs

Table 8 lists the fundamental intrinsic characteristics of the herbal powders, including high bulk density, tapped density, Hausner ratio, Carr's index, and angle of repose. When compared to other botanical compounds, turmeric showed the most flowability.

Table 8. Powder flow properties of individual herbs

Herb	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr's Index (%)	Hausner Ratio	Angle of Repose (°)
Ashwagandha	0.42	0.54	22.2	1.29	32.8
Giloy	0.40	0.53	24.5	1.33	33.6
Turmeric	0.46	0.57	19.3	1.24	31.2
Ginger	0.43	0.55	21.8	1.28	32.5
Amla	0.41	0.54	24.1	1.32	34.1

Qualitative Phytochemical Screening

Qualitative phytochemical screening of individual herbs

Alkaloids, flavonoids, tannins, phenol glycosides, and terpenoids were among the important compounds found in various medicinal plants in varying amounts, according to phytochemical analysis (Table 9). Amla and turmeric were both discovered to contain flavonoids, phenolic compounds, and α-amino acids.

Table 9. Qualitative phytochemical screening of individual medicinal herbs

Phytochemical	Ashwagandha	Giloy	Turmeric	Ginger	Amla
Alkaloids	+++	++	+	+	+
Flavonoids	++	++	+++	++	+++
Tannins	+	++	++	+	+++
Saponins	++	+	–	+	+
Glycosides	++	++	+	+	++
Terpenoids	+++	++	+++	++	+
Phenolics	++	++	+++	++	+++

Symbols: (+++) abundant, (++) moderate, (+) present, (–) absent.

Qualitative Phytochemical Screening of the Final Polyherbal Blend

All significant classes of phytochemicals had favorable responses, according to the qualitative analysis of the polyherbal blend (Table 10). Strong (+++) responses were shown by terpenoids, phenolics, alkaloids, and flavonoids.

Table 10. Qualitative phytochemical screening of the final polyherbal blend

Phytochemical	Test Performed	Observation	Result
Alkaloids	Dragendorff's test	Orange-red precipitate	+++
Flavonoids	Shinoda test	Pink-red colour	+++
Tannins	Test for ferric chloride	Greenish-black colour	++
Saponins	Test of foam	Persistent froth	++
Glycosides	The Keller-Kiliani test	Brown ring	++
Terpenoids	Salkowski examination	Reddish-brown interface	+++
Phenolics	Teste Folin –Ciocalteu	Blue colour	+++

Quantitative Assessment of Phytochemicals

Total Phenolic Content

Amla had a much higher phenolic content (96.8 ± 2.9 mg GAE/g) than the polyherbal blend (88.5 ± 2.4 mg GAE/g), according to quantitative estimation (Table 11, Figure 2).

Table 11. Total Phenolic Content

Sample	Total Phenolic Content (mg GAE/g)
Ashwagandha	54.8 ± 1.6
Giloy	62.5 ± 2.1
Turmeric	78.4 ± 2.5
Ginger	49.6 ± 1.8
Amla	96.8 ± 2.9
Polyherbal Blend	88.5 ± 2.4

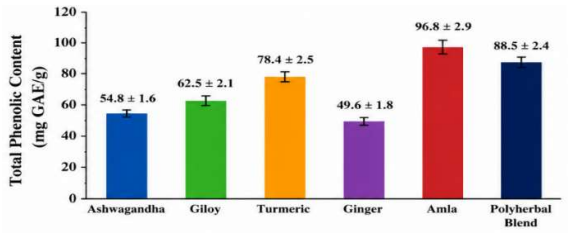

Figure 2. Bar graph of Total Phenolic Content

Total Flavonoid Content

Flavonoid Estimation (Table 12, Figure 3). The highest flavonoid levels were found in amla (58.7 ± 1.9 mgQE/g) and turmeric (61.2 ± 2.0 mgQE/g).

Table 12. Total Flavonoid Content

Sample	Total Flavonoid Content (mg QE/g)
Ashwagandha	28.6 ± 1.1
Giloy	35.8 ± 1.4
Turmeric	61.2 ± 2.0
Ginger	33.4 ± 1.3
Amla	58.7 ± 1.9
Polyherbal Blend	55.6 ± 1.8

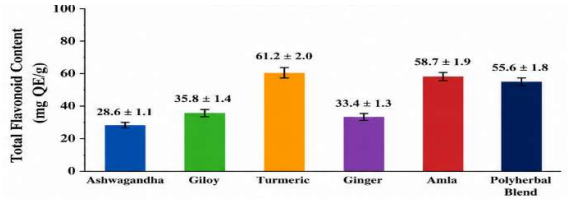

Figure 3. Bar graph of Total Flavonoid Content

Antioxidant Activity

Dose-dependent DPPH radical scavenging activity was found in all samples (Table 13 and Figure 4). The polyherbal extract had the lowest IC50 (15.2 ± 0.5 µg/mL) and the greatest inhibition (95.4 ± 1.3%) among the herbal extracts examined.

Table 13. Radical Scavenging Activity of DPPH

Sample	DPPH Scavenging (%)	IC_{50} (µg/mL)
Ashwagandha	72.8 ± 2.3	46.5 ± 1.4
Giloy	78.4 ± 2.0	39.8 ± 1.2
Turmeric	88.9 ± 1.8	24.6 ± 0.9
Ginger	74.5 ± 2.1	41.7 ± 1.3
Amla	92.3 ± 1.5	18.9 ± 0.7
Polyherbal Blend	95.4 ± 1.3	15.2 ± 0.5
Ascorbic acid	97.6 ± 0.8	11.4 ± 0.4

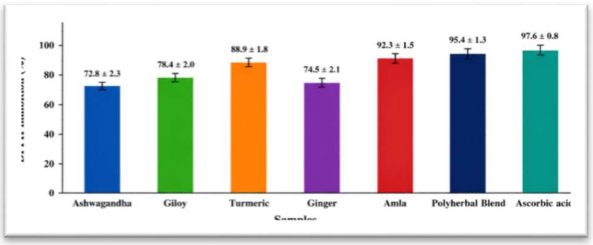

Figure 4. DPPH inhibition (%) of individual herbs, polyherbal blend, and ascorbic acid

Evaluation of Trial Formulations

FTIR Compatibility Study

For every chemical under consideration, the Shimadzu IRAffinity-1S FTIR spectrophotometer produced FTIR spectra that demonstrated the presence of the major functional groups, including distinctive bands resulting from hydroxyl, carbonyl, aromatic, and aliphatic functional groups (Table 14, Figure 5). The lack of notable peak changes or band disappearance during formulation suggests that the polyherbal mix's active ingredients did not interact intermolecularly with the excipients.

Table 14. The various characteristic FTIR peaks of the polyherbal blend and optimized formulation

Functional group	Polyherbal blend (cm^{-1})	Polyherbal blend + excipients (cm^{-1})	Assignment
O–H stretching	3388	3385	Phenolic hydroxyl groups

C–H stretching	2926	2923	Aliphatic hydrocarbons
C=O stretching	1637	1634	Carbonyl compounds
C=C aromatic	1518	1516	Aromatic ring vibration
C–O stretching	1076	1073	Alcohols and ethers

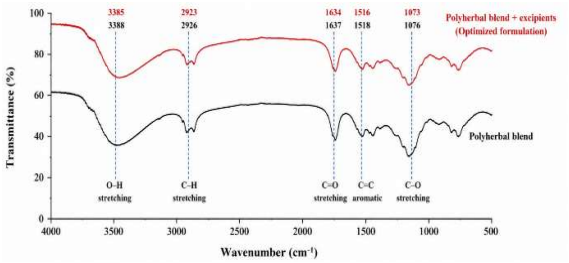

Figure 5. FTIR spectra of the polyherbal blend and optimized polyherbal tablet formulation.

Differential scanning calorimetry Study

The heat flow DSC thermograms (Table 15, Figure 6) showed a second endothermic transition at about 188 °C, which corresponds to the phytoconstituent matrix, and a general endothermic peak at approximately 121 °C (loss of moisture). For the optimized formulation, there are no additional thermal events and only a very tiny shift in the melt peaks.

Table 15. DSC thermal characteristics

Sample	Endothermic peak (°C)	Observation
Polyherbal blend	121.4	Moisture evaporation
Polyherbal blend	187.6	Phytoconstituent melting
Polyherbal blend + excipients	188.9	Slight shift, no incompatibility

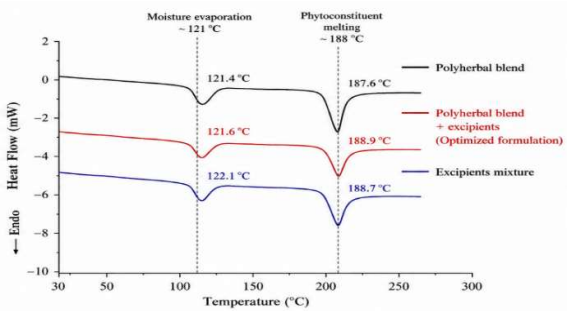

Figure 6. DSC thermograms of the optimized polyherbal tablet formulation and polyherbal blend.

Scanning Electron Microscopy Analysis

A JEOL JSM-IT200 scanning electron microscope was used to capture the SEM images of the tablets of the chosen formulations (Fig. 7). When granules were compressed into tablets, there were no indications that they fractured, aggregated, or had structural flaws.

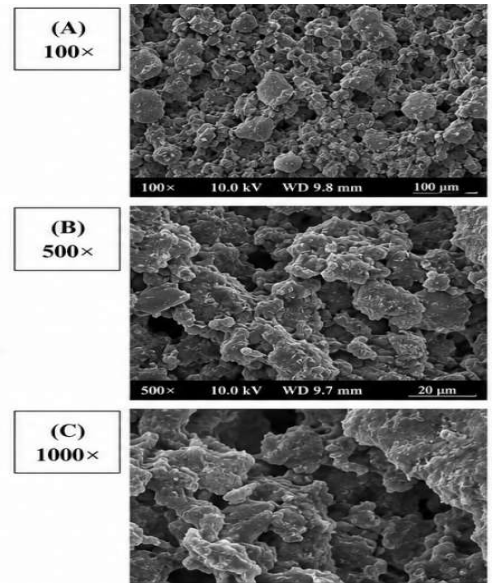

Figure 7. SEM images after compression micrographs at 100×, 500×, and 1000×

Evaluation of Trial Formulations

Pre-compression Evaluation of Trial Formulations

Granules with adequate flow and compressibility were produced by all five formulas. Formulations F3 and F4 were found to have relatively low Carr's index and Hausner ratio values, which suggest excellent flow properties for tablet compression, as shown in Table 16.

Table 16. Granule pre-compression assessment (F1–F5)

Parameter	F1	F2	F3	F4	F5

Bulk density (g/mL)	0.42 ± 0.01	0.44 ± 0.02	0.46 ± 0.01	0.47 ± 0.01	0.45 ± 0.02
Tapped density (g/mL)	0.56 ± 0.01	0.55 ± 0.01	0.57 ± 0.01	0.56 ± 0.01	0.56 ± 0.02
Carr's index (%)	25.0 ± 0.6	20.0 ± 0.5	19.3 ± 0.4	16.1 ± 0.5	19.6 ± 0.5
Hausner ratio	1.33 ± 0.01	1.25 ± 0.01	1.23 ± 0.01	1.19 ± 0.01	1.24 ± 0.01
Angle of repose (°)	34.8 ± 0.7	32.9 ± 0.6	31.4 ± 0.5	30.2 ± 0.4	32.1 ± 0.6

Post-compression Evaluation of Trial Formulations (F1–F5)

Each formula produced tablets with the proper physical characteristics. The tablets had no visible flaws, such as capping, chipping, or lamination, and they were all smooth. According to Table 17, F3 and F4 were the formulations that demonstrated strong mechanical strength, reduced friability, and increased disintegration.

Table 17. Evaluation of experiment formulations after compression (F1–F5)

Parameter	**F1**	**F2**	**F3**	**F4**	**F5**
Appearance	Uniform	Uniform	Uniform	Uniform	Uniform
Thickness (mm)	4.82 ± 0.04	4.86 ± 0.03	4.90 ± 0.02	4.88 ± 0.03	4.85 ± 0.04
Diameter (mm)	12.02 ± 0.03	12.01 ± 0.02	12.00 ± 0.02	12.02 ± 0.03	12.01 ± 0.03
Average weight (mg)	501.8 ± 4.2	499.6 ± 3.9	500.3 ± 3.4	499.8 ± 3.6	500.5 ± 4.1
Hardness (kg/cm)	4.8 ± 0.2	5.2 ± 0.3	6.3 ± 0.2	6.0 ± 0.2	5.4 ± 0.3
Friability (%)	0.92 ± 0.03	0.78 ± 0.02	0.54 ± 0.02	0.60 ± 0.03	0.73 ± 0.03
Disintegration time (min)	18.2 ± 0.6	15.6 ± 0.5	11.4 ± 0.4	12.1 ± 0.5	14.2 ± 0.5

Selection of Optimized Formulation (F1–F5)

Because of its favorable granule flow characteristics, favorable tablet characteristics, and quicker disintegration, formula F3 was determined to be the most acceptable based on a comparative study.

Assay of marker phytoconstituent(s)

The enhanced formulation's drug content was 98.8 ± 1.1% (Table 18), suggesting that the herbal ingredients were dispersed uniformly throughout the tablet's matrix.

Table 18. Drug Content of Optimized Formulation

Parameter	**Result**
Drug content (%)	**98.8 ± 1.1**

In Vitro Dissolution Study

The dissolution study (Table 19, Figure 8) showed a delayed release profile of the medication, demonstrating the successful release of the herbal constituents, with total drug release ranging from 18.4 ± 0.8% at 5 minutes to 96.4 ± 1.6% at 45 minutes.

Table 19. Dissolution profile of optimized formulation (F3)

Time (min)	**Drug Released (%)**
5	18.4 ± 0.8
10	35.6 ± 1.2
15	52.3 ± 1.5
20	68.7 ± 1.8
30	86.2 ± 2.1
45	96.4 ± 1.6

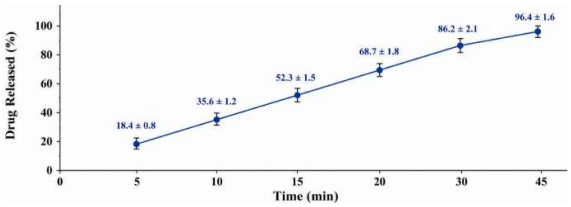

Figure 8. Drug release curve of Optimized formulation (F3)

Drug Release Kinetics

The Korsmeyer–Peppas model (R^2 = 0.993) and the Higuchi model (R^2 = 0.988) provided the best fits for the dissolution data in the release kinetic study (Table 20, Figure 9).

Table 20. Release kinetic analysis

Model	R^2
Zero-order	0.954
First-order	0.981
Higuchi	0.988
Hixson–Crowell	0.972
Korsmeyer–Peppas	**0.993**

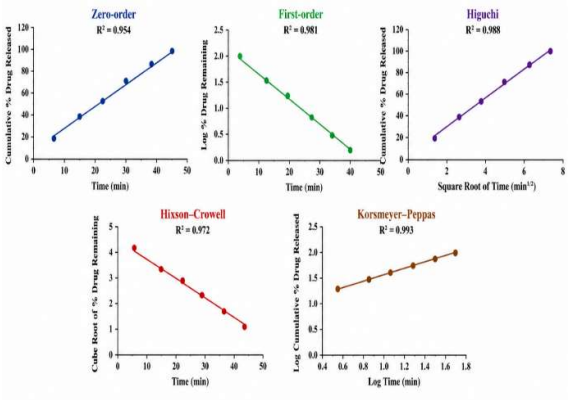

Figure 9. Higuchi plot, zero-order plot, and first-order plot Plots by Hixson and Crowell and Korsmeyer and Peppas

Stability of optimized formulation (F3)

Table 21 displays the results for accelerated stability. Tablets were held for six months under accelerated circumstances, and no significant changes were found in the tablet's physical characteristics, hardness, friability, drug content, or dissolving profile. Throughout the study, the formulation remained steady.

Table 21. Stability evaluation of optimized formulation (F3)

Parameter	Initial	1 Month	2 Months	3 Months	6 Months
Appearance	No change	No change	No change	No change	No change
Hardness (kg/cm²)	6.3 ± 0.2	6.2 ± 0.2	6.2 ± 0.1	6.1 ± 0.2	6.0 ± 0.2
Friability (%)	0.54 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.58 ± 0.03	0.60 ± 0.03
Drug Content (%)	98.8 ± 1.1	98.4 ± 1.0	98.1 ± 1.0	97.8 ± 0.9	97.3 ± 0.8
Dissolution at 45 min (%)	96.4 ± 1.6	96.1 ± 1.5	95.8 ± 1.4	95.5 ± 1.3	95.1 ± 1.2

DISCUSSION

The present study achieved formulation and development of a polyherbal immunity booster tablet combining the herbal substances Withania somnifera, Tinospora cordifolia, Curcuma longa, Zingiber officinale and Phyllanthus emblica L. Herbal raw materials confirmed to the quality specifications as stipulated in pharmacopoeial standards, yielding a total foreign matter of 0.36–0.55%, a loss on drying of 5.46–7.12% and total ash values of 6.24%, thereby demonstrating their potential suitability for pharmaceutical formulation. The World Health Organization, which favors low moisture and ash values to preserve the purity and stability of herbal raw materials, reported the same quality parameters.

Phyllanthus emblica L. had the highest total phenolic content among the tested plants (96.8 ± 2.9 mg GAE/g), followed by F. deltoidea (82.0 ± 15.8 mg GAE/g) and C. longa (81.0 ± 0.6 mg GAE/g), while C. longa (61.2 ± 2.0 mg QE/g) and F. deltoidea (39.4 ±2.1 mg QE/g). Similar findings were reported by Rahim et al., who discovered that the synergistic action of phytoconstituents in polyherbal formulations high in phenol and flavonoid contents resulted in a DPPH radical scavenging activity reaching 90% [41]. Similarly, Cheng et al. noted that

formulations with higher phenolic content were more potent and efficient antioxidants [42].

The F3 (Contact angle 117.45°) formulation demonstrated the best pre-compression flow properties (Carr's index 19.3% and Hausner ratio 1.23) and post-compression characteristics, such as hardness (6.3 ± 0.2 kg/cm²), friability (0.54 ± 0.02%), time to disintegration (11.4 ± 0.4 min), drug content (98.8 ± 1.1%), and cumulative release of drugs (96.4 ± 1.6% at 45 minutes). Diffusion-controlled release was indicated by the release profile fitting the Korsmeyer-Peppas model ($R^2 = 0.993$). Similar results were published by Wu et al. with the tablet hardness varying between 5.8 and 6.5 kg/cm², friability below 1%, drug content above 97%, and dissolution > 95% in 45 minutes [43]. Moreover, the optimized formulation had acceptable stability during six months under ICH Q1A(R2) accelerated conditions, with the drug content decreasing from 98.8% to 97.3% and the dissolution above 95%, which indicates excellent formulation stability.

Conclusion

The wet granulation technique was employed to develop and optimize a standardized polyherbal tablet comprising Withania somnifera, Tinospora cordifolia, Curcuma longa, Zingiber officinale, and Phyllanthus emblica. The pre-compression flow characteristics of F3 were found to be acceptable and to meet quality standards based on the pharmacopeial statement for hardness, friability, weight fluctuation, and disintegration time of the post-compression parameters. The compatibility of the polyherbal blend with the excipients was evaluated using FTIR and DSC, which did not reveal any significant physicochemical interactions. The developed tablet displayed acceptable assay values, reproducible dissolution profiles, and drug release kinetics described by the Korsmeyer–Peppas model. In addition, accelerated stability tests (ICH Q1A(R2) confirmed that the tablets retained their physical appearance, mechanical strength, assay, dissolution, and microbiological quality after six months of storage. In conclusion, the pharmaceutical quality, stability, and manufacturability of the developed polyherbal tablet are generally satisfactory, and the developed polyherbal tablet may therefore be recommended for further product development and scale-up studies.

Conflict of Interest

The author(s) declared no conflict of interest.

Ethical Compliance Standard

NA

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