Journal for Re Attach Therapy and Developmental Diversities
EISSN: 2589-7799
2024 January; 7 (1): 101-112

Design, Development, And Evaluation of a Polyherbal Formulation for Managing Hepatic Disorders

Dipali Kolhe[1*], Ajit Kaur[2]

[1*,2]Department of Pharmaceutical Science, Monad University, Pilkhuwa UP India 245304.

***Corresponding author:** Dipali Kolhe
*Department of Pharmaceutical Science, Monad University, Pilkhuwa UP India 245304,
Email ID: dipalikolhe9@gmail.com

Abstract

This research delves into the comprehensive evaluation of capsules and soft chewable capsules formulated with extracts from five medicinal plants: Swarnakshiri (Argemone Mexicana Linn.), Haridra (Curcuma longa Linn.), Pipali (Piper longum Linn.), Bhringraj (Eclipta alba (L.) Hassk.), and Guduchi (Tinospora cordifolia (Willd.) Hook. f.). The investigation begins with a thorough morphological and microscopical study to authenticate the selected plants. Subsequently, thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) profiles are employed to identify the key phytoconstituents in the formulations. Determination of λmax by UV spectrometry provides insights into the absorption characteristics of the herbal extracts. Additionally, drug-drug interaction studies using Fourier Transform Infra-Red (FTIR) spectroscopy ensure the safety and compatibility of the combined formulations. The evaluation of capsules encompasses appearance, dissolution, disintegration, and absence of heavy metals and microbial contamination. Furthermore, nutritional analysis sheds light on the carbohydrate, protein, and cholesterol content in the formulations. Through this systematic approach, the research aims to elucidate the pharmaceutical potential and safety profile of herbal capsules and soft chewable capsules derived from the aforementioned medicinal plants.

Keywords: Herbal capsules; Phytoconstituents; Formulation development; Heavy metal analysis; Microbial contamination

1.Introduction

Hepatic disorders represent a significant public health concern worldwide, encompassing a spectrum of conditions ranging from fatty liver disease to more severe ailments such as cirrhosis and hepatocellular carcinoma. The prevalence of hepatic disorders continues to rise, driven by factors such as sedentary lifestyles, unhealthy dietary habits, alcohol consumption, viral hepatitis infections, and the increasing prevalence of metabolic syndrome [1].

Despite advances in modern medicine, the management of hepatic disorders remains challenging, often necessitating a multifaceted approach involving lifestyle modifications, pharmacotherapy, and, in severe cases, liver transplantation. However, conventional treatments may be limited by factors such as adverse side effects, incomplete efficacy, and high costs, underscoring the need for alternative therapeutic strategies.

In recent years, there has been growing interest in herbal medicines as potential adjunctive or alternative treatments for hepatic disorders. Herbal formulations offer a rich source of bioactive compounds with diverse pharmacological properties, including antioxidant, anti-inflammatory, and hepatoprotective effects. Moreover, many medicinal herbs have a long history of traditional use in various cultures for the management of liver ailments, providing a valuable foundation for scientific exploration [2].

The present study aims to contribute to this burgeoning field by designing, developing, and evaluating a polyherbal formulation specifically targeted at managing hepatic disorders. Drawing upon the rich tradition of herbal medicine and contemporary scientific methodologies, our research endeavors to harness the therapeutic potential of medicinal plants in combating liver diseases.

Through a comprehensive approach encompassing phytochemical analysis, in vitro assays, animal studies, and human clinical trials, we seek to elucidate the safety and efficacy profile of the polyherbal formulation. By rigorously evaluating its hepatoprotective properties, we aspire to offer a novel therapeutic option that addresses the unmet needs of individuals afflicted by hepatic disorders. This study represents a concerted effort to bridge the gap between traditional knowledge and modern science in the pursuit of effective treatments for hepatic disorders. By harnessing the healing power of nature through the development of a polyherbal formulation, we aim to make tangible strides towards improving the quality of life and clinical outcomes for patients grappling with liver diseases [4].

2. Material and Method

2.1 Material

The herbal formulation for managing hepatic disorders was developed using meticulously selected plant materials and chemicals. Plants such as Swarnakshiri, Guduchi, Haridra, Pipali, and Bhringraj were chosen for their hepatoprotective properties and sourced from reputable suppliers. Additionally, chemicals including jaggery, capsule shells, starch, MCC pH 102, and glycerin were used in the formulation process. State-of-the-art instruments such as extraction units, analytical balances, chromatography systems, and spectrophotometers were employed for extraction, formulation, and analysis. This careful selection of materials and equipment underscores our commitment to maintaining high-quality standards throughout the research process, aiming to optimize the efficacy and safety of the polyherbal formulation.

2.2 Selection of Plant Materials for the Study

The selection of plant materials for this study involved a meticulous process based on several literature surveys and an extensive review of folk medicine and traditional knowledge. After careful consideration, the following plants were chosen: Swarnakshiri, Guduchi, Haridra, Pipali, and Bhringraj. These plants were selected for their reputed hepatoprotective properties and their historical use in traditional medicine systems. To ensure authenticity, each plant was subjected to rigorous authentication procedures.

2.3 Cultivation, Collection, and Authentication Study of Plants

The selected plants underwent systematic cultivation following standard guidelines to ensure uniformity and quality. Upon reaching maturity, the plants were carefully collected and dried under shade to preserve their phytochemical constituents. Additionally, herbarium specimens were prepared for each plant, documenting their morphological characteristics. These specimens were then authenticated by experts from the Department of Botany to confirm their botanical identity and purity, ensuring the reliability and reproducibility of subsequent experiments and analyses [5].

2.4 Morphological and Microscopical Study of Selected Plants

The morphological and microscopical characteristics of the selected plants, including Swarnakshiri, Guduchi, Haridra, Pipali, and Bhringraj, were meticulously examined to ensure their botanical authenticity and quality.

For each plant, various morphological features such as leaf shape, size, color, and venation pattern were observed under a stereomicroscope. Additionally, microscopic examination of plant parts, including stems, leaves, and roots, was conducted to identify diagnostic features such as trichomes, stomata, and glandular structures. Furthermore, histological sections of plant tissues were prepared and examined under a compound microscope to assess cellular structures and arrangements. This analysis helped in confirming the identity of each plant species and ensuring the absence of adulterants or contaminants. The morphological and microscopical studies were essential steps in the authentication process, providing valuable insights into the botanical identity and quality of the selected plants. These analyses helped to establish a foundation of trustworthiness and reliability for subsequent experiments and analyses conducted on the herbal formulation developed for managing hepatic disorders [6].

2.4 Extraction of Selected Plants by Soxhlet Extraction Method

Soxhlet extraction, a classic method in herbal extraction, involves continuous cycling of a solvent through a sample. The process ensures thorough extraction of target compounds from plant material. Swarnakshiri roots, Guduchi stems, Haridra rhizomes, Pipali fruits, and Bhringraj aerial parts were extracted using specific solvents for varying durations. Extracts were then dried and subjected to successive extraction with different solvents to obtain a wide range of secondary metabolites for pharmacological analysis.

2.5 Chromatographic Study of Extract

Chromatographic fingerprint analysis was conducted to identify and quantify marker compounds in plant extracts. Thin-layer chromatography (TLC) plates precoated with silica gel 60F-254 were used, and spotting was done with a CAMAG Linomat V Automatic Sample Spotter. A CAMAG glass twin trough chamber was utilized for chromatography, with a CAMAG TLC scanner 3 linked to WINCATS software for densitometry. Specific mobile and stationary phases were employed for each plant extract. Derivatization using Dragendorff's reagent aided compound visualization.

Chromatogram development involved placing loaded TLC plates in a solvent-saturated glass chamber until 80% development. Plates were then air-dried, and scanning was performed using a CAMAG TLC Scanner-3 at specific wavelengths. Retention factor (Rf) values and area under curve (AUC) were determined using WINCATS software, facilitating marker compound identification and quantification [7].

2.6 Drug-Drug Interaction Analysis
In the formulation process, the components may exhibit either positive or negative interactions with each other, potentially influencing the overall efficacy and safety of the formulation. These interactions could be synergistic, enhancing each other's effects, or antagonistic, counteracting each other's effects. To investigate these interactions, a drug-drug interaction study was conducted using Fourier Transform Infrared (FTIR) spectroscopy [8].

2.6.1 Drug-Excipient Interaction Assessment
Similarly, in the formulation, the drug and excipients may interact positively or negatively, impacting the final product's performance. These interactions can also be synergistic or antagonistic, affecting the overall therapeutic outcome. A thorough investigation of drug-excipient interactions was carried out using FTIR spectroscopy to assess the compatibility and potential impact on formulation stability and efficacy.

2.6.2 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis
FT-IR spectroscopy was utilized for qualitative identification of compounds present in pure extracts and their physical mixtures with various excipients. Spectra were obtained by directly placing the extract and physical mixture on the diamond sampling window of the FT-IR spectrophotometer (Agilent Technologies Cary 650 FT-IR) and analyzing in the range of 4000-650 cm^{-1}. This analysis was crucial for understanding the chemical composition and compatibility of the extracts with formulation excipients, aiding in formulation optimization and ensuring product quality.

2.7 Formulation and Development of Capsule
Capsules containing extracts of Argemone Mexicana, Guduchi, and Piper longum were prepared using the following method:

2.7.1 Preparation of Granules
Granulation of the extracts from the three plants was performed using the wet granulation method. Wet granules were obtained by mixing the solid dry extract (SDE) with 10% (w/w) of MCC PH 102 as a binder in an acetone solution at a concentration of 12.5% (w/v). The mixture was blended thoroughly until achieving the desired consistency for granulation and then passed through a sieve with a nominal aperture of 1 mm. The resulting granules were dried in a circulating air oven at 25 ℃ for 2 hours, screened, and stored for further processing.

2.7.2 Evaluation of Size Distribution and Flow Properties of Granules [9]
The prepared granules were screened and sorted into coarse and fine granules using a 425 μm sieve. The flow properties of the granules intended for encapsulation were assessed using bulk density measurements (Hausner ratio and Car's index) and the angle of repose (determined by the fixed height cone method).

Determination of Granules Particle Size:
Particle size analysis was conducted to assess the uniformity of particle size, which is critical for ensuring optimal therapeutic efficacy. Granules were passed through different sieves with mesh #40 and #60 to obtain an equivalent diameter and interpret the particle size distribution.

Determination of Angle of Repose:
The angle of repose, a measure of the flow properties of powders, pellets, or granules, was determined by pouring the granules onto a level, flat surface to form a conical heap. The included angle with the horizontal was then measured to determine the angle of repose using the formula $\tan(\theta) = h/r$, where h represents the height of the heap and r denotes the radius of the heap.

2.8 Formulation of Soft Chewable Capsules
Soft chewable capsules containing a blend of Argemone Mexicana, Guduchi, and Piper longum extracts were prepared by mixing them with MCC pH 102, starch, talc, jaggery, and glycerin. The extracts were combined in a ratio of 1:0.5:0.1, respectively, and blended to form a uniform paste. After drying in a hot air oven and sieving, starch was added to the granules, followed by lubrication with talc. The granules were then filled into size 1 hard gelatin capsule shells using a hand-filling capsule machine. Different formulations (F1 to F8) were prepared by adjusting the quantities of MCC pH 102, starch, and excipients. Evaluation of physical appearance, weight, and color led to the selection of the optimal batch for further studies [10].

Table 1: Formula for batch preparation of capsule.

Sr. No	Ingredients	Quantity per capsule (mg)							
		F1	F2	F3	F4	F5	F6	F7	F8
1.	**Curcuma longa**	60	60	60	60	60	60	60	60
2.	**Eclipta alba**	30	30	30	30	30	30	30	30
3.	**MCC pH 102**	190	200	90	300	240	150	195	280
4.	**Jaggery**	14000	14000	14000	14000	14000	14000	14000	14000
5.	**Glycerin**	QS	QS	QS	QS	QS	QS	QS	QS

2.9 Evaluation of Capsules

Appearance:

The visual appearance of each formulation was assessed against a black backdrop to determine clarity, an important aspect for liquid filler formulas. Specific gravity, total solids, fat content, and sugar content were also evaluated.

pH Determination:

The pH of the soft chewable capsules was measured using a pH meter to ensure it fell within the range of 2.5 to 7.5, suitable for oral formulations.

Drug Content Determination:

The drug content of the capsules was determined by diluting the formulation with distilled water and measuring the absorbance at 254 nm using a UV-visible spectrophotometer.

Weight Variation Test:

Twenty capsules were randomly selected from the batch, and their individual weights were measured. The average weight was determined and compared to the theoretical weight of each capsule. The percent weight variation was calculated according to USP specifications, ensuring that the weight of each capsule fell within the range of 90 to 110 percent of the theoretical weight.

Disintegration Test:

Six capsules were chosen from the formulation, and their disintegration time was determined using disintegration test equipment. The capsules were immersed in simulated gastric fluid (SGF, pH 1.2) and monitored for the time required to break down into particles small enough to pass through a 10 mesh screen.

Dissolution Test:

The dissolution study was conducted using USP dissolution test apparatus. Selected batches of capsules were tested in pH 6.8 phosphate buffer and 0.1 N HCl at 37.5°C. Samples were collected at various time points, and their absorbance was measured spectrophotometrically to assess dissolution profiles.

Moisture Content Determination:

The capsules were stored under controlled temperature (15-25°C) and relative humidity (45-55%) conditions to maintain moisture content. Moisture levels were monitored to prevent capsules from becoming too flaccid or brittle, which could affect stability [11].

2.10 Chromatographic Study of Formulated Products

In the chromatographic study of the formulated products, aluminum plates coated with Silica Gel 60F254 were used as the stationary phase. These plates, sized 10 × 10 cm with a thickness of 0.2 mm, were prepared by pre-washing with methanol and activation at 60°C for 5 minutes before chromatography. For analysis, 1 g of extract and an equivalent of the final product were weighed and placed in separate iodine flasks. Each flask received 50 ml of methanol and underwent refluxing for an hour. Following filtration, 1-2 cc of the filtrate was concentrated for HPTLC fingerprinting. HPTLC was performed using the concentrated solution, with a single 6 mm band applied via the CAMAG LINOMAT V apparatus at concentrations of 5 and 10 µl for both the extract and final product solution. Plate development occurred for 60 minutes in a CAMAG glass twin-through chamber saturated with the solvent system (methanol:chloroform, 9:1) at 25.2°C and 40% relative humidity, with an 8 cm development distance. Post-development, the plate underwent scanning using the CAMAG TLC Scanner-3 and LINOMAT-V at UV wavelengths of 366 nm and 254 nm. Peak areas and Rf values were recorded for compounds separated on the plate in both raw materials and final products [12].

2.11 Detection of Heavy Metals in the Formulated Product

Atomic absorption spectroscopy, as described in the Indian Ayurvedic Pharmacopoeia for heavy metal detection, was employed to ascertain the presence of heavy metals in the formulated product. Specifically, heavy metals such as lead,

arsenic, cadmium, and mercury were targeted for analysis. This analytical technique allows for the precise quantification of trace amounts of heavy metals present in the formulation, ensuring compliance with safety standards and regulations [13].

2.12 Microbial Analysis of Prepared Formulation

Sample Preparation for Microbial Analysis:

Each gram of the finely ground formulation powder was suspended in 19 ml of sterile saline solution and thoroughly mixed by vortexing for five to ten minutes. Following this, the solution was centrifuged at room temperature at 2000 rpm to remove any remaining raw material fragments, and the resulting supernatant was collected for microbiological analysis [14].

Microbial Analysis by Conventional Plating Method:

The Total Viable Count (TVC) in the supernatant was determined using the pour plate technique and a conventional approach. One milliliter of the supernatant was added to 9 ml of sterile saline solution in screw-capped tubes to create a 101 dilution, which was thoroughly mixed. Subsequently, one milliliter of this dilution was transferred to a new 9 ml of sterile saline solution to create a 102 dilution. This process was repeated to achieve dilutions up to 106 times. For each dilution, 20–25 ml of molten sterile soybean casein digest agar was added to sterile Petri plates, followed by the aseptic transfer of one milliliter of the diluted supernatant. The agar plates were allowed to solidify before being incubated at 35°C for 48 hours. After the incubation period, the plates were examined for colonies, and the number of colonies was counted to determine the microbial load present in the raw material (colony-forming units per gram, cfu/g) [15].

3. Result and Discussion

3.1 Selection of Plant Materials for the Study

Five different plants were carefully selected and authenticated for the study based on extensive literature surveys and in-depth exploration of folk medicine. The selected plants include: Swarnakshiri (Argemone Mexicana Linn.), Haridra (Curcuma longa (Linn.)), Pipali (Piper longum Linn.), Bhringraj (Eclipta alba (L.) Hassk.), and Guduchi (Stem of Guduchi (Willd.) Hook. f.). Each plant was chosen for its potential therapeutic properties and relevance to managing hepatic disorders. These selections were made after thorough review of available literature and traditional medicinal practices, ensuring that the chosen plants possess the desired pharmacological properties for the intended application. The authentication process involved rigorous examination to confirm the identity and purity of the plant materials, thus ensuring the reliability and validity of the subsequent research findings.

3.2 Morphological and Microscopical Study for Selected Plants

The morphological and microscopical characteristics of the selected herbal plants were investigated and mentioned in table 2. Swarnakshiri exhibited a characteristic aroma and taste profile, with a bitter flavor and brown coloration. Haridra, similarly, displayed a characteristic aroma and taste, characterized by bitterness with a pungent and astringent undertone, and a yellow color. Pipali exhibited a pungent flavor profile, with a slightly brown to black coloration. Bhringraj displayed an aromatic flavor profile with a bitter taste and a white color. Guduchi exhibited a complex taste profile, encompassing sweet, sore, bitter, and pungent astringent notes, with a green coloration. Foreign matter content varied among the plants, ranging from 0.30% to 2.56% w/w. Quantitative microscopic parameters were provided for Bhringraj and Guduchi, including vein termination number, stomatal index, and palisade ratio, offering additional insights into their microscopic characteristics. These findings contribute to the comprehensive understanding of the morphological and microscopical attributes of the selected herbal plants, essential for their identification and quality assessment in herbal formulations.

Table 2: Morphological and microscopical study of plants.

Name of Study	Swarnakshiri	Haridra	Pipali	Bhringraj	Guduchi
Aroma	Characteristic	Characteristic	Characteristic	Aromatic	Characteristic
Taste	Bitter	Bitter, Pungent Astringent	Pungent (Spicy Flavor)	Bitter	Sweet, Sore, Bitter, Pungent Astringent
Color	Brown	Yellow	Slightly brown/black	White	Green
Foreign Matter (%w/w)	0.30	2.56	1.044	1.5	1.03
Quantitative Microscopic Study					
Vein islet number	NA	NA	NA	NA	11
Vein termination number	NA	NA	NA	3	4
Stomatal index	NA	NA	NA	16.0	16.8
Palisade ratio	NA	NA	NA	3.8	8–11

3.3 Microscopical and Micro-chemical Tests

Microscopical and micro-chemical tests were performed on selected plant parts. Swarnakshiri root exhibited non-lignified fibers, xylem vessels, calcium oxalate crystals, and starch grains. Haridra rhizome showed a spherical shape with a periderm and wide cortex. Guduchi stem revealed medullary rays, parenchyma, mucilage cells, cork, and vessels. Pipali fruit displayed thin-walled cells, starch grains, and lignified sclerenchymatous cells. Bhringraj leaf had a single-layered epidermis, cortex with collenchymatous cells, and other features. Micro-chemical tests confirmed the presence of specific compounds. Phloroglucinol+ conc. HCl indicated lignified cells, iodine indicated starch, and Ruthenium red indicated mucilage cells. Acetic acid rendered calcium oxalate crystals insoluble, while dilute hydrochloric acid rendered them soluble. These findings contribute to the identification and pharmacological evaluation of plant parts in herbal formulations. Detailed representations are provided in Figure 1 and Table 3.

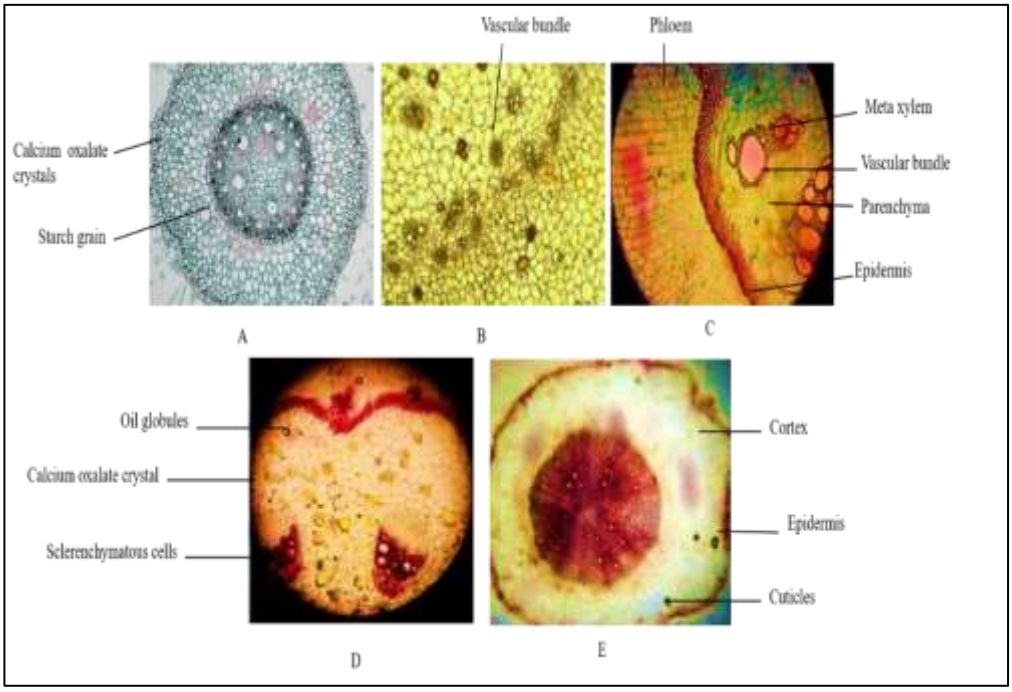

Fig.1: Transverse section of (A) Swarnakshiri rhizomes; (B) Haridra rhizomes; (C) Guduchi stem; (D)Pipali fruit; (E) Bhringraj leaf.

Table 3: Micro-chemical tests.

Reagents	Observation	Characteristics
Phloroglucinol+ conc. HCl (1:1)	Pink	Lignified cells; pericyclic fibres, stone cells, vascular bundles
Iodine	Blue	Starch
Ruthenium red	pink	Mucilage cells
Acetic acid	Insoluble	Prismatic Calcium oxalate crystals
Dil. Hydrochloric acid	Soluble	Calcium oxalate crystals

3.4 Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was conducted on aqueous extracts of the selected plants to detect the presence of phytoconstituents as shown in figure 2. The TLC analysis of Swarnakshiri extract revealed the presence of three distinct spots with Rf values of 0.24 (Dark pink), 0.32 (Light pink), and 0.37 (Pinkish) when visualized under a UV chamber at 254 nm. Guduchi stem extract displayed three spots with Rf values of 0.26 (Dark purple), 0.37 (Purple), and 0.69 (Light purple) under UV chamber at 254 nm. Haridra rhizome extract exhibited three spots with Rf values of 0.24 (Light yellow), 0.42 (Yellow), and 0.62 (Dark brown) under UV chamber at 254 nm. Pipali fruit extract showcased two spots with Rf values of 0.41 (Yellow) and 0.56 (Dark brown) under UV chamber at 254 nm. Bhringraj aerial part extract displayed three spots with Rf values of 0.24 (Light purple), 0.42 (Purple), and 0.56 (Dark purple) under UV chamber at 254 nm. These results indicate the diverse phytochemical profiles of the selected plant extracts, as evidenced by distinct spot patterns observed in TLC analysis.

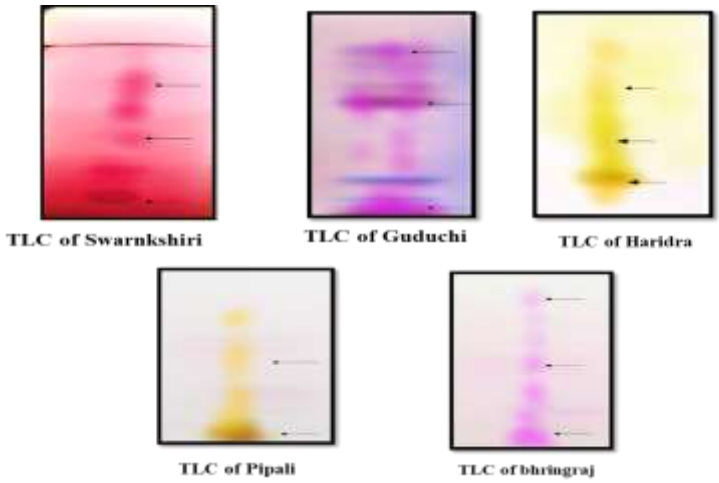

Fig.2: Thin Layer Chromatography (TLC) of selected plants

3.5 Formulation Parameters

3.5.1 Preformulation Study

The Preformulation study involved the assessment of various parameters for different plant extracts, as summarized in Table 4.

Table 4: Preformulation parameters.

Sr. no	Parameters	Swarnakshiri	Haridra	Pipali	Bhringraj	Guduchi
1.	**Particle size**	595 µm	355 µm	592 µm	353 µm	593 µm
2.	**Angle of repose**	24.58°	24.39°	24.48°	24.36°	24.56°
3.	**Solubility**	Soluble in DMSO, ethyl acetate, Insoluble in water	Soluble in methanol, insoluble in water	Soluble in alcohol Insoluble in water	Insoluble in water, soluble in organic solvent	Water soluble, organic solvent soluble
4.	**Hygroscopicity**	10.2%	11.71%	13.4%	12.2%	9.5%

3.5.2 Determination of λmax by UV Spectrometry

The λmax values for different plant extracts were determined using UV spectrometry, revealing their maximum absorption wavelengths. The solution of Swarnakshiri in ethyl acetate exhibited maximum absorption at 226 nm, while the solution of Haridra in methanol displayed maximum absorption at 423 nm. In the case of Pipali, the solution in alcohol demonstrated maximum absorption at 342 nm. Bhringraj and Guduchi extracts, when dissolved in methanol and water respectively, showed maximum absorption at 415 nm and 348 nm. These λmax values provide important information about the specific wavelengths at which these plant extracts absorb light most strongly. The absorption spectra for each extract are depicted in Figure 3.

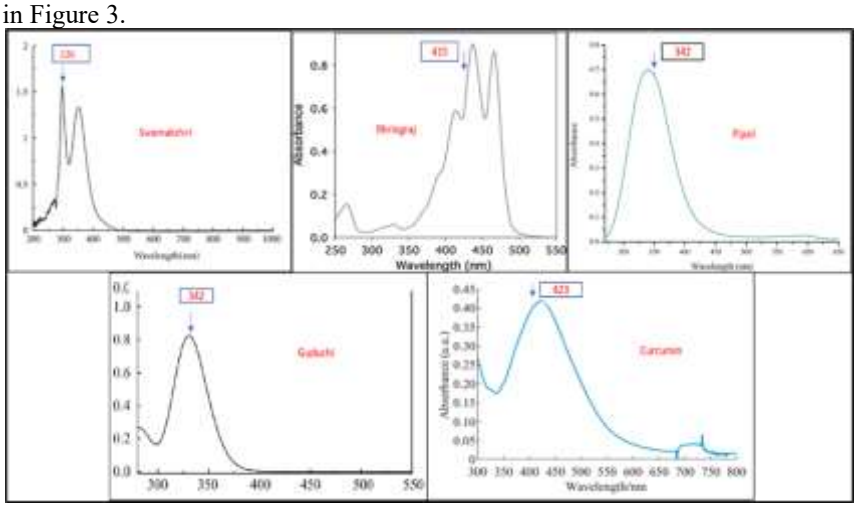

Fig. 3: UV spectrums of extracts

3.5.3 Drug-Drug interaction study

Fourier Transform Infra-Red (FTIR) Spectroscopy

The potential for drug-drug interaction is a critical consideration when formulating dosage regimens involving multiple drugs. In this study, a combination of Haridra and Bhringraj was utilized to formulate soft gelatin capsules, while Swarnakshiri, Guduchi, and Pipali were combined for hard gelatin capsules. To ensure the quality and safety of these formulations, a drug-drug interaction study was conducted using Fourier Transform Infra-Red (FTIR) spectroscopy. The FTIR spectra of Curcumin and Wedelolactone were analyzed, as depicted in Figure 4, while the spectra of Berberine, Piperine, and Tinosporoside were shown in Figure 6.14. The ranges of all drugs studied are presented in Table 6.14. From the data obtained in the drug-drug interaction study, it was observed that there was no significant interaction between the drug mixtures. This finding provides reassurance regarding the compatibility and safety of the formulated capsules. Subsequently, capsules and chewable capsules were formulated using these drug mixtures, as discussed in the following section.

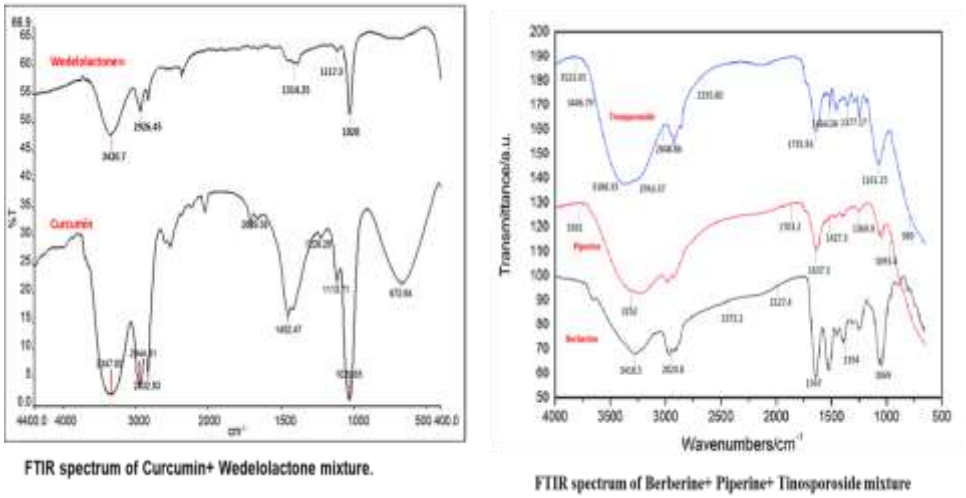

Fig.4: ***Fourier Transform Infra-Red (FTIR) Spectroscopy***

3.6 Formulation and development of capsules

3.6.1 Evaluation parameters of granules

The granules for the Berberine, Piperine, and Tinosporoside mixtures were prepared using the wet granulation method. Subsequently, various evaluation parameters were assessed to ensure the quality and suitability of the granules for capsule formulation. These parameters included moisture content, angle of repose, bulk and tapped density, Hausner ratio, compressibility index, and flow properties. The results of these evaluations are presented in Table 5. The particle size was measured at 356 µm, while the angle of repose indicated freely flowing granules with an angle of 24.58°. The compressibility index fell within the range of 11-13%, suggesting good compressibility. Additionally, the Hausner ratio was calculated at 1.11, indicating good flow properties of the granules. Both bulk and tapped density measurements further supported the suitability of the granules for capsule formulation. Overall, the granules exhibited favorable characteristics, meeting the requirements outlined in the literature, and thus were deemed suitable for further formulation into capsules.

Table 5: Evaluation of granules.

Sr. no	Parameters	Granules
1.	Particle size	356 µm
2.	Angle of repose	24.58°
3.	Flow property	Freely flowing
4.	Compressibility index	11-13%
5.	Haunsar's ratio	1.11
6.	Tapped density	0.46 gm/ml
7.	Bulk density	0.42 gm/ml

3.6.2 *Formulation of Soft chewable capsule*

The 100 capsules each with a nominal weight of Curcuma longa and Eclipta alba prepared with jaggery (14000), MCC pH 102 (150 mg), and glycerin and filled into soft gelatin capsule shells filled using silicon mold. Eight different batches of the capsule were prepared and among these 8 batches, one optimized batch was selected based on weight variation test, dissolution, and disintegration test. A further pharmacological study was performed on the optimized batch.

3.6.2.1 Evaluation of Soft Chewable Capsules

Appearance Assessment:

Liquid-filled batches of curcumin and wedelolactone with jaggery were examined for clarity, color, and precipitation. All formulations exhibited clear, homogeneous solutions without any precipitation which summarized in table 6.

Table 6: Test for capsule filling solution

Sample code	Specific gravity	Total solid content %	Fat content %	Reducing sugar %	Total Sugar%
F1	**3.01**	**30.12**	**7.7**	**1.1**	**21.1**
F2	**2.52**	**34.4**	**11.1**	**0.9**	**24.45**
F3	**2.61**	**29.7**	**8.4**	**1.3**	**23.11**
F4	**3.15**	**31.67**	**8.56**	**1.2**	**30.61**
F5	**4.09**	**26.23**	**9.67**	**0.8**	**34.45**
F6	**2.21**	**26.41**	**7.1**	**1.7**	**23.11**
F7	**1.43**	**22.1**	**6.7**	**0.7**	**12.23**
F8	**1.86**	**32.12**	**8.9**	**1**	**25.5**

The pH of soft chewable capsules

Another crucial element for the composition of liquid fillings is the pH. The pH of liquid fill formulations for soft gels should be between 2.5 and 7.5. While gelatin is hydrolyzed at pH levels below 2.5, causing the soft gel to seep, it may also be tanned (i.e., cross-linked) at pH levels over 7.5, which reduces the solubility of the gelatin shell. The pH of the F7 batch was the most suited for the formulation of capsules in all batches (Table 7).

Table 7: Determination of pH of capsule.

Sample code	**pH**	**SD**
F1	3.01	0.21
F2	2.52	0.35
F3	2.61	0.24
F4	4.5	0.43
F5	4.09	0.34
F6	5.2	0.32
F7	4.7	0.25
F8	6.01	0.32

Drug content and moisture content determination of soft chewable capsule

The drug content was found to be in an acceptable range for all formulations indicating a uniform distribution of the drug. Percent drug content was found to be in the range of 81.32±0.42- 96.51±0.16in all batches. The percent content of the F7 batch was found to be more than other batches i.e 96.51±0.16 which clarifies the F7 batch was more suitable for administration. The moisture absorption was found to be in the acceptable range for all formulations indicating their stability. Percent moisture absorption was found to be in the range of 1.11 ± 0.95 to 3.11± 0. 64 In all batch F7 batch moisture content was found to be 1.11 ± 0.95 which indicate that the F7 batch absorbs less moisture which clarifies the among all batches F7 batch was optimized batch hence the further study was performed on the F7 batch (Table 8).

Table 8: Determination of drug and moisture content.

Sample code	Drug content %	Moisture content %
F1	85.60±0.27	1.17 ± 078
F2	87.24±0.43	2.16 ± 0.67
F3	83.43±0.27	1.78 ± 0.85
F4	81.32±0.42	1.98 ± 0.56
F5	92.34±0.23	3.11± 0. 64
F6	84.84±0.21	2.45 ± 0.23
F7	96.51±0.16	1.11 ± 0.95
F8	93.14±0.22	1.67± 0.86

Determination of viscosity

One of the key variables that is crucial for the optimization of the liquid filling formulation for soft gels is viscosity. Liquid filling formulas for soft gels typically have viscosities between 855 and 3256 cps. Every formulation demonstrated fluid behaviour in the Newtonian sense. Because MCC PH 102 raises the system's flow resistance, it has been demonstrated that an increase in MCC PH 102 concentration greatly raises viscosity. The tendency of MCC PH 102 molecules to orient more in the direction of shear is what causes the reduction in shear viscosity with increasing shear rate. A modification in the concentration of MCC PH 102 caused changes in the viscosity and consistency of liquid fill formulations for soft gels. The F7 batch's viscosity, which was determined to be 19310.33 in all batches and is within acceptable limits, was deemed the best batch to use. On the F7 batch, more research was done (Table 9).

Table 9: Determination of viscosity.

Sample code	Viscosity (CP)
F1	855±0.01
F2	1210±0.11
F3	1185±0.15
F4	1337±0.17
F5	2213±0.22
F6	3256±0.31
F7	1931±0.33
F8	989±0.01

***In-vitro* drug dissolution study for soft chewable capsule**

The in vitro release was carried out for optimized formulation using phosphate buffer pH 6.4 and 0.1 N HCL as a medium. The optimized formulation F7 showed 79.25 % drug release within 120 Min in phosphate buffer and 65.17% in 0.1 N HCL which was satisfactory. The formulated capsule showed a most favorable release within 120 min which followed a steady drug release pattern as shown in figure 5. and table 10 shows the in vitro drug release profile for capsules selected from the optimized batch.

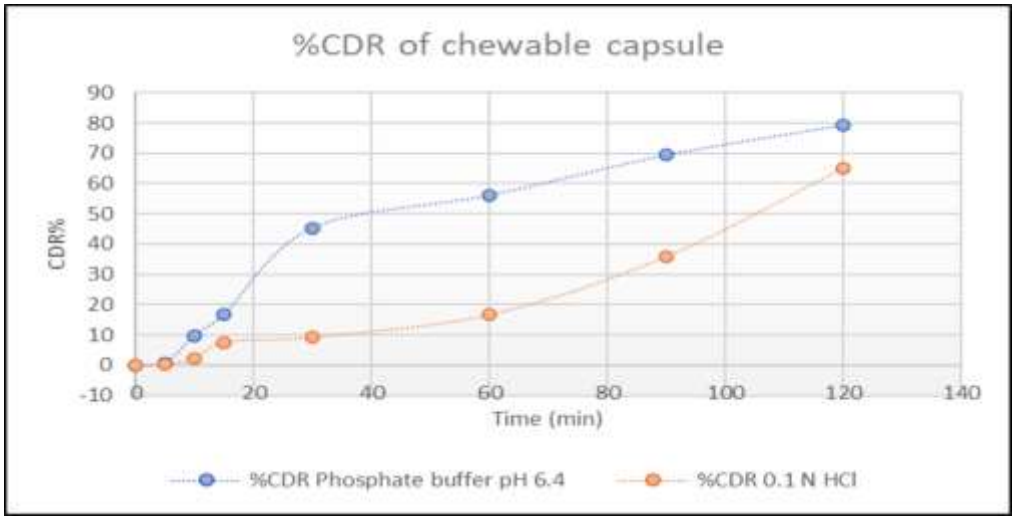

Fig.5: *In vitro* dissolution study of chewable capsules.

3.7 Chromatographic Study of Formulations

HPTLC of Soft Chewable Capsule:

Upon analysis with the same mobile phase, the soft chewable capsule formulation exhibited two primary active constituents: curcumin and wedelolactone, at Rf values of 0.69 and 0.56, respectively. Refer to figure 6.19 for the chromatogram and plate of the formulation.

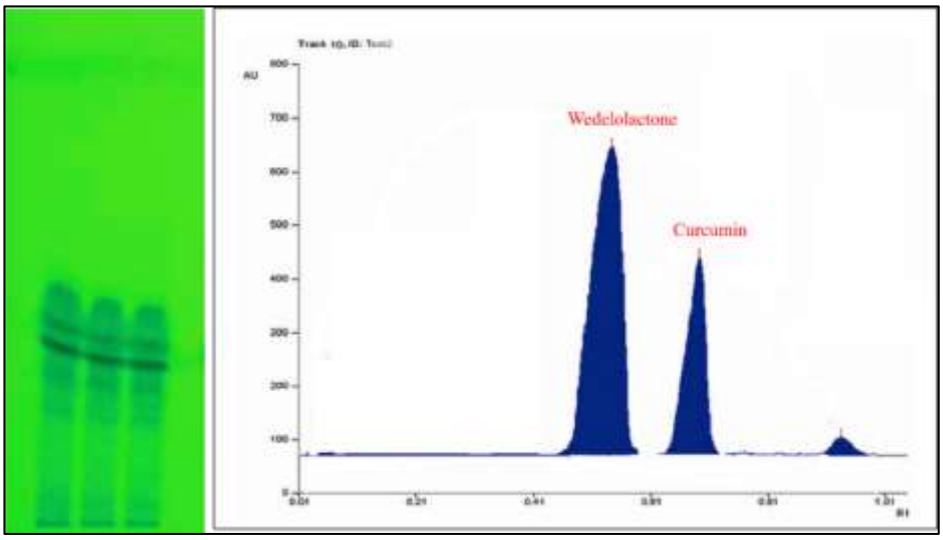

Fig. 6: HPTLC fingerprint and chromatogram of soft chewable capsule.

3.8 Determination of heavy metals, microbial contamination, and nutritional values of formulations (Capsule and soft chewable capsule)

The results obtained from heavy metal analysis, microbial analysis and nutritional value determination are given in table 5.25 Which shows the total absence of heavy metals and microbial contamination capsules.

Table 10: **Data for Heavy Metals, Heavy Metals, Nutritional values.**

Parameters	Hard gelatin capsule	Soft chewable capsule
	Heavy metals	
Lead	Nil	Nil
Arsenic	Nil	Nil
Cadmium	Nil	Nil
Mercury	Nil	Nil
	Microbial contamination	
Total viable count (TVC)	Nil	Nil
	Nutritional value	
Carbohydrate	82.56	86.32
Protein	6.29	7.21
Cholesterol	2.30	2.31

4. Conclusion

In conclusion, the comprehensive evaluation of the formulated capsules and soft chewable capsules reveals promising outcomes regarding their safety, purity, and nutritional content. Heavy metal analysis demonstrated the absence of hazardous elements such as lead, arsenic, cadmium, and mercury, ensuring the products' safety for consumption. Similarly, microbial contamination assessments confirmed the formulations' microbiological purity, with negligible levels of harmful microorganisms detected.

Furthermore, the assessment of nutritional values revealed significant levels of carbohydrates, proteins, and essential nutrients in the capsules, indicating their potential to contribute positively to consumers' nutritional intake. These findings collectively underscore the formulations' suitability for consumption and their potential to promote health and well-being. Overall, the formulated capsules and soft chewable capsules exhibit favorable attributes, positioning them as promising options for individuals seeking safe, pure, and nutritionally enriched supplements to support their health goals.

Acknowledgement

The authors would like to express their gratitude to the Department of Pharmacy at Monad University, Hapur, Uttar Pradesh, for their invaluable support and resources provided throughout the research endeavor. Their assistance and guidance have been instrumental in the successful completion of this study. We extend our sincere appreciation for their unwavering commitment to academic excellence and research advancement.

Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this research article. We have no financial or personal relationships with any individuals or organizations that could inappropriately influence or bias the content of this work.

Refrences:

1. Bisht A, Dwivedi H, Rawat AK. Development and Evaluation of Polyherbal Formulation for Diabetes. Asian Pac. J. Health Sci. 2021;8(4):48-54.
2. Fathima A, Nawaz S, Begum S, Maryam S, Fasahath SA, Begum Z, Mahammed NL. Pharmacological Screening of Polyherbal Formulation For Hepatoprotective Activity On Albino Rats. Asian Journal of Pharmaceutical Research and Development. 2015 Nov 1:1-6.
3. Sheikh ZA, Zahoor A, Khan SS, Usmanghani K. Design, development and phytochemical evaluation of a poly herbal formulation linkus syrup. Chinese Medicine. 2014 May 20;2014.
4. Kushwaha SK, Kori ML. Research Article Development and Evaluation of Polyherbal Tablet from Some Hepatoprotective Herbs. Scholars Academic Journal of Pharmacy. 2014;3(3):321-6.
5. Majhi S, Singh L, Verma M, Chauhan I, Sharma M. In-vivo evaluation and formulation development of polyherbal extract in streptozotocin-induced diabetic rat. Phytomedicine Plus. 2022 Nov 1;2(4):100337.
6. Aladejana EB, Aladejana AE. Hepatoprotective activities of polyherbal formulations: A systematic review. Journal of Medicinal Plants for Economic Development. 2023 Oct 24;7(1):206.
7. Dhumansure RH. *Development and Evaluation of Hepatoprotective Polyherbal Formulation* (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).
8. Singh D, Singh V, Mishra SB, Sharma D, Agarwal V. Evaluation of anti-biofilm, anti-quorum, anti-dysenteric potential of designed polyherbal formulation: in vitro and in vivo study. Journal of Applied Biomedicine. 2022 Jan 1;20(1).
9. Gauttam VK, Kalia AN. Development of polyherbal antidiabetic formulation encapsulated in the phospholipids vesicle system. Journal of advanced pharmaceutical technology & research. 2013 Apr 1;4(2):108-17.
10. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacognosy reviews. 2014 Jul;8(16):73.
11. Jagtap AG, Shirke SS, Phadke AS. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. Journal of ethnopharmacology. 2004 Feb 1;90(2-3):195-204.
12. Jyoti M, Kshitij A, Vivek S, Prem S. Preparation and standardization of a polyherbal formulation. Journal of advanced scientific research. 2012 May 10;3(02):84-5.
13. Dandagi PM, Patil MB, Mastiholimath VS, Gadad AP, Dhumansure RH. Development and evaluation of hepatoprotective polyherbal formulation containing some indigenous medicinal plants. Indian journal of pharmaceutical sciences. 2008 Mar;70(2):265.
14. Gupta A, Upadhyay NK, Sawhney RC, Kumar R. A poly-herbal formulation accelerates normal and impaired diabetic wound healing. Wound repair and regeneration. 2008 Nov;16(6):784-90.
15. Mohanty A, Das C, Dash S, Sahoo DC. Physico-chemical and antimicrobial study of polyherbal formulation. Int J Compr Pharm. 2010;4(4):1-4.