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Research Article

Anti-hemorrhoidal potential of Microspheres containing *Dolichandrone falcata* leaf extract

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Abstract

Hemorrhoids affect a significant portion of the global population, generating a constant need for safe and efficient treatments. Plant-based remedies offer promising alternatives, but their therapeutic efficiency can often be compromised by their poor oral bioavailability. This study investigates the development of a chitosan based sustained release microsphere formulation containing *Dolichandrone falcata* extract for the treatment of hemorrhoids. *In-silico* molecular docking studies were conducted on key flavonoids such as quercetin and rutin, against hemorrhoidal target proteins. The leaf extract of *D. falcata* was prepared using maceration technique and characterized for its phytochemical constituents, revealing a high total flavonoid content. Microspheres were formulated using the emulsion crosslinking method and evaluated for physical characteristics and *in-vitro* drug release. Finally, *in-vivo* anti-hemorrhoidal activity was assessed in a croton oil induced rat model. The docking results indicates strong binding affinities of quercetin and rutin to proteins such as MMP-9, NOS-2 and EGFR. The optimized microsphere formulation exhibited a high percentage yield (79.3%), good entrapment efficiency (73.3%), and a sustained *in-vitro* release profile (73.23% over 9 hrs). *In-vivo* studies demonstrated significant reduction in hemorrhoidal symptoms, comparable to standard treatment. The developed microspheres of *D. falcata* extract provide a promising, sustained-release herbal alternative for hemorrhoid management.

Keywords: *In-silico*, Anti-hemorrhoidal, Flavonoids, Microspheres, Zeta potential, *In-vivo*

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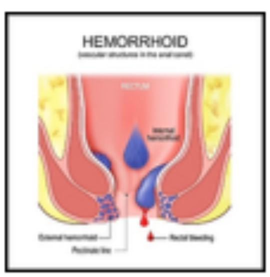
Introduction

Hemorrhoids, commonly known as piles, are a prevalent anorectal disorder characterized by the symptomatic enlargement and distal displacement of the normal anal cushions. (1) These structures are composed of blood vessels, smooth muscle, and connective tissue which are vital for preserving fecal continence. Internal hemorrhoids are usually painless, develop above the dentate line, and frequently cause rectal bleeding. (2) External hemorrhoids are highly innervated, form below the dentate line, and can be extremely painful, especially if they are thrombosed. (3)

Figure 1: Internal and External Hemorrhoids with Rectal Bleeding



The condition affects approximately 4.4 % of the global population, predominantly individuals between 45 and 65 yrs of age. (2) The pathophysiology of hemorrhoids involves an interplay of vascular and mechanical factors. Hemorrhoidal cushions can enlarge and prolapse due to conditions that raise intra-abdominal pressure, such as obesity, pregnancy, persistent constipation, and straining during defecation. (4) Mechanical degeneration of supportive connective tissue due to aging also contributes significantly to disease progression. (1) Current therapeutic approaches include minimally invasive techniques (such as rubber band ligation and sclerotherapy) and surgical therapies (such as hemorrhoidectomy) in addition to conservative dietary and lifestyle modifications. However, these conventional treatments are often expensive, associated with side effects, and have high recurrence rates, creating a demand for safe and long-lasting herbal alternatives. (5)(6)

Dolichandrone falcata (family- Bignoniaceae) is a small deciduous tree endemic to India with recognized traditional uses in treating wounds, inflammatory conditions and hemorrhoids. (7) Numerous bioactive flavonoids, such as quercetin and rutin, which are known to have anti-inflammatory, antioxidant, and veno-protective properties, are found in its leaves, according to phytochemical analyses. These flavonoids suppress inflammatory mediators, increase venous tone, and improve lymphatic drainage. (8)

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Figure 2: *Dolichandrone falcata* tree

Flavonoids have pharmacological potential; however, their quick metabolism and poor oral bioavailability limit their therapeutic usefulness. To address this limitation, novel controlled drug delivery systems like microspheres can be utilized. Polymeric microspheres (1 to 1000 µm in diameter) can encapsulate herbal extracts, providing sustained release, enhanced bioavailability and improved stability. (9)

The objective of this study was to formulate and evaluate a chitosan-based sustained-release microsphere delivery system for *Dolichandrone falcata* leaf extract, investigate the molecular interactions of its phytoconstituents with hemorrhoid-associated proteins using *in-silico* docking, and evaluate the anti-hemorrhoidal activity of the formulation in an *in-vivo* rat model.

Materials and methods

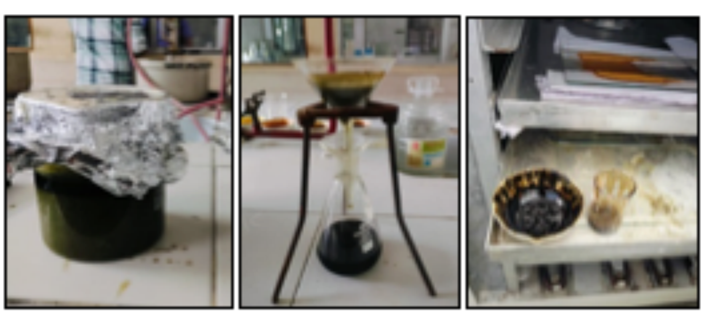
Materials Leaves of *Dolichandrone falcata* were collected from Pusad, Yavatmal, Maharashtra, India, and authenticated by the Department of Botany, R.T.M. Nagpur University. Chemicals including quercetin, rutin, chitosan, light and heavy liquid paraffin, span 80, glutaraldehyde, ethanol, and methanol were procured from standard suppliers.

***In-Silico* Docking Studies** To understand the molecular mechanisms of quercetin and rutin in treating hemorrhoids, *in-silico* molecular docking was performed using Auto-Dock Vina. Target proteins relevant to the pathogenesis of hemorrhoids—including inflammatory mediators (NF-κB, TNF-α, IL-1β, PGE-2), matrix-degrading enzymes (MMP-2, MMP-7, MMP-9), nitric oxide synthases (NOs-2, nNOs), and signal transduction factors (PIK3CA, EGFR, PRKCA)—were retrieved from the RCSB Protein Data Bank. All the twelve protein targets were selected for docking analysis. Protein and ligand preparation, including the removal of native ligands and unwanted chains, and the addition of hydrogen and Gasteiger charges, were executed using UCSF Chimera. Binding interactions were visualized and interpreted using PyMOL and BIOVIA Discovery Studio Visualizer. The toxicity and ADME profile of the compounds were predicted using ADMET Lab 3.0. (10)

Table 1: Properties of Selected proteins

Receptor and Protein PDB ID	Organism	Mutation	Methods	Resolution	R-value	Ramachandran Plot
NF-kB [6M2C]	Homo sapiens	No	X-Ray diffraction	2.70 A^{0}	0.216	Better
TNF-α [7KPA]	Homo sapiens	No	X-Ray diffraction	2.30 A^{0}	0.221	Better
IL-1β [8C3U]	Homo sapiens	No	X-Ray diffraction	1.95 A^{0}	0.209	Better
MMP-9 [1GKC]	Homo sapiens	No	X-Ray diffraction	2.30 A^{0}	0.207	Better
MMP-2 [8H78]	Homo sapiens	No	X-Ray diffraction	2.40 A^{0}	0.268	Worse
MMP-7 [1MMP]	Homo sapiens	No	X-Ray diffraction	2.30 A^{0}	0.180	Worse
NOs-2 [4NOS]	Homo sapiens	No	X-Ray diffraction	2.25 A^{0}	0.199	Worse
nNOs [6AV2]	Homo sapiens	Yes	X-Ray diffraction	2.10 A^{0}	0.183	Better
PIK3CA [5DXT]	Homo sapiens	No	X-Ray diffraction	2.25 A^{0}	0.228	Better
EGFR [3EQR]	Homo sapiens	No	X-Ray diffraction	2.00 A^{0}	0.212	Better
PRKCA [8U37]	Homo sapiens	Yes	X-Ray diffraction	2.48 A^{0}	0.220	Better
PGE-2 [2ZB4]	Homo sapiens	No	X-Ray diffraction	1.63 A^{0}	0.186	Better

Figure 3: Extraction by maceration method



Extraction and Phytochemical Screening Coarsely powdered *D. falcata* leaves (250 g) were subjected to maceration using 60% ethanol and 80% ethanol for seven days. The extract was filtered and the solvent was evaporated using a tray drier. Preliminary phytochemical screening was conducted on the extracts using standard tests for tannins (ferric chloride, dilute nitric acid, bromine water), saponins (foam test), and flavonoids (Shinoda, lead acetate, sodium hydroxide tests). (7)(11)

Thin Layer Chromatography (TLC) and Total Flavonoid Content (TFC) TLC was performed on silica gel G-256 plates using a solvent system of Toluene: Acetone: Formic acid (30:10:10) to identify the presence of flavonoids compared to a standard quercetin reference. The total flavonoid content of the 80% ethanolic extract was estimated utilizing the aluminium chloride colorimetric method, with absorbance measured at 415 nm against a quercetin standard curve. (12)(13)

Fourier Transform Infrared Spectroscopy (FTIR) FTIR spectra of the pure *D. falcata* extract, chitosan, and a physical mixture were recorded (4000–400 cm^{-1}) using the KBr pellet method to investigate potential chemical interactions and compatibility. (14)

Formulation of Chitosan-based Microspheres Microspheres were prepared using the emulsion crosslinking method. In the optimized batch (Batch 3), 300 mg of leaf extract and 450 mg of chitosan were dissolved in 15 ml of 5% acetic acid. This dispersion was added to a mixture of light (100 ml) and heavy (50 ml) liquid paraffin containing 1.5 ml of Span 80. The mixture was stirred at 1200 rpm, and 3 ml of glutaraldehyde (25% aqueous solution) was added as a crosslinking agent. The resulting microspheres were recovered by decantation, washed with n-hexane to remove oil traces, rinsed with water, and dried at 40°C for 24 hours. (15)

Table 2: Preparation of Microsphere by Emulsion Crosslinking Method

Sr. No.	Ingredients	Batch-1	Batch-2	Batch-3
1	Leaf Extract	150mg	150mg	300mg
2	Chitosan	300mg	450mg	450mg
3	5% Acetic acid	15ml	15ml	15ml
4	Glutaraldehyde	3ml	3ml	3ml
5	Span 80	1.5ml	1.5ml	1.5ml
6	Liquid paraffin oil	150 ml	150 ml	150 ml

Evaluation of Microspheres

Percentage Yield: The total weight of the drug and polymer used in the formulation was measured before the preparation of microspheres. The final weight of the dried microspheres was then measured accurately using an analytical balance. The percentage yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of dried microspheres obtained}}{\text{Total weight of drug and polymer used}} x\,100$$

Percentage Entrapment Efficiency

To determine the percentage entrapment efficiency of the prepared microspheres, an accurately weighed quantity of 25 mg microspheres was crushed and dispersed in 100 ml of phosphate buffer (pH 6.8). The dispersion was sonicated for 20 minutes to ensure complete disruption of the microspheres and release of the entrapped drug. This was followed by continuous stirring on a magnetic stirrer for 6 hours to achieve thorough extraction of the drug into the buffer medium. The resulting dispersion was then filtered to remove particulate matter, and the filtrate was analysed for drug content using a UV-Visible spectrophotometer at 266 nm. The percentage entrapment efficiency was calculated using the following formula:

$$\% \text{ Entrapment Efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} x\,100$$

Zeta Potential Determination

Zeta Potential was determined using LITESIZER 500 at Balpande College of Pharmacy, Nagpur. Zeta potential was measured by electrophoretic light scattering, which assessed the particle's speed in the presence of electronic field. How fast the particles move depends on the surface charge, or zeta potential, of the particles. In general, the higher the zeta potential, the more stables the colloid.

Surface Morphology Determination

Scanning Electron Microscopy (SEM) was used to characterized the surface morphology of the prepared microspheres. Microsphere formulation was mounted on a clear – glass stub, air-dried, gold coated and visualized under SEM Machine model JEOL JSM 6380A by VNIT, Department of Metallurgical Materials Engineering, Nagpur.

***In-vitro* drug release study of microspheres:** The *in-vitro* release study of the microspheres was evaluated using the USP Type II (paddle) dissolution apparatus. Microspheres equivalent to 250 mg of extract were accurately weighed and transferred into a dissolution vessel containing 500 mL of phosphate buffer (pH 6.8), pre-equilibrated to 37 ± 0.5°C. The paddle was rotated at a speed of 50 rpm, and the temperature of the dissolution medium was maintained at 37 ± 0.5°C throughout the study. At predetermined time intervals, aliquots were withdrawn, filtered, and analyzed for drug release using a UV-Visible spectrophotometer at 266 nm. After each sampling, the same volume of fresh pre-warmed buffer was added to maintain sink conditions. (15)

***In-Vivo* Anti-hemorrhoidal Activity**: The anti-hemorrhoidal potential was assessed in healthy Wistar albino rats (150-250 g), divided into four groups (Normal, Negative control, Formulation, Standard Daflon 500mg). Hemorrhoids were induced by a 30-second recto-anal application of a croton oil preparation (deionized water, pyridine, diethyl ether, and croton oil in a 1:4:5:10 ratio) daily for 5 days. Test formulations were administered orally via gavage at a dose of 200 mg/kg twice daily for 7 days. The therapeutic impact was evaluated through daily monitoring of physical appearance using a visual analogue scale, hemorrhoid size (Digital Williams Probe), and subsequent histopathological examination of isolated recto-anal tissues. (16)(17)

Table 3: Visual analogue scale

Score	Specification
1	Healthy pink tissue with no swelling
2	Reddish pink tissue with mild swelling and no bleeding
3	Red tissue with mildly severe swelling and no bleeding
4	Brown tissue with moderately severe swelling, and presence or absence of bleeding
5	Dark brown tissue with severe swelling and bleeding

Results and Discussion

Toxicity and Docking Analysis ADMET Lab predictions indicated that quercetin possessed moderate toxicity across mutagenicity, carcinogenicity, and cytotoxicity parameters, while rutin showed lower toxicity in most parameters despite high mutagenicity. Molecular docking revealed that both flavonoids exhibited strong binding affinities toward crucial hemorrhoidal target proteins. Quercetin demonstrated high binding scores with MMP-9 (-9.4 kcal/mol), PIK3CA (-9.2 kcal/mol), and EGFR (-9.8 kcal/mol). Rutin displayed robust interactions with NOS-2 (-11.2 kcal/mol) and PGE-2 (-10.1 kcal/mol). The formation of conventional hydrogen bonds, pi-pi stacking, and hydrophobic contacts visualized via Discovery Studio highlights their capacity to suppress inflammatory responses, oxidative stress, and matrix degradation pathways.

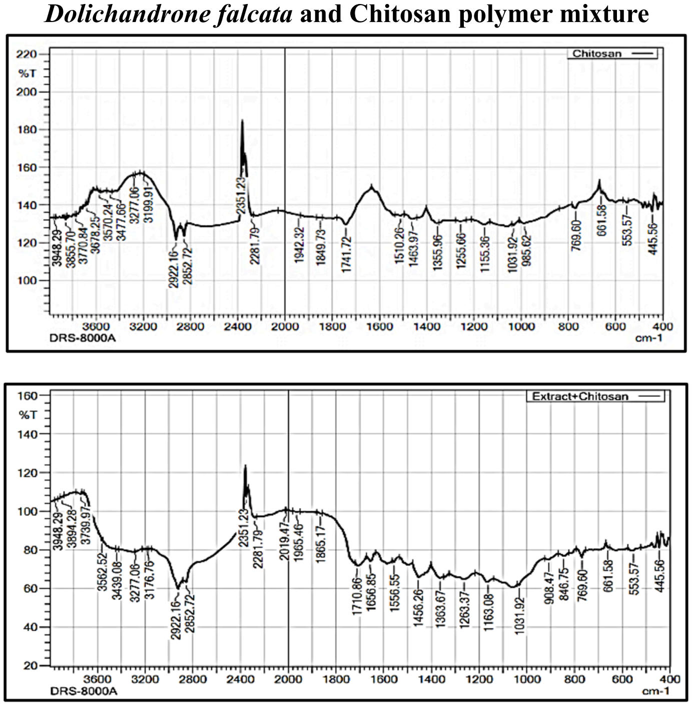
Table 4: Binding Interaction Analysis of ligands with Selected Hemorrhoidal Proteins

Sr.no	Receptors [PDB Ids]	Docking score with Quercetin	Docking score with Rutin	Native Ligand	No. of conventional hydrogen bonds with Quercetin	No. of conventional hydrogen bonds with Rutin
1	NF-kB [6M2C]	-5.9	-5.4	Zn	1	1
2	TNF-α [7KPA]	-5.9	-6.7	D84	2	5
3	IL-1β [8C3U]	-7.2	-7.4	T9C	1	4
4	MMP9 [1GKC]	`-9.4	-8.6	NFH	5	4
5	MMP-2 [8H78]	-9.1	-9.0	L2U	4	1
6	MMP7 [1MMP]	-7.4	-8.6	ZN	2	7
7	NOs-2 [4NOS]	-9.1	-11.2	HEM	5	6
8	nNOs [6AV2]	-8.9	-11.0	HEM	2	4
9	PIK3CA [5DXT]	-9.2	-9.8	5H5	4	6
10	EGFR [3EQR]	-9.8	-10.4	T74	5	6
11	PRKCA [8U37]	-8.5	-8.0	V5U	2	1
12	PGE-2 [2ZB4]	-9.0	-10.1	NAP	2	7

Extraction, Phytochemical Analysis, and TFC The 80% ethanolic extraction provided an optimal balance, yielding 5.68% of a thick, greenish-black viscous extract. Preliminary screening of this extract confirmed the abundant presence of tannins, saponins, and flavonoids (positive Shinoda and sodium hydroxide tests). The TLC plate displayed a major spot with an Rf value of 0.76, strictly correlating to the standard quercetin Rf of 0.75, confirming the presence of quercetin-like flavonoids. Using the colorimetric method, the total flavonoid content was estimated to be 19.47 µg/ml, translating to 19.47 mg/g of quercetin equivalents.

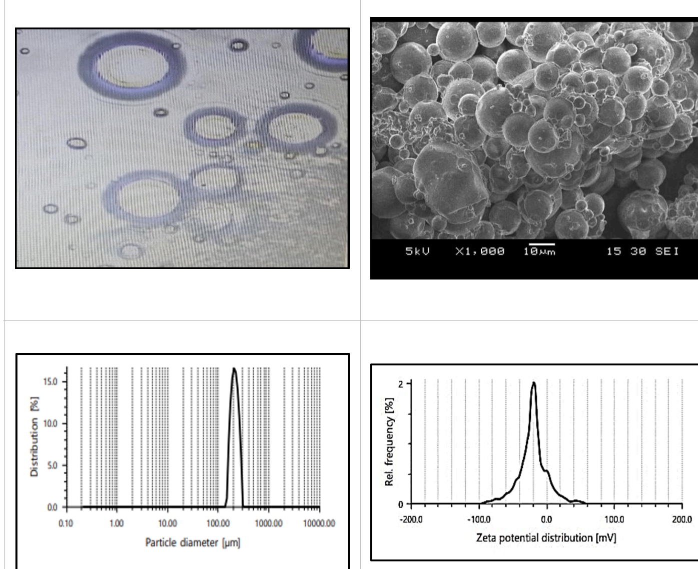
FTIR Interaction Analysis The FTIR spectrum of the pure *D. falcata* extract exhibited characteristic broad peaks for O–H and N–H stretching (3925.14 – 3292.48 cm^{-1}), C=O stretching (1714.72 cm^{-1}), and C–O stretching (1031.92 cm^{-1}). The spectrum of the extract combined with the chitosan polymer successfully retained these characteristic peaks without any significant shift or disappearance of functional groups.

Figure 4: FTIR Spectra of (a) *Dolichandrone falcata* (b) *Dolichandrone falcata* and Chitosan polymer mixture



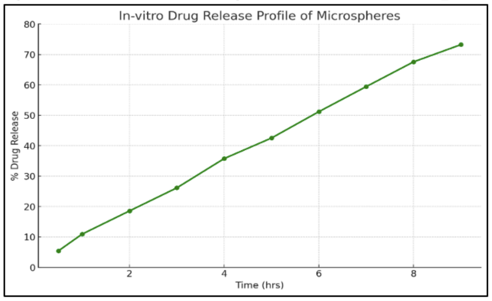
Characterization of Microspheres Among the prepared trial batches, Batch 3 (formulated via emulsion crosslinking with 450 mg chitosan and 3 ml glutaraldehyde) emerged as the optimized formulation. It provided an excellent percentage yield of 79.3% and a high drug entrapment efficiency of 73.3%. Microscopic evaluation and dynamic light scattering indicated that the microspheres were predominantly spherical with a mean particle size of 255 µm. Zeta potential analysis revealed a value of -19.6 mV, indicating sufficient surface charge for stability against aggregation. SEM analysis further corroborated these findings, displaying distinct spherical microparticles with a highly smooth surface topology.

Figure 5: Microsphere (a) Under motic (b) SEM (c) Particle diameter (d) Zeta potential



***In-Vitro* Drug Release** The *in-vitro* dissolution profile demonstrated that the chitosan microspheres successfully achieved sustained release of the *D. falcata* extract. An initial burst release of 10.92% was observed within the first hour, likely attributed to the drug adsorbed on the particle surface. This was followed by a controlled and gradual release, ultimately reaching 73.23% over 9 hours.

Figure 6: *In-vitro* drug release of microsphere



***In-Vivo* Anti-Hemorrhoidal Efficacy** In the croton oil-induced rat model, treatment with the *D. falcata* microsphere formulation led to a marked alleviation of hemorrhoidal symptoms. External visual assessments using the established visual analogue scale documented progressive healing throughout the 7-day treatment. Visual analogue scale observations demonstrated improvement in inflammation and swelling compared to the negative control group. The therapeutic benefits observed matched closely with those of the standard drug, Daflon 500 mg, proving the effectiveness of the optimized herbal microspheres in mitigating acute hemorrhoidal pathogenesis.

Table 5: Visual analogue scale

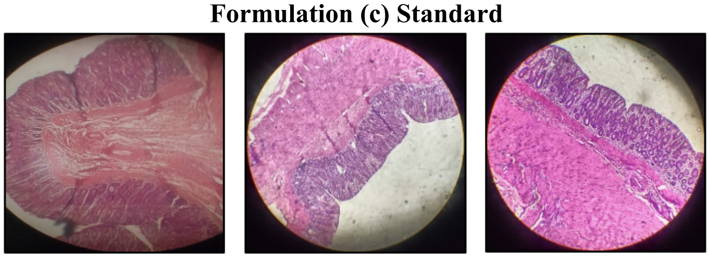
	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Negative Control	4.75±0.5	3.25±0.96	4±1.41	2.75±1.5	3±1.41	3.5±1.73	3.5±1.73
Formulation	4.75±0.5	3±0.82	3.75±1.5	2.5±0.58	1.75±0.5	1.25±0.5	1±0.0
Standard	4.5±1	4.25±0.96	4±0.82	2.5±0.58	2.75±0.5	1.75±0.96	1.25±0.5

Table 6: Changes in hemorrhoids size

	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Negative Control	4.75±1.26	4.25±0.96	4.25±0.96	3.25±2.22	4±2.83	3.75±2.63	4.25±2.87
Formulation	4.5±1.29	4±0.82	4.25±2.06	3.25±0.96	2.5±0.58	1.25±1.5	0±0.0
Standard	5±0.82	3.25±0.5	4.25±0.96	3.75±1.71	4±0.82	2.5±0.58	1.25±1.5

Histopathological examination revealed noticeable differences among the experimental groups. The negative control group showed severe tissue damage and inflammatory changes, whereas the formulation-treated group demonstrated marked tissue recovery and restoration of normal architecture. Histological findings were comparable to those observed in the standard treatment group.

Figure 6: Histopathological Results (a) Negative Control (b) Formulation (c) Standard



Discussion

The docking findings suggest that quercetin and rutin may contribute to anti-hemorrhoidal activity through modulation of inflammatory mediators, nitric oxide pathways, and extracellular matrix remodeling proteins. Strong interactions with MMPs, NOS isoforms, EGFR, and PGE-2 support a multi-target mechanism of action.

The phytochemical profile and flavonoid content corroborate previous reports describing *Dolichandrone falcata* as a rich source of bioactive polyphenols. Flavonoids such as quercetin and rutin are known to possess antioxidant, venotonic, anti-inflammatory, and vascular protective properties, which are relevant to hemorrhoid management.

FTIR compatibility studies demonstrated that incorporation of the extract into the chitosan matrix did not alter the chemical integrity of the active constituents. This compatibility is important for maintaining formulation stability and therapeutic performance.

The optimized microspheres exhibited favorable physicochemical properties, including high entrapment efficiency and acceptable particle size distribution. The observed zeta potential suggests adequate stability of the formulation, while SEM findings confirmed successful microsphere formation.

The sustained-release profile obtained from the chitosan matrix may improve residence time and maintain prolonged availability of phytoconstituents. Such controlled release could reduce dosing frequency and enhance therapeutic effectiveness.

In-vivo evaluation demonstrated significant improvement in hemorrhoidal symptoms compared with untreated controls. The reduction in inflammation, edema, and hemorrhoid size, together with favorable histopathological findings, indicates meaningful therapeutic benefit. The comparable performance to Daflon 500 mg further highlights the potential of the formulation as a herbal alternative for hemorrhoid management.

Overall, the findings demonstrate that *Dolichandrone falcata*-loaded chitosan microspheres combine the pharmacological benefits of flavonoids with controlled drug delivery, providing a promising strategy for the treatment of hemorrhoidal disease.

Conclusion

The current study successfully bridges the traditional therapeutic utility of *Dolichandrone falcata* with modern sustained-release drug delivery systems. The identified flavonoids, notably quercetin and rutin, exhibited compelling *in-silico* affinities for major protein targets responsible for hemorrhoid pathogenesis, offering a molecular rationale for their anti-inflammatory and tissue-healing properties. The formulation of chitosan-based microspheres using an emulsion crosslinking method achieved an optimal particle size, high entrapment efficiency, and a controlled drug release profile. Furthermore, robust *in-vivo* testing confirmed significant anti-hemorrhoidal activity matching established standard therapies. Consequently, *D. falcata* extract-loaded microspheres represent a highly viable, safe, and cost-effective oral herbal alternative to conventional treatments for managing internal and external hemorrhoids.

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