

Formulation, Development and *In Vitro* Evaluation of Herbal Plant Extract of *Pedilanthus Tithymaloides* Microsphere Gel

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Date of Submission: 23-04-2026

Date of Acceptance: 03-05-2026

ABSTRACT

The present study aimed to develop and evaluate a microsphere-based gel formulation containing *Pedilanthustithymaloides* leaf extract for topical applications. Leaves were collected, and extracts were prepared using petroleum ether and methanol, with methanol yielding a higher percentage of bioactive constituents. Phytochemical screening of the methanolic extract revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenolics, saponins, and triterpenoids, while quantitative analysis confirmed substantial phenolic (55 mg/g GAE) and flavonoid (53 mg/g rutin equivalent) content, indicating strong antioxidant, anti-inflammatory, and wound-healing potential. The extract was formulated into microspheres to enhance stability and control release. Particle size analysis showed sizes ranging from 176.34 nm to 304.7 nm, with F2 identified as the optimized formulation due to its small size (176.34 nm) and low polydispersity index (17.7%), ensuring uniformity and improved bioavailability. Zeta potential analysis revealed negative charges between -24.4 mV and -37.4 mV, with F2 exhibiting the highest stability (-37.4 mV). SEM imaging confirmed smooth and spherical morphology. Microspheres were incorporated into a gel, which demonstrated favorable organoleptic properties, uniformity, skin-compatible pH (6.0–6.3), suitable viscosity (2659–2710 cps), and good spreadability (14.93 gm·cm/sec). Stability studies over 90 days under both normal and accelerated conditions indicated minimal changes in pH and viscosity, confirming physical and chemical stability. Overall, the developed *Pedilanthustithymaloides* microsphere gel demonstrated effective encapsulation of bioactive compounds, excellent stability, and optimal physicochemical properties for topical use. This study highlights its potential as a novel herbal therapeutic system for skin applications.

Keywords: *Pedilanthustithymaloides*, microspheres, herbal gel, phytochemicals, phenolic content.

I. INTRODUCTION

The plant *Pedilanthustithymaloides* said to possess the wide range of medicinal properties which were confirmed through previous studies. The present study was to determine its antimicrobial activity using its leaves extract and also analysing whether their phytochemical constituents are responsible for its anti-microbial activities(Karimi *et al.*, 2019). *Pedilanthustithymaloides* leaves extract was obtained and tested for antimicrobial activities and analysed for the presence of chemical constituents by preliminary phytochemical analysis and by FTIR analysis(Gugala *et al.*, 2019). The antimicrobial susceptibility studies were conducted against gram (-) bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and gram (+) bacteria such as *Staphylococcus aureus*. This supports the medicinal use of the leaf which acts as an antimicrobial agent. However further studies are needed to isolate the active compound from the leaf and to study the antimicrobial activity of that active compound. *Pedilanthustithymaloides* leaves are widely used in Indian medicine to heal wounds, burn, mouth ulcers. However, systematic evaluation of these activities is lacking (Mahato *et al.*, 2025).

The different parts of *P. tithymaloides* are known to contain a variety of bioactive phytochemicals and secondary metabolites with significant medicinal potential. These include saponins, tannins, phenolic compounds, glycosides, flavonoids, resins, steroids, terpenoids, nicotinamide, rutin, quercitrin, pyrogallol, gluconic acid, palmitic acid, docosenoic acid, and hexeicosanoic acid, among others(Awuchi, 2020). Given that plants of the same species can exhibit variations in secondary metabolite composition due to differences in geographical and climatic conditions, it is essential to investigate these variations to understand their impact on pharmacological efficacy. Among the various reported pharmacological activities, the antioxidant,

antimicrobial, and anti-inflammatory properties of *P. tithymaloides* have been studied in samples collected from Bihar, West Bengal, Madhya Pradesh, and other regions of India. Additionally, studies have established its strong antibacterial, antioxidant, and anti-inflammatory properties in samples from different parts of the world (Mahato *et al.*, 2025).

Microspheres are spherical, free-flowing particulate carriers ranging from 1 to 1000 μm in size, capable of encapsulating bioactive compounds and providing controlled and sustained drug release. They offer several advantages, including protection of the drug from degradation, improved stability, enhanced bioavailability, and targeted delivery (Gurung and Kakar 2020). Incorporation of herbal extracts into microspheres can significantly enhance their therapeutic efficacy by controlling the release profile and improving penetration through biological barriers. Topical drug delivery systems, particularly gels, have gained considerable importance due to their ease of application, patient compliance, and ability to deliver drugs directly to the site of action (Patil *et al.*, 2019). Gels provide a suitable base for incorporating microspheres, resulting in a microsphere-loaded gel system that combines the advantages of both controlled release and convenient topical application. Such systems are especially beneficial in the treatment of skin infections, inflammation, and wound healing, where localized and sustained drug delivery is required (Sayed *et al.*, 2015).

The formulation of microsphere gel involves careful selection of polymers, solvents, and processing parameters to achieve optimal particle size, drug entrapment efficiency, and release characteristics. Various techniques such as solvent evaporation, ionotropic gelation, and emulsion cross-linking are commonly employed for the preparation of microspheres. These parameters play a crucial role in determining the physicochemical properties and performance of the final formulation (Dhaddeet *al.*, 2021).

Therefore, the present study is aimed at the formulation, development, and in vitro evaluation of microsphere gel containing *Pedilanthustithymaloides* extract. The study focuses on optimizing formulation parameters, characterizing the microspheres, and evaluating the performance of the microsphere-loaded gel in terms of physicochemical properties, drug release, and stability.

II. MATERIALS AND METHODS

2.1 Chemicals

Petroleum ether were obtained from Vizag Chemical, a reputable supplier of analytical reagents. Purosolv provided the Methanol. GACL provided the Sodium hydroxide. Bodal Chemicals Ltd provided the Conc. Sulphuric acid. Uma Chemicals provided the Copper sulphate. Lead acetate were obtained from Ava Chemicals while, Mercuric chloride provided by Gurjar Chemicals Pvt. Ltd. Agro Phos India Limited provided the Ammonium sulphate. Sukha Chemical Industries provided the Ferric chloride. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Meru Chem Pvt. Ltd, Deepak Nitrite Limited, Sihauli Chemicals Pvt. Ltd., Dhruv Chemicals, Vishal Laboratories, Belami Fine Chemicals Pvt. Ltd and CDH Fine Chemical.

2.2 Plant Collection

Fresh and healthy leaves of *Pedilanthustithymaloides* were selected as plant material. Leaves were collected from local area Bhopal. The collected leaves were washed thoroughly with distilled water to remove dirt, dust, and other contaminants. Leaves were shade-dried at room temperature to preserve heat-sensitive phytochemicals. Dried leaves were pulverized into a coarse powder using a mechanical grinder. The powdered material was stored in airtight, amber-colored containers at room temperature to prevent degradation. Plant authenticity was confirmed by a qualified botanist.

2.3 Extraction Process

1. Plant material: 200 g of powdered leaves of *Pedilanthustithymaloides* were used.

2. Solvents:

- **Polar solvent:** Methanol
- **Non-polar solvent:** Petroleum ether

3. Soxhlet Extraction:

➤ **Petroleum Ether Extraction (Non-Polar):**

200 g of powdered *Pedilanthustithymaloides* leaves were placed in Soxhlet thimble. 500 mL of petroleum ether was added to Soxhlet apparatus. Extraction was carried out for 6–8 hours until the siphon solvent became nearly colorless, indicating complete extraction of non-polar compounds. The petroleum ether extract was filtered and concentrated under reduced pressure using a rotary evaporator, then dried and stored in an airtight amber-colored container.

➤ **Methanol Extraction (Polar):**

After completion of petroleum ether extraction, the marc (residue) was collected, dried, and reloaded into a Soxhlet thimble for methanolic extraction. Approximately 500 mL of methanol was used as the solvent, and extraction was carried out for 6–8 hours

until the siphon tube showed nearly colorless solvent, indicating exhaustive extraction of polar constituents. The obtained methanolic extract was filtered through Whatman No. 1 filter paper to remove insoluble impurities and then concentrated under reduced pressure using a rotary evaporator at 40–50 °C. The concentrated extract was further dried in a hot air oven or desiccator to obtain a semi-solid or solid mass, which was finally stored in airtight amber-colored containers at room temperature for further phytochemical and pharmacological evaluation.

Percentage yield calculation:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of initial plant material}} \times 100$$

2.4 Quantitative Estimation of Phytoconstituent

2.4.1 Estimation of Total Phenolic Content (TPC)

The total phenolic content of *Pedilanthustithymaloides* leaf extract was quantified using spectrophotometric method based on Folin–Ciocalteu reagent. A calibration curve was generated by preparing a series of gallic acid standard solutions of known concentrations. An aliquot of extract was mixed with Folin–Ciocalteu reagent, followed by the addition of sodium carbonate solution. The reaction mixture was allowed to stand at room temperature to develop blue chromophore. The intensity of the color was measured at 760 nm using a UV–Visible spectrophotometer. The phenolic content was calculated from the gallic acid standard calibration curve and expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g). The Folin–Ciocalteu assay measures reducing capacity, with phenolic compounds contributing significantly to the observed response (Babbar *et al.*, 2011).

2.4.2 Estimation of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric assay was used to assess the extract's total flavonoid content. Standard solutions of rutin were prepared to construct a calibration curve. An aliquot of plant extract was combined with 2% Aluminumchloride solution and incubated at ambient temperature to allow complex formation between flavonoid compounds and aluminum ions, resulting in a yellow-colored complex. The absorbance of this complex was then measured at 510 nm using UV–Visible spectrophotometer. The flavonoid concentration was calculated from rutin standard calibration curve and

expressed as milligrams of rutin equivalents per gram of dried extract (mg RE/g) (Ghafar *et al.*, 2017).

2.5 Solubility study

The solubility of the dried leaf extract was evaluated in various polar and non-polar solvents to identify suitable media for formulation development. About 10 mg of extract was separately added to 1 mL of each solvent, including methanol, ethanol, distilled water, petroleum ether, chloroform, acetone, and DMSO. The mixtures were shaken for 2–3 minutes and observed visually for degree of dissolution. Solubility was categorized as freely soluble, soluble, sparingly soluble, slightly soluble, or insoluble based on clarity and residue formation. The observations were recorded and tabulated to compare solvent compatibility and to assist in selecting appropriate solvents for further formulation studies (Jager *et al.*, 2007).

2.6 Formulation of microspheres by Solvent Evaporation Method

Five microsphere formulations of *Pedilanthustithymaloides* extract were prepared using varying ratios of HPMC and Ethyl Cellulose (EC) to study the effect of polymer concentration on formulation characteristics. The accurately weighed extract and polymers were dissolved in a 1:1 mixture of ethanol and dichloromethane to form the organic phase. This phase was then slowly added into an aqueous phase containing 0.01% Tween-80 maintained at 30–40 °C under continuous stirring at 300 rpm. The emulsion was stirred for 45 minutes to allow solvent evaporation and microsphere formation. The formed microspheres were collected by filtration, washed with distilled water to remove residual solvent and surfactant, and dried in a hot air oven at 37 °C until constant weight. The same procedure was repeated for all five formulations with different polymer ratios to evaluate their influence on particle size, yield, and drug release behaviour (Kim *et al.*, 2002).

Table 1: Composition of Microsphere formulation

Formulation	<i>Pedilanthus</i> Extract (1gm)	HPMC (mg)	Ethyl Cellulose (mg)	Ethanol (mL)	Dichloromethane (mL)	Tween-80 (0.01% w/v in 250mL water)
F1	1.0	300	50	10	10	250 mL
F2	1.0	250	100	10	10	250 mL
F3	1.0	200	150	10	10	250 mL
F4	1.0	150	200	10	10	250 mL
F5	1.0	100	250	10	10	250 mL

2.7 Evaluation parameter of extract loaded Microsphere

2.7.1 Particle size

The particle size of *Pedilanthustithymaloides* extract-loaded microspheres was measured using a dynamic light scattering particle size analyzer (e.g., Malvern Zetasizer)(Dubey and Parikh 2004).

2.7.2 Zeta potential

The surface charge and stability of *Pedilanthustithymaloides* extract-loaded microspheres were evaluated by measuring their zeta potential using a Zetasizer instrument (e.g., Malvern Zetasizer Nano ZS)(Saxena and Shaikh, 2021).

2.7.3 Scanning Electron Microscopic (SEM)

The morphological properties of *Pedilanthustithymaloides* extract-loaded microspheres were evaluated using a scanning electron microscope (SEM). The dried microspheres were first mounted on aluminium stubs using double-sided carbon tape and then coated with thin conductive layer of gold-palladium (2–20 nm) using vacuum-sputter coater. Upon exposure to the electron beam of the SEM, secondary electrons, including Auger electrons, were emitted from the microsphere surface. These electrons were scattered at 90° and collected by the detector. The signals were processed according to Rutherford and Kramer’s law, producing high-resolution images that revealed the surface morphology, particle shape, and texture of microspheres. This method provides detailed insight into microsphere’s structural integrity, smoothness, and any aggregation, which are crucial parameters affecting drug release and stability(Gupta *et al.*, 2016).

2.8 Formulation of *Pedilanthustithymaloides* Microsphere-loaded gel

The microsphere-loaded gel of *Pedilanthustithymaloides* extract was formulated using Carbopol 934 and carboxymethyl cellulose (CMC) as gelling agents. Both polymers were dispersed in distilled water and allowed to hydrate completely for 1–2 hours. Propylene glycol was added as a humectant and solubilizer, followed by

the incorporation of methyl paraben and propyl paraben as preservatives. The dried extract-loaded microspheres were then slowly dispersed into the gel base under gentle stirring to ensure uniform distribution without damaging the microspheres. The pH was adjusted to 6–6.5 using triethanolamine to obtain a smooth, homogeneous gel suitable for topical application. The prepared formulation was stored in airtight containers at room temperature for further evaluation of physicochemical properties such as appearance, viscosity, spreadability, pH, and stability. This method allows the microspheres to maintain controlled-release characteristics while providing an easily applicable and patient-friendly topical gel system(Shah *et al.*, 2023).

Table 2: Composition of extract loaded microsphere Gel formulation

Excipients	Quantity
Carbopol 934	1.00 gm
Carboxymethyl cellulose	1.00 gm
Propylene glycol	0.5 mL
Methyl paraben	0.2 mL
Propyl paraben	0.2 mL
Microspheres	1.0%
Triethanolamine	q.s
Distilled water	100 mL

2.9. Characterization of Microsphere gel formulation

2.9.1 Physical properties

The physical properties of the *Pedilanthustithymaloides* extract-loaded microsphere gel were evaluated to assess its suitability for topical use. The gel was visually examined for smoothness, consistency, and any presence of aggregates, while colour uniformity was checked to detect possible degradation(Tournier *et al.*, 2017).

2.9.2 Determination of Viscosity

The viscosity of *Pedilanthustithymaloides* extract-loaded microsphere gel was evaluated to determine its rheological behavior and suitability for topical administration. The measurement was carried out using a Brookfield viscometer equipped with an appropriate spindle selected based on the consistency of the gel (Priyanka *et al.*, 2019).

2.9.3 Measurement of pH

The pH of the *Pedilanthustithymaloides* extract-loaded microsphere gel was measured to ensure skin compatibility and formulation stability. A sample of the gel was diluted with distilled water to form a uniform dispersion, and the digital pH meter was calibrated using standard buffer solutions before recording the final pH value (Jadhav *et al.*, 2016).

2.9.4 Spreadability study

The spreadability of the *Pedilanthustithymaloides* extract-loaded microsphere gel was evaluated to determine its ease of application and uniform distribution on the skin. A fixed amount of gel was placed between two glass slides, and a standard weight was applied for a specific time to allow even spreading. Afterward, the extent of spread was

measured to calculate spreadability. The results indicated that good spreadability ensures smooth application, uniform distribution of microspheres, improved patient compliance, and effective topical delivery, making it an important parameter for formulation performance (Al-Barghouthy *et al.*, 2025).

2.10 Stability Study

The stability of the *Pedilanthustithymaloides* extract-loaded microsphere gel was evaluated to assess its physical integrity and performance during storage. The formulation was stored in airtight containers under different conditions: room temperature ($25 \pm 2^\circ\text{C}$), refrigerated conditions ($4 \pm 2^\circ\text{C}$), and accelerated conditions ($40 \pm 2^\circ\text{C}$ with 75% RH) for 90 days. Samples were analyzed at predetermined intervals (0, 30, 45, 60, and 90 days) for changes in pH, viscosity, and spreadability. The results were compared with initial values to identify any significant variations. The absence of notable changes indicated that the formulation remained stable, consistent, and suitable for prolonged topical use (Abd-Allah *et al.*, 2010).

III. RESULT AND DISCUSSION

3.1 Plant Collection

Table 3: *Pedilanthustithymaloides* Plant collection

Plant name	Plant part used	Weight
<i>Pedilanthustithymaloides</i>	Leaves	200 gm

3.2 Percentage Yield

Table 4: Percentage Yield of crude extracts of *Pedilanthustithymaloides* extract

Plant name	Solvent	Color of extract	Theoretical weight	Yield (gm)	% yield
<i>Pedilanthustithymaloides</i>	Pet ether	Brownish-yellow	200	4.2	2.1 %
	Methanol	Greenish-brown	190	12.5	6.57 %

3.3 Preliminary Phytochemical Study

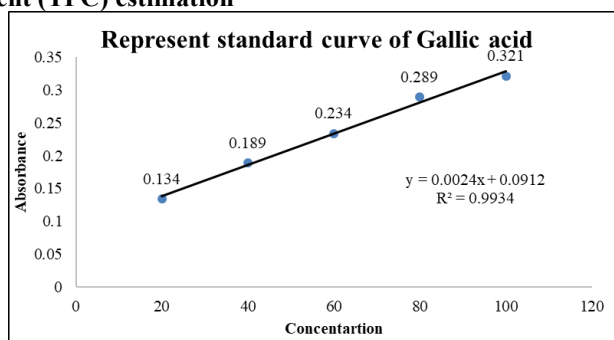
Table 5: Phytochemical testing of *Pedilanthustithymaloides* leaf extract

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Methanolic extract
Alkaloids		
Dragendorff's test	Absent	Present
Mayer's reagent test	Absent	Present
Wagner's reagent test	Absent	Present
Hager's reagent test	Present	Present
Glycoside		
Borntrager test	Absent	Present
Killer-Killani test	Absent	Present
Carbohydrates		
Molish's test	Present	Present
Fehling's test	Present	Present
Benedict's test	Present	Present
Barfoed's test	Present	Present

Iodine Test	Present	Present
Flavonoids		
Lead acetate	Absent	Present
Alkaline reagent test	Absent	Present
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Lead Acetate Test	Absent	Present
Gelatin Test	Absent	Present
Saponin		
Foam test	Present	Present
Froth Test	Present	Present
Test for Triterpenoids and Steroids		
Salkowski's test	Present	Present
Liebermann-Burchard's test	Present	Absent

3.4 Quantitative Estimation of Phytoconstituents

3.4.1 Total Phenolic content (TPC) estimation



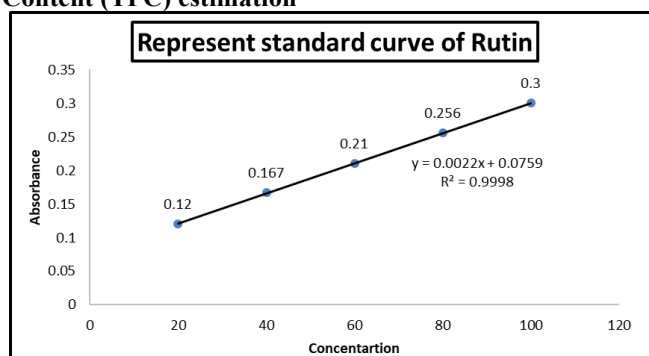
Graph 1: Represent standard curve of Gallic acid

3.4.1.1 Total Phenolic Content

Table 6: Total Phenolic Content in *Pedilanthusthymaloides* extract

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.138	55 mg/gm
0.190	
0.275	

3.4.1.2 Total Flavonoid Content (TPC) estimation



Graph 2: Represent standard curve of Rutin

3.4.1.3 Total Flavonoid Content (TFC) estimation

Table 7: Total Flavonoid Content in *Pedilanthustithymaloides* extract

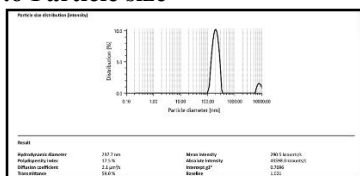
Absorbance	TFC in mg/gm equivalent of Rutin
0.120	53 mg/gm
0.182	
0.241	

3.5 Organoleptic Properties

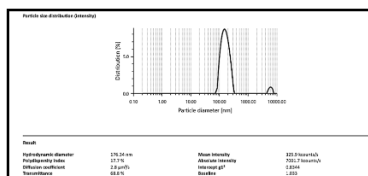
Table 8: The Organoleptic Studies of *Pedilanthustithymaloides* extract

Physical parameter	Study
Colour	Light yellowish-pale yellow
Odour	Nutty to mild herbaceous aroma
Appearance	Semi-solid viscous extract / soft paste

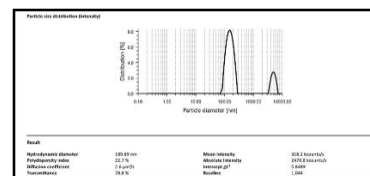
3.6 Particle size



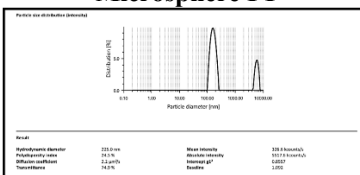
Graph 3: Particle size of Microsphere F1



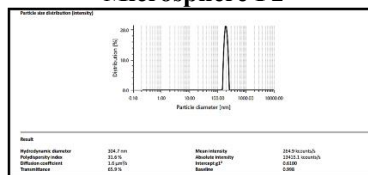
Graph 4: Particle size of Microsphere F2



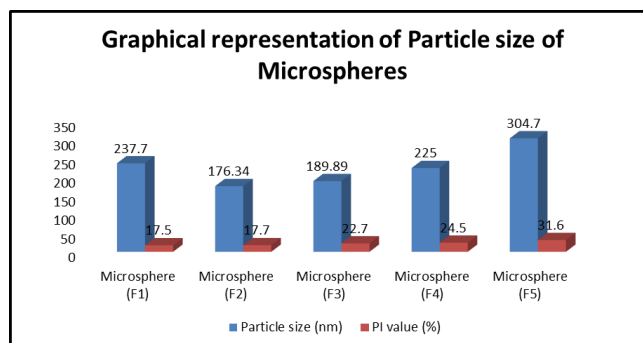
Graph 5: Particle size of Microsphere F3



Graph 6: Particle size of Microsphere F4

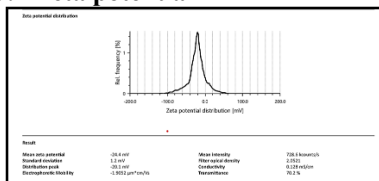


Graph 7: Particle size of Microsphere F5

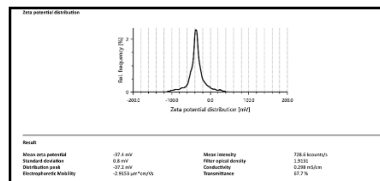


Graph 8: Graphical representation of Particle size of Microspheres

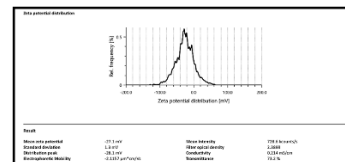
3.7 Zeta potential



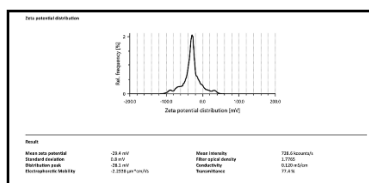
Graph 9: Zeta potential Microsphere F1



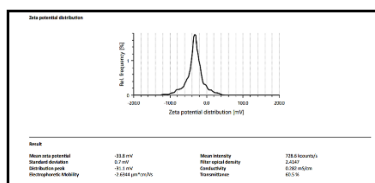
Graph 10: Zeta potential Microsphere F2



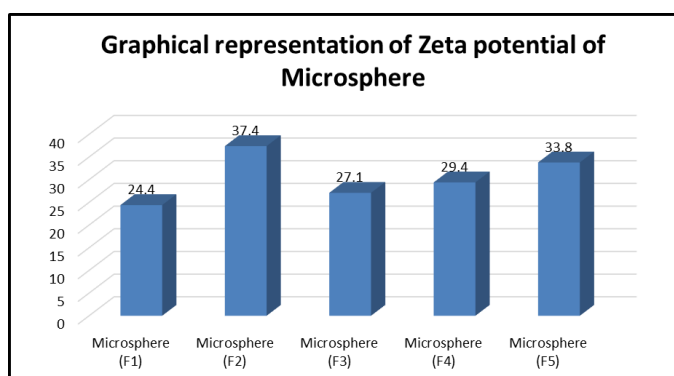
Graph 11: Zeta potential Microsphere F3



Graph 12: Zeta potential Microsphere F4



Graph 13: Zeta potential Microsphere F5



Graph 14: Graphical representation of Zeta potential of Microsphere

3.8 SEM analysis of Optimized formulation

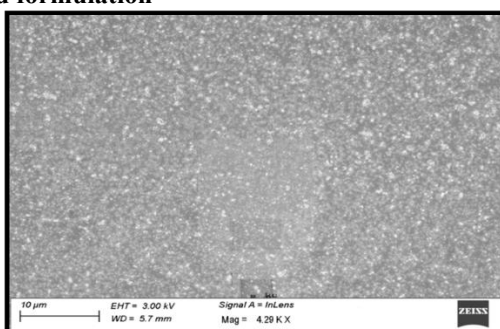


Figure 1: Scanning electron microscope (SEM) and microscopy image

3.9 Evaluation Parameter of gel formulation

3.9.1 Organoleptic Properties

Table 9: Organoleptic properties of Extract loaded Microsphere gel

Parameters	Results
Physical appearance	Smooth, soft gel
Colour	Light yellowish
Homogeneity	Uniform, free from lumps or aggregates



Figure 2: Microsphere gel

3.9.2 Measurement of pH, Viscosity and Spreadability test

Table 10: pH, Viscosity and Spreadability test of *Pedilanthustithymaloides* extract loaded Microsphere gel

Formulation	pH	Viscosity determination (cps)	Spreadability test (gm.cm/sec)
Gel formulation	6.05	4659±0.67	14.93

3.10 Stability study

Table 11: Stability Study of Microsphere gel formulation

Time (Days)	25 °C ± 2 °C & 60 ± 5% RH			40 °C ± 2 °C & 70 ± 5% RH		
	pH	Viscosity (cps)	Colour	pH	Viscosity (cps)	Colour
0	6.05	4659 ± 0.67	Light yellowish	6.05	4659 ± 0.67	Light yellowish
30	6.03	4672 ± 0.70	Light yellowish	6.02	4690 ± 0.72	Light yellowish
45	6.06	4685 ± 0.72	Light yellowish	6.01	4708 ± 0.75	Light yellowish
60	6.02	4697 ± 0.74	Light yellowish	6.00	4725 ± 0.77	Light yellowish
90	6.01	4710 ± 0.76	Light yellowish	5.99	4750 ± 0.80	Light yellowish

Discussion

The present study demonstrated that *Pedilanthustithymaloides* leaves are a rich source of bioactive phytoconstituents suitable for formulation development. Methanolic extraction yielded a higher percentage (6.57%) compared to petroleum ether (2.1%), confirming methanol as a more efficient solvent due to its ability to extract polar compounds such as phenolics, flavonoids, alkaloids, and glycosides. Phytochemical screening further supported this, showing a wider range of active constituents in the methanolic extract. Quantitative analysis revealed significant levels of total phenolic content (55 mg/g GAE) and total flavonoid content (53 mg/g rutin equivalent), indicating strong antioxidant and therapeutic potential. Organoleptic and solubility studies confirmed the extract's predominantly polar nature, supporting its suitability for pharmaceutical formulation.

Microsphere formulation studies showed that particle size ranged from 176.34–304.7 nm, with formulation F2 exhibiting the smallest size and lowest polydispersity, indicating uniform distribution. Zeta potential values (-24.4 to -37.4 mV) confirmed good colloidal stability, with F2 again showing the highest stability. SEM analysis

revealed well-formed, uniformly distributed microspheres. The optimized microsphere gel demonstrated desirable topical properties including near-physiological pH (6.05), appropriate viscosity, good spreadability, and uniform appearance. Stability studies under accelerated and room conditions showed no significant changes in pH, viscosity, or colour over 90 days, confirming formulation stability.

Overall, the findings indicate that *Pedilanthustithymaloides*-loaded microsphere gel is a stable and promising topical delivery system with strong phytochemical and therapeutic potential, particularly for wound healing and anti-inflammatory applications.

IV. CONCLUSION

In conclusion, the study successfully developed a stable, effective, and biocompatible microsphere gel formulation containing *Pedilanthustithymaloides* leaf extract for potential topical applications. The methanolic extract provided rich source of therapeutic phytochemicals, including flavonoids and phenolics, which were effectively encapsulated within microspheres to ensure sustained release and improved stability.

Among the prepared formulations, F2 was identified as the most optimized microsphere due to its small, uniform particle size and high negative zeta potential, reflecting excellent stability and controlled delivery potential. The final gel formulation demonstrated favourable physicochemical properties, including smooth texture, uniformity, appropriate pH, viscosity, and spreadability, making it suitable for direct application to skin. Moreover, stability studies confirmed that the formulation retained its integrity and functional properties over time under both standard and accelerated conditions. Overall, this research highlights the potential of *Pedilanthustithymaloides* extract-loaded microsphere gel as promising herbal therapeutic system that combines the medicinal benefits of the plant with enhanced delivery, stability, and patient-friendly application. The developed formulation may serve as basis for further pharmacological studies and clinical evaluation in topical therapy.

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