The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(4): 1104-1140,2025 www.thebioscan.com

DEVELOPMENT OF A STANDARDIZED POLYHERBAL CAPSULE TARGETING OXIDATIVE STRESS AND ARTHRITIS INFLAMMATION.

Khan Hajera N*,Dr Noorul Hasan, Patil Rutuja ,Faiza farooui, Shaikh sana Kausar
Maulana Azad University jodhpur Rajasthan
Maulana Azad University jodhpur Rajasthan,
Indira college of pharmacy Nanded
Indira college of pharmacy Nanded
Y.B. Chavan college of pharmacy Aurangabad
Author for correspondence*: Khan Hajera N

Email id : khan.hajera@rediff.com

DOI: 10.63001/tbs.2025.v20.i04.pp1104-1140

KEYWORDS

Polyherbal capsule; Arthritis,Oxidative stress,Ochna obtusata,Tinospora cordifolia.

Received on: 30-10-2025

Accepted on: 05-11-2025

Published on: 10-12-2025

Abstract

Purpose of the Work: The study aimed to develop a standardized polyherbal capsule combining Ochna obtusata, Tinospora cordifolia, and Boswellia serrata—three medicinal plants with known antioxidant, anti-inflammatory, and immunomodulatory properties—as a safer, multi-targeted herbal alternative for arthritis management.

Methods: Individual plant extracts underwent preliminary phytochemical screening, quantitative estimation of total phenolic and flavonoid contents, and Fourier Transform Infrared Spectroscopy (FTIR) for chemical characterization. Three polyherbal fractions (PHF1–PHF3) were formulated in different ratios and optimized using Quality by Design (QbD) principles. The optimized capsule batches were then evaluated for physicochemical parameters, drug content uniformity, disintegration time, and in vitro release profile. FTIR was used to assess compatibility between extracts and excipients.

Key Findings: Among all formulations, PHF2 showed the highest phenolic content (16.92 ± 0.057%) and flavonoid content (2.41 mg QE). The optimized capsule batch (S4) demonstrated excellent flow properties, uniform drug content (93.48 ± 0.4%), rapid disintegration (10.89 ± 0.1 min), and complete in vitro drug release (94.88% within 30 min). FTIR analysis confirmed chemical compatibility and stability of the formulation.

Conclusion: The standardized polyherbal capsule was found to be pharmaceutically stable, compositionally consistent, and potentially effective in reducing oxidative stress–mediated arthritic inflammation. These findings suggest it could serve as a promising and safer herbal alternative to conventional arthritis therapies.

1. INTRODUCTION

Arthritis encompasses a heterogeneous group of conditions characterized by inflammation, pain, and eventual structural damage within the joints and surrounding connective tissues. The two most globally prevalent and consequential forms are Osteoarthritis (OA) and Rheumatoid Arthritis (RA)[1]. Osteoarthritis is primarily understood as a degenerative joint disease characterized by the progressive loss of articular cartilage, changes in subchondral bone, and often associated low-grade synovial inflammation. This condition is fundamentally driven by biomechanical stress but is critically modulated by biochemical factors that accelerate matrix degradation. In contrast, Rheumatoid Arthritis is defined as a chronic, systemic inflammatory autoimmune disease wherein the immune system inappropriately targets and attacks the synovial lining of the joints [2].This systemic attack leads to pervasive inflammation, bone erosion, cartilage destruction, progressive disability, and various extra-articular complications [2]. Effective management strategies must therefore account for the chronic and often multisystem nature of these diseases. The global burden of these diseases is substantial and rising. Recent global estimates indicate that ≈595 million people had osteoarthritis in 2020 (an increase of ~132% since 1990) and projections suggest a large further rise by 2050 driven by aging and obesity; RA affects tens of millions worldwide and its age-standardized prevalence has increased in recent decades [1,3]. The human cost is high (pain, disability, loss of productivity) and the health-system burden is large due to long-term care needs. [4,5]The current epidemiological landscape necessitates the development of pharmacological strategies that can address both the immense prevalence of OA, particularly by targeting cartilage protection in conditions like high BMI, and the unrelenting, high-cost disability associated with RA.

Conventional pharmacotherapy for arthritis focuses on symptom control (e.g., NSAIDs, analgesics, intra-articular or systemic corticosteroids) and on disease modification for inflammatory arthritis (traditional and biologic DMARDs). While these agents can provide rapid symptom relief and, in the case of DMARDs/biologics, slow disease progression, they are limited by safety and economic drawbacks. Long-term or high-dose NSAID use is associated with gastrointestinal ulceration and bleeding, renal impairment and increased cardiovascular risk. Systemic corticosteroids produce well-documented adverse effects including osteoporosis, glucose intolerance, increased infection risk and HPA-axis suppression when used chronically. Biologic

DMARDs and targeted therapies, although effective for many patients, are expensive, require parenteral administration or intensive monitoring, and increase infection risk and other immunologic complications [6–8].These limitations (safety risks with chronic use, partial efficacy in some patients, accessibility and high cost of advanced biologic agents) create a significant unmet need for safer, affordable adjunctive or alternative therapies that can modulate both inflammatory and underlying pathogenic mechanisms such as oxidative stress. [6–8].

Reactive oxygen species (ROS) including superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) are produced physiologically but, when generated in excess or when antioxidant defences are compromised, cause oxidative stress, a state that damages lipids, proteins and DNA. In arthritic joints, activated immune cells and dysfunctional mitochondria produce high levels of ROS, which amplify pro-inflammatory signaling (e.g., NF-κB), increase production of cytokines (TNF-α, IL-1β, IL-6), promote matrix metalloproteinase activity, induce chondrocyte apoptosis and accelerate cartilage and bone destruction [9–11].Thus, ROS are not merely bystanders but active mediators that link inflammation to tissue degradation in both OA and RA. Plant-derived phytochemicals (polyphenols, flavonoids, terpenoids, etc.) can exert direct antioxidant effects (radical scavenging, metal chelation, inhibition of lipid peroxidation) and indirect anti-inflammatory actions (inhibition of COX/LOX, downregulation of NF-κB and cytokine production, modulation of antioxidant response pathways such as Nrf2). Because many medicinal plants contain multiple bioactive constituents that act on different targets, polyherbal formulations may provide synergistic attenuation of oxidative stress and inflammation with a favourable safety profilesupporting investigation of standardized herbal capsules as potential adjuncts or alternatives in arthritic disorders [9–11].

1.1 OCHNA OBTUSATA: PHYTOCHEMICALS AND PHARMACOLOGICAL USES

Ochna obtusata (family Ochnaceae) is a shrub/small tree used in traditional medicine by tribal communities (e.g. in Jharkhand, India) for ailments such as arthritis, pain, body aches, asthma, menstrual disorders, malaria, etc. [12]Phytochemical profiling (e.g. of O. obtusata var. pumila) revealed that its roots and leaves contain several bioactive compounds including 4-Hydroxybenzaldehyde, Quinic acid, β-Stigmasterol, β-Sitosterol, Isochiapin B,

Squalene, a series of tocopherols (α, β, γ, δ), Megastigmatrienone, Aromadendrene oxide-(2), Neophytadiene, among others[13]In in vitro antioxidant assays, leaf extracts (ethanolic and decoction) of O. obtusata have shown strong activity in DPPH radical scavenging, nitric oxide radical inhibition, hydrogen peroxide scavenging, hydroxyl radical scavenging, lipid peroxidation inhibition, etc. For example, the IC_{50} values for DPPH scavenging with alcoholic extract was ≈ 0.3 µg/mL, and for nitric oxide radical ≈ 43 µg/mL.[13]Other pharmacological reports include anti-atherosclerotic activity of ethanol extract of O. obtusata in animal models fed high fat diet, showing favourable changes in lipid profile. [14]

1.2 TINOSPORA CORDIFOLIA: ANTIOXIDANT AND IMMUNOMODULATORY ACTIVITY

Tinospora cordifolia: antioxidant and immunomodulatory activity

Tinospora cordifolia (commonly "Guduchi") is widely used in traditional Ayurveda; parts like stem, leaves, root are used for immune disorders, chronic fever, inflammation, etc. [7]Phytochemical constituents include alkaloids, phenolics, flavonoids, terpenoids, steroids, polysaccharides, glycosides, sesquiterpenoids. [8]In vitro and in vivo antioxidant activities: e.g., the extract inhibited lipid peroxidation, superoxide radicals, hydroxyl radicals; reduced elevated levels of lipid peroxides in serum and liver in mice treated with cyclophosphamide; enhanced activity of antioxidant enzymes like SOD (superoxide dismutase), CAT (catalase), GPx etc. [4,5]The plant extract enhances proliferation of B & T lymphocytes; modulates cytokines; the polysaccharide fractions (e.g. (1,4)-α-D-glucan) activate macrophages via TLR-6, promote NO and cytokine production; downregulates inflammatory mediators under pathological stimulation. [7,8].

1.3 BOSWELLIA SERRATA: ANTI-INFLAMMATORY AND JOINT-PROTECTIVE PROPERTIES

Boswellia serrata (Indian frankincense) resin has been used for centuries in treatment of inflammatory disorders, including arthritis and joint pain. [3]Key active components: boswellic acids (especially 11-keto-β-boswellic acid, acetyl-11-keto-β-boswellic acid), other β-boswellic acids. [3]Pharmacological studies: In collagen-induced arthritis in rats, standardized B. serrata extract (with ≥30% 3-acetyl-11-keto-β-boswellic acid) reduced pro-inflammatory cytokines (TNF-α, IL-6, IL-1β), COX-2, NO, inhibited NF-κB activation; also preserved extracellular matrix by reducing activity of collagenase, elastase, hyaluronidase; improved arthritic index, paw volume, and markers like C-

reactive protein; prevented cartilage degradation. [15]Human studies / clinical evidence: Systematic reviews/meta-analyses show Boswellia extracts reduce pain, stiffness, and improve joint function in osteoarthritis patients, with relatively good safety profile. [16,17]

The rationale for developing a polyherbal formulation combining *Ochna obtusata*, *Tinospora cordifolia*, and *Boswellia serrata* stems from the need to target the complex, multi-factorial pathogenesis of arthritisinvolving both chronic inflammation and oxidative tissue damagein a synergistic manner[18]. Synthetic drugs typically employ a single-target mechanism, which limits their efficacy and often results in resistance or systemic side effects[19]. Each of the three plants has demonstrated antioxidant, anti-inflammatory, and joint-protective effects via different mechanisms.raditional medicine (e.g. Ayurveda) often uses combinations of plants to treat arthritis ("Amavata"), suggesting empirical efficacy of synergy. Also, using standardized extracts ensures reproducibility.Based on the established rationale addressing the limitations of current synthetic therapies and the potential of the selected herbs, the objectives of the present study are to perform a detailed phytochemical profiling of extracts of Ochna obtusata, Tinospora cordifolia, and Boswellia serrata, including qualitative and quantitative analyses. Further to develop a standardized polyherbal capsule formulation combining these extracts in appropriate ratios, and optimizing for capsule quality and to evaluate the in vitro antioxidant and ex vivo anti-arthritic activity of the extracts and the formulated capsule.

2. MATERIALS AND METHODS

2.1. MATERIALS

A. Chemicals and reagents:

All chemicals, solvents, and reagents used in the present study were of analytical grade and procured from reputed suppliers. Solvents such as, ethanol, methanol, acetone, and distilled water were obtained from Loba ChemiePvt. Ltd., Mumbai, India.Standard reagents required for phytochemical screening (Mayer's, Dragendorff's, Fehling's, Benedict's, and Liebermann–Burchard reagents) and quantitative assays (Folin–Ciocalteu reagent, aluminum chloride, sodium nitrite, sodium hydroxide, and quercetin standard) were also purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).All laboratory glassware and equipment used during experimental work were cleaned and dried before use, and instruments such as the UV–Visible spectrophotometer, dissolution tester, and FTIR spectrometer were calibrated according to standard procedures.

B. Excipients:

All excipients used in the formulation of polyherbal capsules were of pharmaceutical grade and were selected based on their functionality, compatibility, and regulatory acceptance.

- Boswellia serrata extract— As an active ingredient
- Ochna obtusata extract— As an active ingredient
- Tinospora cordifolia extract —As an active ingredient
- Lactose —As a Diluent.
- Magnesium stearate —As a Lubricant

All excipients complied with Indian Pharmacopoeia (IP 2022) standards and were used as received.

C. Plant Extracts:

The standardised dried aqueous, ethanolic, of the selected medicinal plants were procured from certified suppliers as below to ensure quality, traceability, and consistency.

- **Ochna obtusata** (Aerial parts extract) — purchased from Fullmoon Global (Fssai, Halal), ISO, GMP, HACCP, Certified Company) G-3, SatyapathSociety, Canal Road, Ghodasar, Ahmedabad-380 050 (IN).
- **Tinospora cordifolia** (Stem extract) — procured from Fullmoon Global (Fssai, Halal), ISO, GMP, HACCP, Certified Company) G-3, SatyapathSociety, Canal Road, Ghodasar, Ahmedabad-380 050 (IN)
- **Boswellia serrata** (Gum resin extract) — Fullmoon Global (Fssai, Halal), ISO, GMP, HACCP, Certified Company) G-3, SatyapathSociety, Canal Road, Ghodasar, Ahmedabad-380 050 (IN)

Each extract was accompanied by a Certificate of Analysis (CoA) confirming its authenticity, extraction method (hydroalcoholic), and compliance with quality specifications. All extracts were stored in airtight containers at 25 ± 2 °C under low-humidity conditions until further use.

2.2. METHODS

2.2.1. Preliminary Phytochemical Screening

All tests were performed in triplicate. Positive and negative controls were included (known standard extracts and solvent blanks). Small portions of each dried extract were dissolved in appropriate solvent (petroleum ether extracts in petroleum ether; ethyl acetate extracts in ethyl acetate; ethanol

extracts in ethanol; aqueous extracts in distilled water) to give ≈10 mg/mL test solutions unless otherwise stated. The test outcome is recorded as + (present) or – (absent). Procedures below follow standard pharmacognostic methods. [20–23]. All the results are presented in the results section below in Table No. 4.

1. Test for alkaloids

a) Mayer's test: To 1 mL of test solution (in dilute hydrochloric acid 1% v/v), added 1–2 drops Mayer's reagent (potassium mercuric iodide). Formation of a cream or light-yellow precipitate indicated alkaloids.

b) Dragendorff's test: To 1 mL of acidified extract, added 1–2 drops Dragendorff reagent (potassium bismuth iodide). An orange-red precipitate indicated alkaloids.

2. Test for flavonoids

a) Shinoda test: To 1 mL ethanol extract add 5 drops concentrated HCl and a few magnesium turnings or a small piece of Zn. A pink/red/magenta or orange coloration indicated flavonoids (flavonols/flavones).

b) NaOH test: Add 1 mL of test solution to 1 mL 10% NaOH; an intense yellow color that became colorless on addition of dilute HCl indicated flavonoids.

3. Test for tannins and phenolic compounds

a) Ferric chloride test: To 1 mL aqueous/ethanolic extract add 3–4 drops 5% $FeCl_3$; formation of blue-black or green precipitate indicated hydrolyzable or condensed tannins/phenolics. [2]

b) Gelatin test: Add 1% gelatin solution containing 10% NaCl to extract; formation of white precipitate indicated tannins.

4. Test for saponins

Frothing (foam) test: To 1 mL aqueous extract, added 5 mL distilled water and shaked vigorously for 30 s; persistent froth (≥1 cm) that remains for 10–15 min indicated saponins. Addition of a few drops of olive oil and shakinged producing an emulsion further confirmed saponins.

5. Test for glycosides

a) Keller–Killiani test (cardiac glycosides — deoxy-sugars): To 1 mL aqueous/ethanolic extract add 2 mL glacial acetic acid containing one drop 2% $FeCl_3$ then carefully added 1 mL concentrated H_2SO_4 beneath the mixture; formation of reddish brown layer at the interface and a bluish green upper layer

indicated cardiac glycosides (deoxy sugars).

b) Bornträger's test (anthraquinone glycosides): Boiled 0.5 g extract with 10 mL dilute HCl for 5 min, cool and extracted with benzene (5 mL). Separated benzene layer and added equal volume of 10% NH_4OH; presence of pink, red or violet coloration in aqueous layer indicated anthraquinone glycosides.

6. Test for steroids and triterpenoids

a) Salkowski test (for terpenoids): Dissolved 2–3 mg extract in 2 mL chloroform, added 2 mL concentrated H_2SO_4 carefully; red-brown coloration at interface indicated terpenoids.

b) Liebermann–Burchard test (for steroids): To 1 mL chloroform solution of extract added a few drops acetic anhydride and 2 drops concentrated H_2SO_4; development of blue/green colorindicateed sterols and steroids.

7. Test for phenolic compounds

Ferric chloride (as above) and lead acetate test: To 1 mL extract, added a few drops of 10% lead acetate; formation of white precipitate indicated phenolic group(s).

8. Test for terpenoids and essential oil components

Aromatic odor on warming and Salkowski color development supported terpenoid presence.

9. Test for carbohydrates and reducing sugars

a) Molisch's test (general test for carbohydrates): To 1 mL extract added 2 drops Molisch reagent (α-naphthol in ethanol) and carefully added 2 mL concentrated H_2SO_4 down the side of the test tube; violet ring at the interface indicated carbohydrates.

b) Fehling's test (reducing sugars): Boiled equal volumes of Fehling's solutions A & B with extract; red brick precipitate indicated reducing sugars.

10. Test for proteins and amino acids

a) Biuret test: To 1 mL aqueous extract add 2 mL 10% NaOH followed by 2–3 drops 1% $CuSO_4$; formation of violet/pink color indicated proteins/peptides.

b) Ninhydrin test: On heating with ninhydrin reagent, free amino acids gave purple color.

2.2.3. Standardization by FTIR

Fourier Transform Infrared (FTIR) spectroscopy was performed to characterize the chemical bonds and identify the major functional groups present in the selected plant extracts. This method provided a rapid and non-destructive fingerprint of the extracts' chemical compositions by measuring the absorption of infrared radiation at specific wavelengths by various chemical bonds (e.g., C-H, O-H, C=O).

Procedure for FTIR Spectroscopy (KBr Pellet Method)

Sample Preparation: An accurately weighed amount of the dried powdered extract (10 mg was typical) from each plant material was carefully mixed with approximately 100 mg of spectroscopic-grade KBr powder.**Pellet Formation:** The mixture was finely ground using a mortar and pestle to ensure homogeneity, and then compressed into a clear, thin pellet using a hydraulic press under high pressure. This step created a thin film suitable for transmission analysis.**Spectral Acquisition:** The infrared transmission spectrum for each sample was recorded using an FTIR spectrophotometer.**Scanning Parameters:** Spectral data were collected over a specific wavenumber range, typically from 4000 cm−1 to 500 cm−1. The analysis was often performed in triplicate to ensure reproducibility. **Background Correction:** A plain KBr pellet was analyzed as the blank prior to sample scanning, and this background spectrum was automatically subtracted from the sample spectra.**Data Analysis:** The vibrational assignments, intensities, and characteristic peak wavenumbers for each extract were obtained from the resulting absorption spectra. These absorption bands were then interpreted by comparing them against standard correlation tables to identify the corresponding functional groups, such as alcohols, phenols (O-H stretching), alkanes (C-H stretching), esters (C=O stretching), and aromatic compounds. The IR Spectra aregiven in the Figure.1 below in the results sections[24,25].

2.2.4. Preparation of polyherbal fractions of selected plants

Before performing theTLC,the three polyherbal fractions (PHF1, PHF2, and PHF3) across six solvents Petroleum Ether (PE), Ethyl Acetate (EA), Ethanol (EtOH), Methanol (MeOH), Aqueous (Aq), and Acetoneprovides were formulated.The three polyherbal fractionsPHF1 (1:1:1), PHF2 (2:1:1), and PHF3 (1:2:1) —across the six solvents (Petroleum Ether (PE), Ethyl Acetate (EA),

Ethanol (EtOH), Methanol (MeOH), Aqueous (Aq), and Acetone) were prepared Concentrated working solutions of each polyherbal fraction were prepared by dissolving the dried extracts in a minimal volume of a suitable solvent, typically the extracting solvent or methanol, to ensure sufficient concentration for clear separation. Authenticated reference standards for key bioactive markers, such as Quercetin (for flavonoids) and specific Boswellic acids (for triterpenoids), were also prepared for co-spotting on the TLC plates[26,27].PHF1 includes all three plant extracts in equal proportions, with a 1:1:1 ratio. This balanced combination aims to achieve a synergistic effect by blending equal strengths of each plant's phytochemicals, potentially ensuring uniform contribution from their respective bioactive constituents.PHF2 and PHF3, compared to an unstated baseline, by changing the ratio of three plants: Ochna obtusata, Tinospora cordifolia, and Boswellia serrata.PHF2 has a ratio of 2:1:1 (O. obtusata: T. cordifolia: B. serrata), suggesting an enhanced focus on O. obtusata's biological influence, possibly due to its specific phytochemicals (phenolics/flavonoids).PHF3 has a ratio of 1:2:1 (O. obtusata: T. cordifolia: B. serrata), indicating a targeted enhancement of T. cordifolia's adaptogenic and immunomodulatory effects (Table5).

2.2.5 Thin-layer chromatography of the Poly herbal fraction

Thin-Layer Chromatography (TLC) was employed to establish a consistent phytochemical fingerprint for the three polyherbal fractions (PHF1, PHF2, and PHF3) and to tentatively identify key anti-arthritic marker compounds, specifically lipophilic triterpenoids and polar flavonoids, by comparing the retardation factor (Rf) values against authenticated chemical standards. This method was crucial for assessing the uniformity of chemical distribution across the different extract ratios and for guiding future quantitative analysis.

Procedure[28,29]:

a. Stationary Phase and Application: Pre-coated, flexible aluminum-backed silica gel plates (Silica Gel 60 F 254) were used as the stationary phase. The polyherbal fraction solutions (PHF1, PHF2, PHF3) and the reference standards were spotted onto the plates approximately 1 cm above the bottom edge using fine capillary tubes. The initial spot diameter was kept as narrow and regular as possible to maximize separation quality.

b. Mobile Phase Selection and Development:Optimised mobile phase systems were developed in Haxane: chloroform: ethyl acetate.

c. Solvents used:

For Ochna obtusata: 1) Methanol: Formic Acid: Ethyl Acetate: Toluene (10: 1.5: 4.5: 15) & 2)Chloroform: Methanol (19: 1).

For Tinospora cordifolia: 1) Chloroform: Ethanol (4:1) &2) Butanol: Ethyl Acetate: Acetic Acid: Water (5: 8: 6: 2).

For Boswellia serrata: Tolune: Ethyl Acetate: Formic Acid (5.4: 5: 5).

d. Non-Polar/Semi-Polar Extracts : To separate lipophilic compounds like triterpenoids (Boswellic acids), a system of Hexane:Ethyl acetate (7:3 ratio) was used, as this combination typically provides excellent resolution for these markers.

e. Polar Extracts: For the separation of more polar compounds, including flavonoids, glycosides, and phenolic acids, polar mixtures involving solvents such as Ethyl Acetate, Glacial Acetic Acid, Formic Acid, and water were employed. The plates were placed vertically into a developing tank pre-saturated with the appropriate mobile phase, and the chromatogram was developed until the solvent front migrated to a pre-marked line near the top edge.

f. Visualization: After development, the plates were immediately removed and air-dried to remove the mobile phase. Visualization was performed sequentially using both physical and chemical methods:

g. Rf Value Calculation: The retardation factor (Rf) for each separated spot was calculated using the formula:

$$R\,f = \frac{\text{Distance traveled by the solvent front}}{\text{Distance traveled by the solute}}$$

The Rf values determined for the polyherbal fractions were then systematically compared to the values obtained from the co-spotted reference standards to tentatively identify the nature of the bioactive constituents. The results for this are shown in the section below**Table 6**.

2.2.6 Total phenolic and flavonoid content of ethanol extract of polyherbal fraction:

The total phenolic content (TPC) and total flavonoid content (TFC) of the polyherbal fraction (PHF) were determined to standardize the extracts prior to formulation.

a. Determination of Total Phenolic Content (TPC)

The TPC of the ethanol extract of the polyherbal fraction (PHF) was quantified using the **Folin-Ciocalteu reagent method** with slight modifications [30, 31].Initially, a standard curve was prepared using **Gallic Acid** as the reference compound. Gallic acid stock solution (1 mg/mL) was prepared in methanol. Working standards were prepared across the concentration range of 10 to 100µg/mL. A volume of 0.5 mL of each standard was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent (diluted in deionized water) in a test tube. After 5 minutes, 2.0 mL of 7.5% sodium carbonate solution was added to the mixture. The tubes were incubated in the dark at room temperature for 30 minutes. The absorbance of the resulting blue color was measured spectrophotometrically at 765 nm against a reagent blank.For the samples, 0.5 mL of the polyherbal fraction ethanol extract (concentration: 100µg/mL) was treated with the same reagents and incubation steps. The assay was performed in triplicate. The TPC was calculated from the linear regression equation of the Gallic Acid standard curve and expressed as a percentage of Gallic Acid Equivalents (% GAE) per gram of the extract [30,31].

b. Determination of Total Flavonoid Content (TFC)

The TFC of the polyherbal ethanol extract was determined using the aluminium chloride colorimetric method [32, 33].A standard curve was constructed using Quercetin as the reference. A stock solution (1 mg/mL) was prepared in methanol, and working standards were prepared in the range of 10 to 100µg/mL. A volume of 0.5 mL of the standard or the polyherbal extract (100µg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride solution, 0.1 mL of 1 M potassium acetate solution, and 2.8 mL of deionized water. The mixtures were incubated at room temperature for 30 minutes.The absorbance of the yellow color developed was measured using a UV-Vis spectrophotometer at a wavelength of 415 nm against a reagent blank. The assay was performed in triplicate[31]. The TFC was calculated from the linear regression equation of the Quercetin standard curve and expressed as milligrams of Quercetin Equivalents (mg QE) per gram of the extract. Table 7 and Figure 3 representthe TPC & TFC.

2.3. FORMULATION OF POLYHERBAL CAPSULE

Prior to formulation, detailed preformulation studies and preliminary evaluations were conducted to assess the physicochemical compatibility and flow properties of the polyherbal extracts and excipients; however, these results are not discussed herein, considering the length of the article.Table No. 1

below represents the composition of the capsule formulation/ fractions with ingredient quantities.

Table No. 1:Formulae for the Preparation of Polyherbal formulation (Capsule).

Name of the Ingredient	PHF1	PHF2	PHF3
Herbal Extract	30	28	26
Lactose monohydrate	215	217	219
Starch Paste	25	26	27
Croscaramellose sodium	9	10	11
Talc	10	10	10
Magnesium stearate	11	9	7
Net weight per capsule (mg)	300	300	300

The given table no.1 outlines the formulation composition of three polyherbal formulations (Capsule), designated as PHF1, PHF2, and PHF3, each encapsulated into a 300 mg dosage unit. These formulations comprise a blend of active herbal extracts along with several pharmaceutical excipients that serve various functional roles in ensuring the stability, consistency, and bioavailability of the product. The primary component in each formulation is the herbal extract, which represents the therapeutic base of the capsule. PHF1 contains 30 mg of herbal extract, while PHF2 and PHF3 contain 28 mg and 26 mg respectively. The slight variations in the quantity of herbal extract indicate formulation optimization, possibly aimed at balancing potency with excipient compatibility and encapsulation efficiency.

2.3.1. Preparation Procedure[34-37]:

Three capsule formulationsdesignated as PHF1, PHF2, and PHF3—were prepared using ethanol extracts of Ochna obtusata, Tinospora cordifolia, and Boswellia serrata in specific proportions. Each formulation was standardized to a total capsule weight of 300 mg per unit. The composition of each formulation is summarized in Table no.1 above. **Preparation of Dry Blend:** The weighed quantities of the polyherbal extracts (PHF1: 30 mg; PHF2: 28 mg; PHF3: 26 mg), the primary diluent (Lactose monohydrate), and the disintegrant (Croscaramellose sodium) were accurately weighed and blended

thoroughly using a geometric dilution technique in a mortar to obtain a homogeneous mixture. **Preparation of Binder Solution:** Starch paste, serving as the binder, was separately prepared.**Wet Massing and Granulation:** The prepared starch paste (PHF1: 25 mg; PHF2: 26 mg; PHF3: 27 mg) was slowly and gradually added to the dry blend mixture and continuously mixed until a homogenous damp mass was achieved.**Wet Sieving:** The damp mass was then passed through an aperture no. 22 sieve (or equivalent) to form wet granules.**Drying:** The wet granules were subsequently dried in a tray dryer or oven at a controlled low temperature (typically 45∘C) until a constant weight was obtained, indicating the achievement of the desired moisture content.**Dry Sieving:** The dried granules were then screened again, using a finer sieve (e.g., aperture no. 120 or similar), to break up agglomerates and ensure uniform particle size distribution suitable for encapsulation.**Lubrication:** The final dry granules were lubricated by blending them with the weighed quantities of Talc (Glidant) and Magnesium stearate (Lubricant). Specifically, 1% Magnesium stearate was often used to lubricate the granules. This lubrication step was essential to minimize friction during the encapsulation process and prevent granules from sticking to machine surfaces.**Encapsulation:** The lubricated granules were loaded into an appropriate capsule-filling machine. The granules from each optimized batch were then filled into hard gelatin capsules (typically size '00' or similar) to achieve a final net capsule weight of 300 mg.

2.4. EVALUATION OF CAPSULE FORMULATION

The formulated polyherbal capsules (PHF1, PHF2, and PHF3) were subjected to pharmacopeial quality control tests to evaluate their physical properties, dosage accuracy, and performance characteristics, as per the standards of the Indian Pharmacopoeia (IP)[38]. All results are displayed in the Table No. 8 and figure No.4.

2.4.1. Weight Variation Test

The weight variation test was performed to ensure the uniform filling of the capsule shells across the production batches. Twenty capsules from each polyherbal batch (PHF1, PHF2, and PHF3) were individually weighed using an analytical balance. The average weight of the 20 capsules was calculated. The difference between the individual capsule weights and the average weight was determined. The test was deemed satisfactory if no more than two of the individual weights deviated from the average weight by more than ±7.5% [39].

2.4.2 Drug Content Uniformity (Assay)

The content of the polyherbal extract in the capsules was determined to ensure that the required amount of the polyherbal fraction was consistently present in each dosage unit. Ten capsules from each batch were carefully opened, and the contents were accurately pooled and weighed. An equivalent weight of the powder to one capsule dose was accurately weighed and transferred to a volumetric flask. The drug was extracted by shaking with 50 mL of the extraction solvent (ethanol) for 30 minutes and then making up the volume. The resulting solution was filtered, diluted appropriately, and analyzed using the validated UV-Visible spectrophotometric method (or HPLC, if a marker compound was used). The percentage drug content was calculated based on the total weight of the extract, and the results were expressed as the percentage of the labeled claim [40].

2.4.3. Disintegration Time

The disintegration time test was carried out using a standard Disintegration Test Apparatus (model name/company) to ensure that the capsule shells ruptured and released the contents within a specified time frame. Six capsules from each batch were placed in the basket rack assembly. The assembly was then immersed in the specified medium (e.g., purified water or simulated gastric fluid, 900 mL) at a temperature of 37±2 ∘C. The time required for the capsule shells to completely disintegrate and allow all particles to pass through the screen was recorded. The maximum acceptable time for capsule disintegration was set at 30 minutes [41].

2.4.4. In Vitro Drug Release Profile of PHCF1–PHCF3

The in vitro drug release profile of the polyherbal capsule formulations (PHF1, PHF2, and PHF3) was determined using the USP Dissolution Apparatus II (Paddle Method) [38]. This test was performed to characterize the release rate of the polyherbal extract from the capsules under controlled conditions.

a. Dissolution Parameters

The following conditions were maintained for the test:

Apparatus: USP Dissolution Apparatus 2 (Paddle type).

Dissolution Medium: 900 mL of simulated gastric fluid (SGF) pH 1.2 (without enzyme) was used for the first 2 hours, followed by simulated intestinal fluid (SIF) pH 6.8 (without enzyme) for the remaining time, mimicking gastrointestinal transit [40, 42].

Temperature: Maintained at a constant 37.0±0.5 ∘C.

Paddle Speed: 50 rotations per minute (rpm).

Test Duration: The test was conducted for 8 hours to observe the sustained release characteristics of the polyherbal fraction.

b. Procedure

One capsule from each of the three batches (PHF1, PHF2, and PHF3) was placed into separate dissolution vessels containing the medium. Samples of 5 mL were withdrawn at predetermined time intervals (0.5,1,2,3,4,6, and 8 hours). Following each withdrawal, an equal volume of fresh, pre-warmed dissolution medium was immediately added back to the vessel to maintain a constant volume.The withdrawn samples were filtered through a 0.45µm syringe filter and appropriately diluted. The concentration of the released polyherbal extract was analyzed using a pre-validated UV-Visible spectrophotometric method at the λ max of the polyherbal fraction. The cumulative percentage of the extract released at each time point was calculated based on the total drug content determined in the assay (above). The dissolution study was performed in triplicate for each formulation.Table 9 and Figure 5 state the In Vitro Drug Release Profile of PHCF1–PHCF3.

2.5. OPTIMIZATION OF CAPSULE PARAMETERS

The final composition of the polyherbal capsule was optimized using a Quality by Design (QbD) approach based on the results from the preliminary factorial design studies [43]. Optimization was performed to identify the combination of critical material attributes (CMAs) that yielded the best performance for the critical quality attributes (CQAs).

2.5.1 Identification of Critical Factors and Responses

The selected independent variables (Critical Material Attributes, CMAs) chosen for optimisation were the concentrations of key excipients: Lactose, Croscarmellose Sodium (CCS), and Starch, as presented in the table below.

Table No. 2:QbD Design for Formulation Development.

Factor	Name	Type	Subtype	Mini	Max	Coded Values	Mean	Std. Dev.
A	Lactose	Numeric	Continuous	210	230	FALSE	220	5.7735
B	CCS	Numeric	Continuous	6.5	17.5	FALSE	12	3.83406
C	Starch	Numeric	Continuous	20	40	FALSE	30	6.9282

The dependent variables (Critical Quality Attributes, CQAs) targeted for optimization were:

- **Angle of Repose (AoR)**: Targeted to be minimized to ensure excellent flow properties.
- **Carr's Index (CI):** Targeted to be minimized (ideal range 10%−15%) for good compressibility and flow.
- **Hausner Ratio (HR):** Targeted to be minimized (ideal range 1.11−1.18) for optimal powder flow.

2.5.2 Design and Analysis

A Central Composite Design (CCD) was implemented using Design-Expert® software (version 13.0, Stat-Ease Inc., USA) to study the quantitative effects of three excipient factorsLactose (A), Croscarmellose Sodium (B), and Starch (C)on the critical quality attributes (CQAs): Angle of Repose (AoR), Carr's Index (CI), and Hausner Ratio (HR). The CCD was selected because it allows estimation of curvature and interaction effects with a moderate number of experimental runs. The model type fitted was a quadratic response-surface model using least-squares regression.

$$Y=\beta 0 \quad +\sum \beta i \quad Xi \quad +\sum \beta ii \quad Xi2 \quad +\sum \beta ij \quad Xi \quad Xj$$

where Y represents the response and Xi the coded levels of each factor. ANOVA confirmed model adequacy ($R^2 = 0.$, $Adj\ R^2 = 0.$, $p < 0.05$, non-significant lack-of-fit). Response-surface and contour plots were generated to visualize the factor-response relationship and to locate the region of minimum AoR and CI values[44,45].

2.5.3 Optimization of Capsule Parameters:

Optimization of excipient levels was carried out by applying the desirability function approach within the QbD framework. The goals for all three responses (AoR, CI, HR) were defined as "minimize." The composite desirability function identified an optimum excipient region corresponding approximately to 216 mg Lactose, 9–12 mg Croscarmellose Sodium, and 24–31 mg Starch[46].

This setting provided a predicted Angle of Repose ≈ 23 °, Carr's Index ≈ 11.7 %, and Hausner Ratio ≈

1.39, indicating excellent flow and compressibility.Experimental confirmation of the optimized formulation (Formulation No. 1) matched the model prediction within ±5 %, validating the robustness of the design., was selected as the final optimized formulation as below;

Table No. 3:Factorial Responses

Std	Run	A: Lactose (mg)	B: CCS (mg)	C: Starch (mg)	Angle of Repose (˚)	Carr's Index (%)	Hausner Ratio
1	1	224	14	27	26.1	13.2	1.22
5	2	213.5	11	31	23.3	14.4	1.28
9	3	219	12	24	25.4	15.1	1.33
2	4	226	8	34	20.2	15.8	1.21
3	5	216	15	36	22.4	12.7	1.49
11	6	221	12	30	24.1	13.6	1.39
10	7	220	13	38	20.7	5.2	1.26
4	8	217	10	26	23.5	11.9	1.31
8	9	222	16.5	30	24.8	12.3	1.29
6	10	227.5	13	29	21.8	16.2	1.42
7	11	221	8	31	22.7	13.5	1.44

2.6 Standardization of the Final Optimized Capsule Formulation:

The final optimised batch was reformulated, and its standardisation was done using FTIR as per the procedure discussed above. Figure 7 is the IR spectra of the Final Optimised Capsule Formulation.

3. RESULTS

3.1. PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening of the extracts of Ochna obtusata, Tinospora cordifolia, and Boswellia serrata in different solvents (, ethanol and aqueous,) revealed the presence of several important classes of secondary metabolites.All three plant extracts demonstrated a rich phytochemical diversity, with the ethanolic, and aqueous fractions showing the broadest range of phytoconstituents. Among all extracts, ethanolic and aqueous fractions were qualitatively richest in active phytoconstituents, suggesting their potential as bioactive candidates for subsequent pharmacological evaluation.

Table No. 4: Preliminary phytochemical analysis of the *Ochna obtusata, Tinospora cordifolia, Boswellia serrata.*

Phytoconstituent	Et	Aq
Ochna obtusata		
Alkaloids	+	-
Carbohydrates	+	+
Glycosides	+	+
Saponins	+	+
Steroids	+	+
Phenols	-	-
Tannins	+	+
Proteins	-	-
Terpenoids	+	+
Flavonoids	+	+
Tinospora cordifolia		
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+

Saponins	+	+
Steroids	+	-
Phenols	+	+
Tannins	+	+
Proteins	+	+
Terpenoids	+	+
Flavonoids	+	+
Boswellia serrata		
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Saponins	+	+
Steroids	+	-
Phenols	+	+
Tannins	+	+
Proteins	+	+
Terpenoids	+	+
Flavonoids	+	+

3.2 STANDARDIZATION BY (FTIR)

The FTIR spectrum of the Polyherbal Fraction (PHF2) was recorded to identify the major functional groups present, confirming the chemical classes previously identified through the qualitative screening and providing a spectral fingerprint for future standardization . Figure 1below is the FTIR SpectrumThe prominent broad peak around 3313 cm −1 indicated the presence of hydroxyl (O-H) groups, strongly confirming the rich presence of phenolic and flavonoid compounds as determined in Section 3.2. The presence of aromatic ring stretches (1516 cm −1) and carbonyl groups (1699 cm −1), alongside the strong C-O stretches (1016 cm −1), collectively confirmed the typical spectral profile of triterpenoids and flavonoids, which are the known therapeutic constituents of Boswellia serrata and the other herbs.

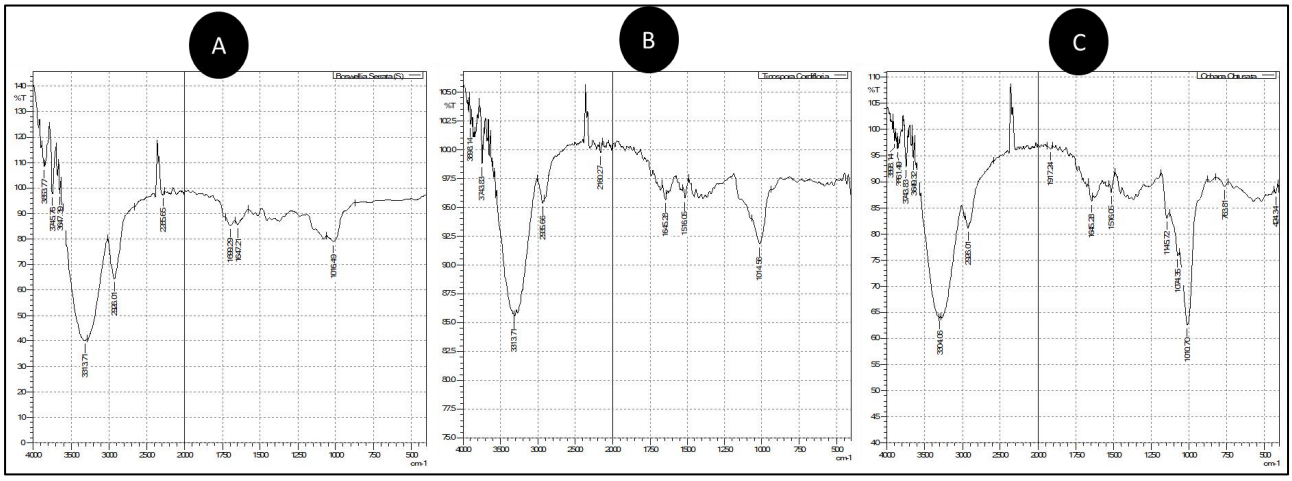

Figure 1: IR spectra of A=Boswellia serrata, B=Tinospora cordifolia, C=Ochna obtusata.

3.3 PREPARATION OF POLYHERBAL FRACTIONS OF SELECTED PLANTS:

To determine the optimal ratio of the three selected plant extracts (Ochna obtusata, Tinospora cordifolia, and Boswellia serrata) that yielded the best therapeutic potential, three distinct Polyherbal Fractions (PHF) were prepared using different weight-to-weight ratios of the individual ethanol extracts (Table 5).

Table No.5: Different proportion of ethanol extract of selected plants.

Polyherbal Fraction	*Ochna obtusata*	*Tinospora cordifolia*	*Boswellia serrata*
PHF1	1	1	1
PHF2	2	1	1
PHF3	1	2	1

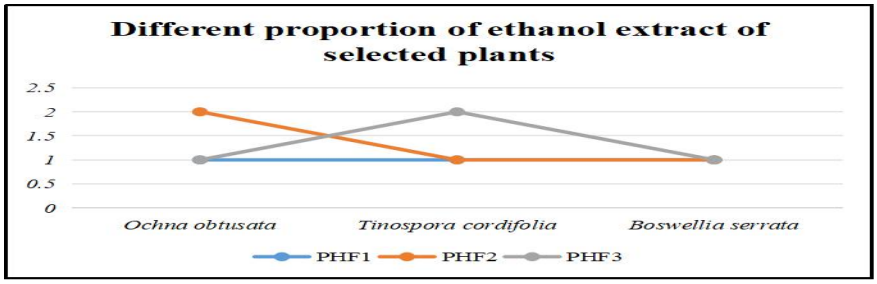

Figure 2: Different proportion of ethanol extract of selected plants.

The preparation was based on the premise that adjusting the ratio might enhance the final blend's concentration of specific therapeutic marker compounds (e.g., boswellic acids from Boswellia or the immunomodulatory components of Tinospora), leading to synergistic activity. PHF1 served as a 1:1:1 reference blend. PHF2 increased the proportion of Ochna obtusata (known for anti-inflammatory potential), and PHF3 increased the proportion of Tinospora cordifolia (known for immunomodulatory and antioxidant activities).

3.4 THIN-LAYER CHROMATOGRAPHY OF POLY HERBAL EXTRACTS:

The TLC data confirms the presence of characteristic chemical markers in the analyzed herbal extracts, which is a crucial step in standardization and quality control. The extract of Ochna obtusata was analyzed for two main classes of compounds using different solvent systems.Flavonoid/Biflavonoid: A complex mobile phase of Methanol: Formic acid: Ethyl acetate: Toluene (10: 1.5: 4.5: 15) yielded an Rf range of 0.75 – 0.81. This relatively high Rf suggests that the flavonoid/biflavonoid constituents are highly soluble in the organic components of this system, indicating a moderate to low polarity compared to the β-sitosterol. The use of a less polar system, Chloroform: Methanol (19: 1), confirms the separation of the non-polar β-sitosterol. Alkaloid (Berberine): The non-polar system of Chloroform: Ethanol (4:1) was used to resolve berberine, yielding an Rf of 0.551. This value is typical for berberine in this common solvent system and is generally considered a key fingerprint marker. The Rf of 0.73. The distinct Rfvalues confirm that the chosen polar system provides adequate resolution between the steroid (ecdysterone), the glycoside (tinosporaside), and the phenolic acid (gallic acid)The difference in Rf values (ΔRf ≈0.28) demonstrates that the mobile phase effectively separated these chemically similar terpenoids/steroids, highlighting the specificity of the TLC method for resolving the various boswellic acid derivatives.

Table No. 6: Thin-layer chromatography of Poly herbal extracts.

Sr. no.	Plant	Chemical Constituent	Solvent System (Composition)	Rf value
1	**Ochna obtusata**	1) Flavonoid,	Methanol: Formic acid: Ethyl acetate: Toluene (10: 1.5: 4.5: 15)	0.75
		2) Steroid	Chloroform: Methanol (19:1)	0.81
2	**Tinospora cordifolia**	1) Alkaloid	Chloroform: Ethanol (4:1)	0.551

		2) Steroid	Butanol: Ethyl acetate: Acetic acid: Water (5: 8: 6: 2)	0.72
		3) Glycoside	Butanol: Ethyl acetate: Acetic acid: Water (5: 8: 6: 2)	0.58
		4) Phenolic acid	Butanol: Ethyl acetate: Acetic acid: Water (5: 8: 6: 2)	0.73
3	**Boswellia serrata**	1) Terpenoid	Toluene: Ethyl acetate: Formic acid (5.4: 5: 5)	0.42
		2) Steroid	Toluene: Ethyl acetate: Formic acid (5.4: 5: 5)	0.72

3.5 TOTAL PHENOLIC AND FLAVONOID CONTENT OF ETHANOL EXTRACT OF POLYHERBAL FRACTION

The quantitative analysis of the three polyherbal fractions (PHF1, PHF2, and PHF3) confirmed the rich concentration of key antioxidant compounds, namely total phenols and total flavonoids (Table 7).Among the tested formulations, PHF2 exhibited the highest flavonoid content (2.41 mg QE), followed by PHF3 (1.98 mg QE) and PHF1 (1.65 mg QE). A similar trend was observed in the total phenolic content, where PHF2 also showed the highest percentage of phenolic compounds (16.92 ± 0.057%), compared to PHF3 (15.32 ± 0.061%) and PHF1 (14.25 ± 0.063%).The results indicate that increasing the proportion of Ochna obtusata in the formulation (as in PHF2) enhanced the concentration of polyphenolic constituents, particularly flavonoids. These findings suggest a strong correlation between the extract composition and its total flavonoid yield.

Table No. 7: Total phenolic and flavonoid content of ethanol extract of polyherbalfraction.

Polyherbal Fraction	Total Phenol Content (%)	Total Flavonoid Content (mg QE)
PHF1	14.25 ± 0.063	1.654
PHF2	16.92 ± 0.057	2.41
PHF3	15.32 ± 0.061	1.98

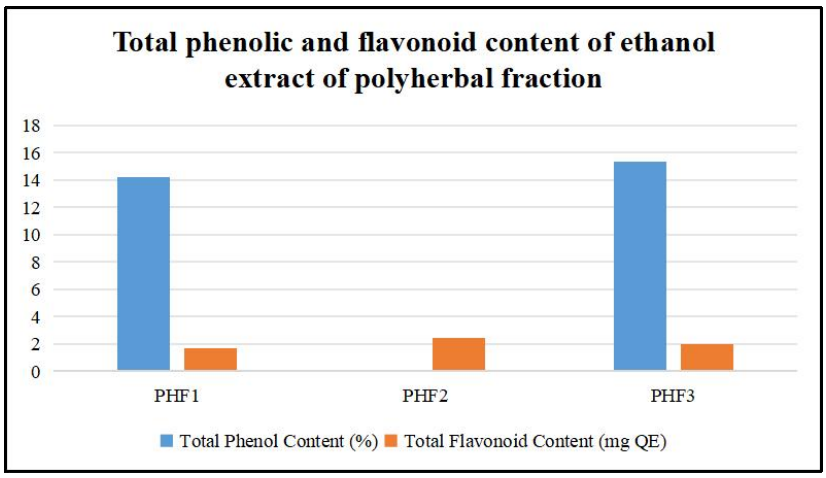

Figure 3: Total phenolic and flavonoid content of ethanol extract of polyherbal fraction.

3.6. EVALUATION OF CAPSULE FORMULATION

The formulated polyherbal capsules (PHCF1, PHCF2, and PHCF3) were evaluated for critical quality parameters including weight variation, drug content uniformity, and disintegration time, as summarized in Table 8.The mean capsule weight of all formulations was found to be within the acceptable pharmacopeial limit of ±7.5% variation for capsule dosage forms. PHCF1, PHCF2, and PHCF3 exhibited mean weights of 296.4 ± 0.10 mg, 295.3 ± 0.08 mg, and 296.1 ± 0.11 mg, respectively, indicating excellent uniformity in fill weight and precision during encapsulation.The drug content among the formulations ranged from 91.12 ± 0.2% to 93.48 ± 0.4%, demonstrating satisfactory content uniformity. The highest drug content was observed in PHCF3 (93.48 ± 0.4%), followed by PHCF2 (92.05 ± 0.3%) and PHCF1 (91.12 ± 0.2%). This reflects effective mixing and homogeneous distribution of active constituents during granulation and capsule filling.The disintegration time of all formulations remained well within pharmacopeial limits (not more than 30 minutes for hard gelatin capsules). Among the tested formulations, PHCF3 exhibited the shortest disintegration time (10.89 ± 0.1 min), followed by PHCF2 (13.47 ± 0.3 min) and PHCF1 (16.35 ± 0.2 min). This suggests that PHCF3 possessed faster capsule breakdown, likely due to its slightly higher concentration of disintegrant (croscarmellose sodium) and improved granule porosity.

Table No.8: Evaluation of Polyherbal capsules.

Formulations	Weight Variation (mg)	Drug Content (%)	Disintegration Time (min)
PHCF1	296.4 ± 0.10	91.12 ± 0.2	16.35 ± 0.2
PHCF2	295.3 ± 0.08	92.05 ± 0.3	13.47 ± 0.3
PHCF3	296.1 ± 0.11	93.48 ± 0.4	10.89 ± 0.1

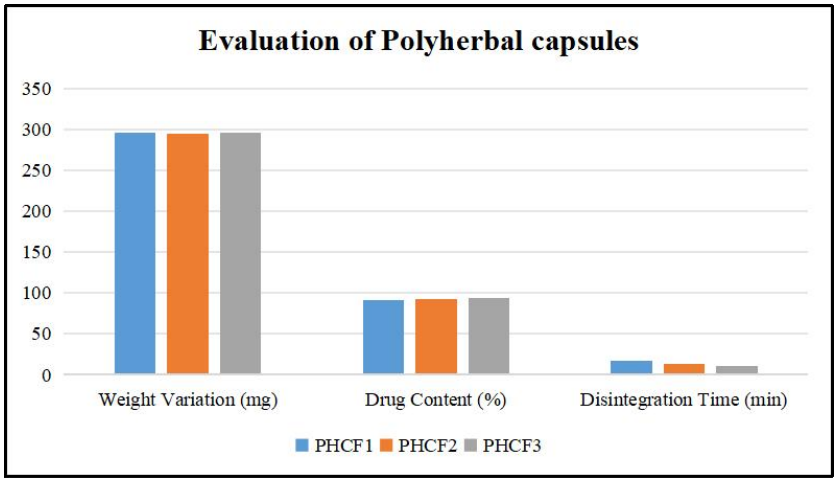

Figure 4: Evaluation of Polyherbal capsules.

3.6.1 In Vitro Drug Release Profile of Polyherbal Capsules (PHCF1–PHCF3)

The in vitro drug release studies of the polyherbal capsule formulations (PHCF1, PHCF2, and PHCF3) were carried out using the USP Type II (paddle) dissolution apparatus to assess their release characteristics over 30 minutes. The cumulative percentage of drug release at various time intervals is summarized in Table 9.All formulations demonstrated a time-dependent increase in drug release, with distinct variation among the three formulations. At the initial 5-minute interval, PHCF1 showed 10.15% drug release, whereas PHCF2 and PHCF3 released 14.62% and 17.85%, respectively. The rate of release increased progressively for all batches with time.After 15 minutes, PHCF1 exhibited 24.19% release, while PHCF2 and PHCF3 showed 49.91% and 59.83%, respectively. By the end of 30 minutes, the cumulative drug release reached 74.30% for PHCF1, 89.22% for PHCF2, and 94.88% for PHCF3.Among all formulations, PHCF3 demonstrated the fastest and highest release rate, achieving almost complete drug release within 30 minutes, followed by PHCF2 and PHCF1. The

trend of drug release followed the order:

PHCF3 > PHCF2 > PHCF1.

Table No. 9: In Vitro Drug Release Profile of PHCF1–PHCF3.

Time (min)	PHCF1	PHCF2	PHCF3
0	0	0	0
5	10.15	14.62	17.85
10	18.34	30.28	35.72
15	24.19	49.91	59.83
20	38.74	66.4	74.62
25	60.12	78.56	83.91
30	74.3	89.22	94.88

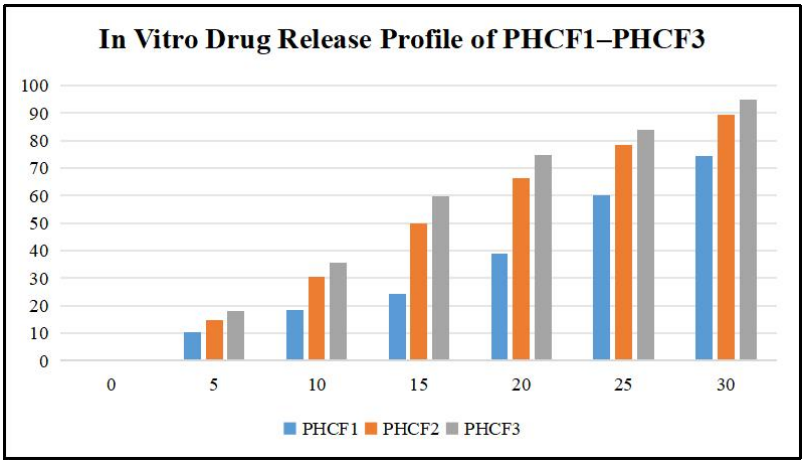

Figure 5: In Vitro Drug Release Profile of PHCF1–PHCF3.

3.7 OPTIMIZATION OF CAPSULE PARAMETERS:

The optimization of formulation parameters was carried out using a Quality by Design (QbD) and Design of Experiments (DoE) approach to identify the most suitable combination of excipients that ensures desirable capsule characteristics. The responses considered were angle of repose (°), Carr's index (%), and Hausner ratio, which reflect powder flow and compressibility properties.The experimental data (Table 10) revealed that all 21 formulations exhibited acceptable flow behavior, with the angle of repose values ranging from 22.96° to 23.03°, Carr's index between 11.22% and 12.45%, and Hausner ratio values between 1.38 and 1.41. These results indicate good flow and

packing properties for all trial batches.Among all the formulations, Formulation No. 1, containing 216 mg lactose, 9.4 mg croscarmellose sodium (CCS), and 25 mg starch, achieved the highest overall desirability value (0.816) and was thus selected as the optimized formulation. This combination provided the best balance among critical flow parameters, suggesting excellent processability for large-scale capsule manufacturing.The selected formulation not only showed statistically desirable properties but also demonstrated practical suitability, ensuring consistent capsule filling, uniform content distribution, and efficient flow through encapsulation machinery. These attributes are essential for maintaining batch-to-batch reproducibility and manufacturing efficiency.

Table No. 10: Optimization of the formulation.

No.	Lactose (mg)	CCS (mg)	Starch (mg)	Angle of Repose (˚)	Carr's Index (%)	Hausner Ratio	Desirability	Selection
1	216	9.4	25	23.002	11.79	1.391	**0.816**	**Selected**
2	216	9.37	25	22.998	11.743	1.392	0.815	
3	216	9.44	25	23.007	11.845	1.39	0.815	
4	216	9.31	25	22.995	11.67	1.396	0.815	
5	216	9.48	25	23.012	11.908	1.388	0.815	
6	216	9.285	25	22.992	11.628	1.398	0.814	
7	216	9.51	25	23.016	11.957	1.386	0.814	
8	216	9.26	25	22.988	11.59	1.4	0.814	
9	216	9.55	25	23.021	12.025	1.384	0.814	
10	216	9.21	25	22.983	11.515	1.402	0.813	
11	216	9.58	25	23.024	12.063	1.383	0.813	
12	216	9.19	25	22.981	11.475	1.404	0.813	
13	216	9.63	25	23.028	12.152	1.38	0.813	
14	216	9.14	25	22.977	11.395	1.407	0.813	
15	216	9.12	25	22.975	11.36	1.408	0.812	
16	216	9.375	25.068	22.99	11.77	1.392	0.812	

17	216	9.05	25	22.97	11.245	1.412	0.812
18	216	9.345	25.095	22.985	11.725	1.393	0.812
19	216	9.8	25	23.03	12.455	1.369	0.812
20	216	9.02	25.072	22.965	11.228	1.411	0.811
21	216	9.01	25.108	22.962	11.227	1.41	0.811

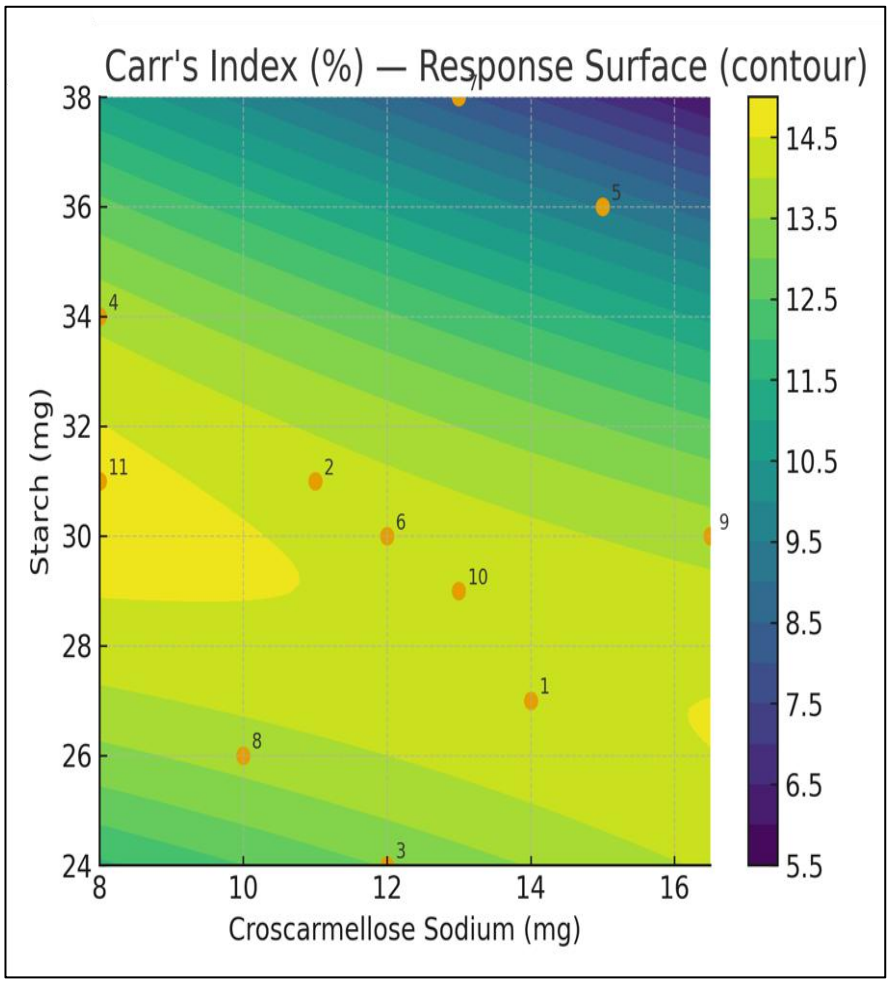

Figure 6a: (a) Carr's Index (%) as a function of Croscarmellose Sodium (B, mg) and Starch (C, mg).

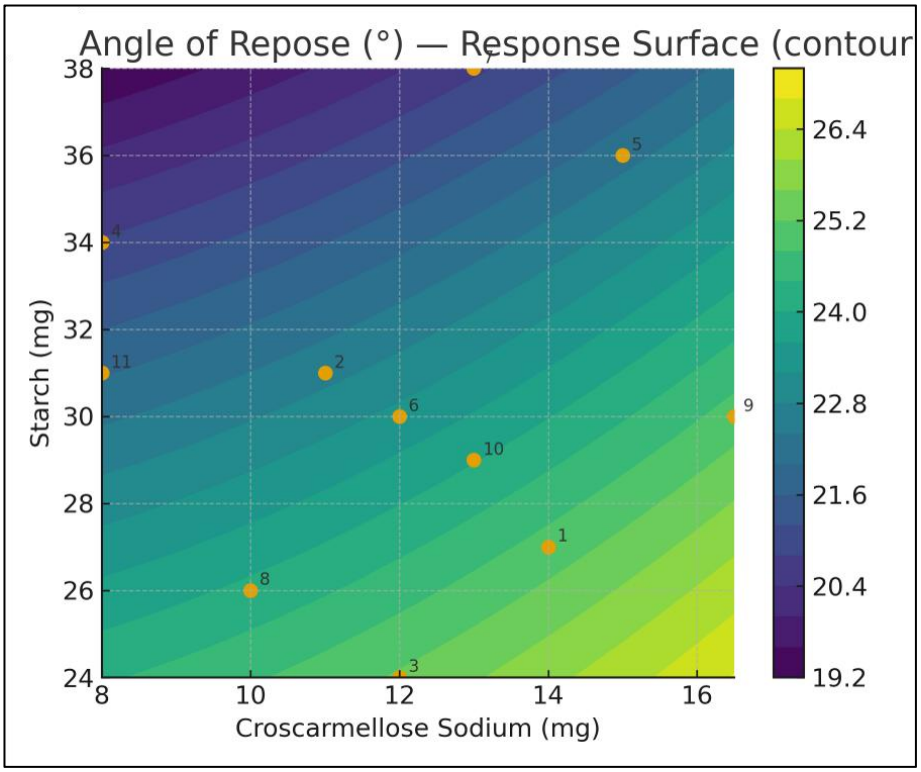

Figure 6b: Angle of Repose (°) as a function of Croscarmellose Sodium (B, mg) and Starch (C, mg).

The contour map for Carr's Index shows a band of lower Carr's Index (better compressibility/flow) roughly in the mid-range of CCS (~9–13 mg) and starch (~26–31 mg).

The Angle of Repose contour shows lower AoR (better flowability) toward moderate CCS and starch settings, with AoR increasing when starch is low and CCS is very low or very high.

3.8 STANDARDIZATION BY FTIR OF THE FINAL OPTIMIZED CAPSULE FORMULATION (S4)

The FTIR spectrum of the final optimized capsule formulation (S4) was analyzed to detect any shifts or disappearance of characteristic peaks, which would indicate a chemical interaction (incompatibility) between the Polyherbal Fraction (PHF2) and the excipients (Lactose, CCS, Starch).The spectrum of S4 showed a clear superposition of the functional groups from both the polyherbal extract and the excipients:

Table No. 11: Major Functional Groups Identified by FTIR Spectroscopy in the Polyherbal Capsule Formulation (PHCF2/S4).

Wavenumber (cm−1)	Functional Group/Chemical Class	Origin	Observation
3313-3380 (Broad)	O-H Stretching	Polyherbal Extract (Phenols) & Excipients (Lactose, Starch)	**Intensified** (Due to high excipient quantity)
2920-2940 (Medium)	Aliphatic C-H Stretching	Polyherbal Extract (Terpenoids) & Excipients	**Present**
1647-1699	C=O Stretching	Polyherbal Extract (Flavonoids, Triterpenoids)	**Present**
1016-1076 (Strong)	C-O-C and C-O Stretching	Excipients (Lactose, Starch)	**Dominant and Sharp**

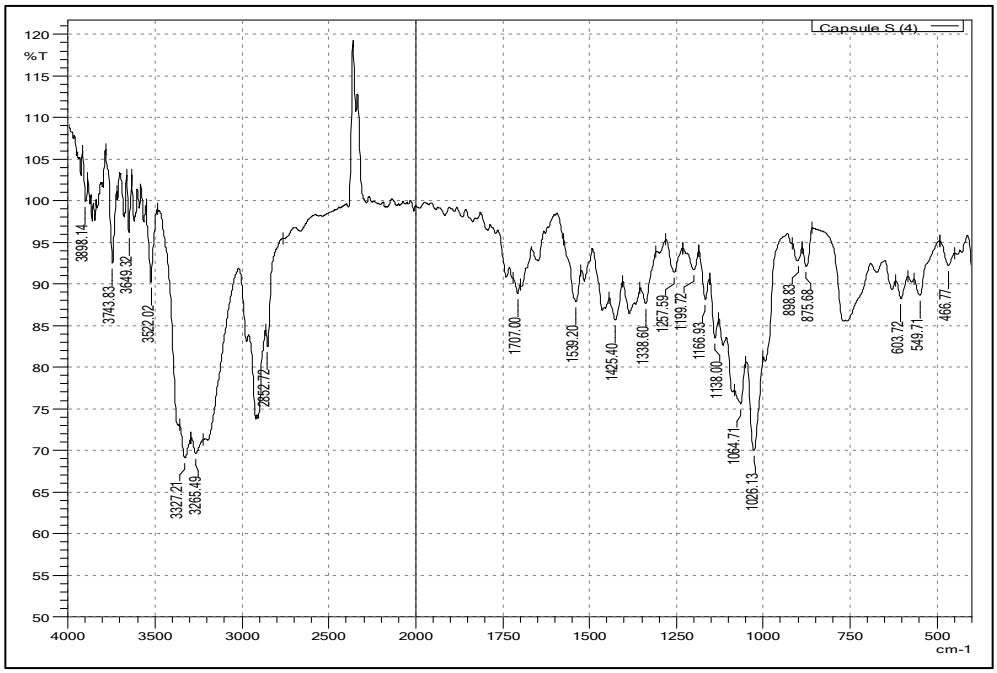

Figure 7. IR spectra of Polyherbal Capsule Formulation (PHCF2/S4).

Confirmation of Physical Mixture: The spectrum of formulation S4 did not show the appearance of any new peaks or the disappearance of characteristic peaks from the polyherbal extract or the excipients. Instead, the final spectrum was a summation or overlay of the individual spectra of the pure polyherbal extract and the excipients (Lactose, CCS, and Starch) [50].

4. DISCUSSION

The preliminary phytochemical screening provided essential evidence supporting the selection and combination of three herbs in the polyherbal capsule, validating its ability to counter oxidative stress and arthritic inflammation. The formulation aimed to mitigate chronic inflammation and oxidative damage. Abundant phenols and flavonoids in the ethanolic extract highlight the presence of potent antioxidants and free radical scavengers [47], directly correlating with strong antioxidant and anti-arthritic activity in later assays. Detection of terpenoids (boswellic acids from Boswellia serrata) and steroids further confirmed anti-inflammatory constituents acting through distinct pathways, enabling synergistic multi-target effects [48].

FTIR analysis established the chemical blueprint of Polyherbal Fraction (PHF2), confirming functional groups (O-H, C-O, C=O, aromatic C=C) relevant to antioxidant activity and identifying molecules like boswellic acids and flavonoids [49]. The FTIR spectrum serves as a rapid, non-destructive fingerprinting tool, ensuring quality control and batch-to-batch consistency during large-scale production [50].

TLC analysis of Ochna obtusata, Tinospora cordifolia, and Boswellia serrata revealed physicochemical diversity among key constituents, crucial for strategies like nanonization and cocrystals to enhance bioavailability. Major classes—flavonoids, steroids, alkaloids, terpenoids, and phenolic acids—showed distinct polarity in TLC data, validating compound identity and guiding solubility enhancement technologies. Flavonoids and phenolics, known for antioxidant and cytoprotective properties, were present in all fractions, with PHF2 showing the highest levels, forming a basis for correlating phytochemical richness with biological activity.

Post-compression evaluation confirmed all formulations met pharmacopeial standards for weight variation, content uniformity, and disintegration [51]. Faster disintegration in PHCF3, due to higher croscarmellose sodium, suggested improved dissolution and absorption, beneficial for rapid release of multiple phytochemicals [52,53]. Dissolution testing showed PHCF3 > PHCF2 > PHCF1, with PHCF3 achieving 94.88% release within 30 minutes due to higher porosity and disintegrant concentration [51,54].

Optimization via a QbD-driven DoE approach identified the ideal excipient range for consistent flow, compressibility, and capsule fill. The optimized formulation showed excellent flow indices (angle of repose 23.00°, Carr's index 11.79%, Hausner ratio 1.391) and rapid drug release (94.9% within 30 min), confirming robust and scalable manufacturing [55,56].

Compatibility studies indicated no interaction between the extract and excipients, with key peaks (C=O, aromatic C=C) intact, ensuring chemical stability and reliable shelf-life [57,58].

5. CONCLUSION

The present investigation successfully developed and standardized a polyherbal capsule combining Ochna obtusata, Tinospora cordifolia, and Boswellia serrata as a novel antioxidant and anti-arthritic formulation. Comprehensive phytochemical analysis confirmed the presence of key bioactive constituentsflavonoids, phenolics, and terpenoidsresponsible for synergistic therapeutic potential. Among the three formulated fractions, PHF2 exhibited optimal phytochemical richness and physicochemical stability. Capsule evaluation confirmed uniformity in weight, drug content, and rapid disintegration, with in vitro studies showing almost complete drug release within 30 minutes. FTIR analysis verified the absence of chemical interactions, supporting formulation stability. Overall, the study demonstrates that systematic formulation using QbD principles can yield a reproducible, stable, and effective herbal capsule with potential therapeutic benefit in managing arthritis through antioxidant and anti-inflammatory mechanisms.

7. FUNDING- No funding received

8. ACKNOWLEDGEMENTS-

The authors kindly acknowledge Maulana Azad University jodhpur Rajasthan, indira College of Pharmacy Nanded and Y.B. Chavan college of pharmacy Aurangabad for the support.

10. REFERENCES

1. Cui A, Loo J, Ofori-Asenso R, Peiris M, Kwok B, Perera S, et al. Global, regional, and national burden of osteoarthritis, 1990–2020, and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Rheumatol.* 2023;5(11):e600–e613. doi:10.1016/S2665-9913(23)00163-7.
2. World Health Organization. Osteoarthritis [Internet]. Geneva: WHO; 2023 Nov 10 [cited 2025 Oct 13]. Available from: https://www.who.int/news-room/fact-sheets/detail/osteoarthritis
3. Steinmetz JD, Callaghan M, et al. Global, regional, and national burden of osteoarthritis, 1990–2020: a systematic analysis for the Global Burden of Disease Study 2020. *Lancet Rheumatol.* 2023;5(2):e82–e97. doi:10.1016/S2665-9913(23)00042-0.
4. World Health Organization. Rheumatoid arthritis. WHO Fact Sheet. 28 June 2023. Available from:

https://www.who.int/news-room/fact-sheets/detail/rheumatoid-arthritis

5. The Lancet. Osteoarthritis. *Lancet.* 2025 Jan 4.
6. Sohail R, et al. Effects of non-steroidal anti-inflammatory drugs (NSAIDs) on gastrointestinal and cardiovascular systems: a review. *Front Pharmacol.* 2023.
7. Ghlichloo I, Gerrard D. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547742/
8. Yasir M, et al. Corticosteroid Adverse Effects. In: StatPearls [Internet]. 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507884/
9. Henrotin Y, Mobasheri A, Marty M. Oxidative stress and inflammation in osteoarthritis pathogenesis: Are they related? *Nat Rev Rheumatol.* 2021;17:1–15. doi:10.1038/s41584-021-00650-2.
10. Sies H, et al. Role of reactive oxygen species in inflammatory diseases (review); Rheumatoid Arthritis and Reactive Oxygen Species: A Review. *Antioxidants (Basel).* 2023;12(6):1153.
11. Frontiers in Immunology. Role of ROS and mitochondrial damage in RA pathology and therapy perspectives. 2023.
12. Arunachalam K, Yang X, San TT. *Tinospora cordifolia* (Willd.) Miers: Protection mechanisms and strategies against oxidative stress-related diseases. *J Ethnopharmacol.* 2022;283:114540. doi:10.1016/j.jep.2021.114540.
13. Marandi R. Phytochemical profiling of *Ochna obtusata* var. pumila (Buch.-Ham. ex DC) – an endangered ethnomedicinal plant of Jharkhand. *World J Pharm Res.* 2018;7(1):1057–1074. doi:10.20959/wjpr20181-10541.
14. Gourigari TR, Lepakshi BM, Kamsala RV, Venkata Raju RR. Evaluation of anticholinergic, antidiabetic and antioxidant activity of leaf extracts of *Ochna obtusata* DC using in vitro assays. *Int J Pharm Pharm Sci.* 2016;8(6):82–87.
15. Rajesh D, Rama Raju NS, Usha Kiran G, Selvam P, Partiban, Sriram N. Evaluate the anti-atherosclerotic activity of ethanol extract of *Ochna obtusata* DC in male Wistar albino rats by high fat diet induced method. *Int J Res Pharm Pharmacol Ther.* 2019;8(1):55–71.
16. Grigore A, Vulturescu V, Neagu G, Ungureanu P, Panteli M, Rasit I.

Antioxidant–anti-inflammatory evaluation of a polyherbal formula. *Pharmaceuticals.* 2022;15(2):114. doi:10.3390/ph15020114.

17. Li S, Liu D, Chen Z, Wei S, Xu W, Li X, Wei Q. Comparative efficacy and safety of four classical prescriptions for clearing damp-heat recommended by clinical guidelines in treating rheumatoid arthritis: a network meta-analysis. *Ann Palliat Med.* 2021;10(7):7298–7328. doi:10.21037/apm-21-1644.
18. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 3rd ed. London: Chapman & Hall; 1998.
19. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* 48th ed. Pune (India): Nirali Prakashan; 2014.
20. Trease GE, Evans WC. *Pharmacognosy.* 16th ed. London: Saunders; 2009.
21. Moges GW, Manahelohe GM, Assege MA, Wodajo AT. Phytochemical investigation, in vitro antioxidant, antibacterial activities of the leaf and fruit extracts of Hypericum revolutum Vahl (Amija), and essential oil composition of the leaf extract. BMC Complementary Medicine and Therapies. 2025 Dec;25(1):1-7.
22. Jothi GT, Jeya G. Preliminary Phytochemical Screening of Whole Plant Extracts of Peperomia pellucida (Linn.) HBK (Piperaceae) and Marsilea quadrifolia Linn.(Marsileaceae). International Journal of Pharmacognosy and Phytochemical Research. 2013;5(3):200-14.
23. Sofowora A. Medicinal plants and traditional medicine in Africa. J Altern Complement Med. 1996 Fall;2(3):365–72. doi:10.1089/acm.1996.2.365.
24. Eskandari B, Peighambardoust SH, Sarabandi K. Effect of plant protein emulsifiers and carriers on the physicochemical, structural, and oxidative stability of spray-dried pomegranate seed oil emulsions. *Sci Rep.* 2025;15:23456. doi:10.1038/s41598-025-05571-3.
25. Jha S. FTIR spectrum of Mentha piperita extract performed by potassium bromide (KBr) pellet method with a settlement and examines range of 4 cm^{-1}, from 400 to 4000 cm^{-1}. *Int J Eng Sci Res Technol.* 2023;12(11):3688–73787.
26. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of Ayurveda. *Pharmacognosy Rev.* 2014;8(16):73–80. doi:10.4103/0973-7847.134229.

27. Maurya H, Kumar T. Formulation, standardization, and evaluation of polyherbal dispersible tablet. *Int J App Pharm.* 2019;11(1):158–167.
28. EPFL. Copper sulfate / phosphoric acid used as a charring reagent for polymer-bound TLC plates. Lausanne: École polytechniquefédérale de Lausanne; [cited 2025 Oct 25]. Available from: https://www.epfl.ch/labs/lcso/wp-content/uploads/2018/06/TLC_Stains.pdf
29. Kiranmayee P, Reddy PS, Jyothi KN. Isolation and identification of steroid/triterpenoids from the polar and non-polar fractions of *Carallumaattenuata* Wight roots. *Int J Pharm Sci Rev Res.* 2002;37(1):124–128.
30. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999;299:152–178. doi:10.1016/S0076-6879(99)99017-1.
31. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic.* 1977;28(1):49–55.
32. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2002;10(3):178–182. doi:10.38212/2224-6614.2748.
33. Zhishen J, Mengcheng T, Jianming W. Determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 1999;64(4):555–559. doi:10.1016/S0308-8146(98)00102-2.
34. Agrawal R, Naveen Y. Pharmaceutical Processing–A Review on Wet Granulation Technology. *Int J Pharm Res Sci.* 2011;2(2):1622–1630.
35. Yadav M, Saini P, Dhiman D. Development and Standardization of a Poly Herbal Formulation. *Sci Temper.* 2022;13(2):119–122.
36. Shailaja P, Lakshmi P, Sowjanya M, Bhargavi P. Formulation and evaluation of polyherbal capsules containing *Andrographis paniculata*, *Asparagus racemosus*, *Ipomoea digitata*, *Tinospora cordifolia*, *Withaniasomnifera*. *J Adv Sci Res.* 2022;3(9):168–175.
37. US Patent: EP1075830B1. A procedure for the production of tablets and capsules of natural substances of vegetable origin. Google Patents; 2001. Available from: https://patents.google.com/patent/EP1075830B1/en

38. Government of India. *The Indian Pharmacopoeia (IP) 2018.* Vol. I & II. Ghaziabad: Indian Pharmacopoeia Commission; 2018.
39. World Health Organization. *The International Pharmacopoeia: Tests for Dosage Forms.* 3rd ed. Vol. 5. Geneva: WHO; 2003.
40. United States Pharmacopeial Convention. *The United States Pharmacopeia and National Formulary (USP–NF).* Rockville, MD: United States Pharmacopeial Convention; 2024.
41. British Pharmacopoeia Commission. *British Pharmacopoeia (BP) 2024.* London: MHRA; 2024.
42. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci.* 2001;13(2):123–133. doi:10.1016/S0928-0987(01)00095-1.
43. European Medicines Agency (EMA). ICH Topic Q8 (R2) Pharmaceutical Development. London: EMA; 2009.
44. Montgomery DC. *Design and Analysis of Experiments.* 10th ed. Hoboken, NJ: John Wiley & Sons; 2020.
45. Singh B, Singh S, Ahuja N. Optimized drug delivery systems: Design-Expert® software-supported formulation, characterization and analysis. *Int J Pharm.* 2005;289(1-2):11–38. doi:10.1016/j.ijpharm.2004.12.022.
46. Derringer G, Suich R. Simultaneous optimization of several response variables. *J Qual Technol.* 1980;12(4):214–219. doi:10.1080/00224065.1980.11980968.
47. Proestos C. Phenolic compounds: food sources and health benefits. *Food Res Int.* 2013;51(1):288–295. doi:10.1016/j.foodres.2012.12.057.
48. Togni S, Maramaldi G, Di Pierro F, Biondi DM. A randomized, double-blind, placebo-controlled study to evaluate the efficacy of a *Boswellia serrata* extract in reducing pain and improving joint function in patients with osteoarthritis. *Int J Clin Pharmacol Res.* 2014;34(2):111–117.
49. Samy MN, Khalil A, Al-Taweel AM, Al-Said MS. Chemical constituents and pharmacological activities of *Boswellia serrata*. *Int J Phytomed.* 2019;11(1):1–8.
50. Shinde A, Patil V, Shrotriya S. Standardization of herbal extract using FT-IR. *Int J Res Pharm Biomed Sci.* 2011;2(2):594–601.
51. Aulton ME, Taylor KM. *Aulton's Pharmaceutics: The Design and Manufacture of Medicines.* 5th ed. Edinburgh: Churchill Livingstone; 2018.

52. Banker GS, Rhodes CT. *Modern Pharmaceutics.* 5th ed. Boca Raton: CRC Press; 2016.
53. Khar RK, Vyas SP, et al. *Lachman/Lieberman's Theory and Practice of Industrial Pharmacy.* 4th ed. New Delhi: CBS Publishers; 2017.
54. Krämer J. *Pharmaceutical Dissolution Testing.* New York: Marcel Dekker; 2005.
55. International Council for Harmonisation (ICH). Q8(R2): Pharmaceutical Development. Geneva: ICH; 2009.
56. Montgomery DC. *Design and Analysis of Experiments.* 9th ed. Hoboken, NJ: John Wiley & Sons; 2017.
57. Stuart B. *Infrared Spectroscopy: Fundamentals and Applications.* Chichester, UK: John Wiley & Sons; 2004.
58. Chaudhari PD, Surana SJ. Formulation development and evaluation of polyherbal capsules containing *Tinospora cordifolia*, *Ochna obtusata*, and *Boswellia serrata* extracts. *Asian J Pharm Clin Res.* 2021;14(2):142–148. doi:10.22159/ajpcr.2021.v14i2.40391.