LEX LOCALIS-JOURNAL OF LOCAL SELF-GOVERNMENT
ISSN:1581-5374 E-ISSN:1855-363X
VOL. 23, NO. S6(2025)

FORMULATION, EVALUATION AND IN-VITRO DRUG RELEASE STUDIES OF POLYHERBAL-LOADED CHITOSAN SCAFFOLD FOR TREATING AND MANAGING FROSTBITE CONDITIONS

Lahari Priya M[1], Shanmugam R*[1] , Ramya Gade[2] , Subhajit Dutta[1], Vijayaraj Surendran[3], L Priyanka Dwarampudi[2], D. Nagasamy Venkatesh[4]

[1]TIFAC CORE in HD & Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Nilgiris, Ooty, Tamil Nadu, India.
[2]Department of Pharmacognosy, JSS College of Pharmacy, Ooty, JSS Academy of Higher Education and Research, Nilgiris, Ooty, Tamil Nadu, India.
[3]Department of Pharmaceutical Analysis, Dr. Kalam College of Pharmacy, Thanjavur, Tamil Nadu, India.
[4]Department of Pharmaceutics, JSS College of Pharmacy, Ooty, JSS Academy of Higher Education and Research, Nilgiris, Ooty, Tamil Nadu, India.

***Corresponding Author:** Dr. R. Shanmugam,
*TIFAC CORE in HD & Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Nilgiris, Ooty, Tamil Nadu, India
shanmugam_55555@yahoo.co.in

Abstract
Frostbite is a cold injury that occurs at temperatures below 0°C, leading to the formation of ice crystals within and between cells, which causes considerable damage to the endothelium. This research focused on the development and characterization of a polyherbal scaffold using chitosan with the incorporation of genistein and capsaicin, primarily for topical application in frostbite conditions. A polyherbal scaffold formulation was developed using the freeze-drying technique and evaluated for its linearity, FT-IR, tensile strength, porosity, water absorption test, SEM analysis and *In-vitro* drug release studies. FTIR analysis indicated that there were no chemical interactions between the polymer, drugs and the formulation. The tensile strength reveals that the average stress range is 0.015 ± 0.004 N/m2 and the average strain range is 34.7 ± 11.50. The porosity was found to be 78.8% and the water absorption test was found to be 33.04%. The SEM analyses revealed that the formulated polyherbal scaffold is Porous. The *In-vitro* drug release studies reveal that capsaicin shows a faster release compared to genistein in all time intervals. The results indicate that this polyherbal chitosan-loaded scaffold shows potential for effectively treating frostbite through its ability to reduce inflammation, enhance wound healing and promote tissue regeneration. The incorporation of genistein and capsaicin in the chitosan-loaded scaffold provides a complementary strategy for frostbite-affected areas.

Keywords: Frostbite, Polyherbal Scaffold, Genistein, Capsaicin,

Introduction

Frostbite is a type of cold injury that occurs at temperatures below 0°C, which develops ice crystals within and between cells, resulting in significant damage to the endothelium. Frostbite symptoms include vasoconstriction, hemorrhage, and decreased blood flow to the affected area, which can potentially lead to ischemic perfusion injury. (1) Frostbite can affect military personnel who are working in cold climatic conditions, homeless personnel, sports personnel, mountaineers, high-altitude personnel and people participating in recreational activities (skiing, hiking, climbing, and ice climbing). Frostbite mainly occurs on the ears, nose, cheeks, fingers, hands, toes, and feet. (2) The pathophysiology of frostbite includes direct phase due to ice crystal formation and the indirect phase includes microvascular thrombosis and inflammatory responses, leading to heightened tissue ischemia and potential necrosis. Frostbite severity is from mild to severe—First-degree which means injury to the skin with redness and swelling. Second-degree burns induce full-thickness damage and clear blisters. Third-degree burns generate bleeding blisters and blue-gray discolouration. Fourth-degree which means damage to muscles, bones, and tendons. Complications encompass paresthesia, cold sensitivity, gangrene, and persistent pain. (3)

Scaffold-based biomaterials have become effective alternatives for treating tissue damage caused by frostbite because they can accelerate the healing and regeneration in cases of cold-induced injury.

Frostbite causes necrosis, reduced blood flow, and damaged cellular repair, developing biomaterial scaffolds necessary for repairing the structure and function of damaged tissues. (4) These scaffolds act as supporting matrices that mimic the extracellular matrix (ECM) and help fibroblasts grow, new blood vessels form, and the skin heal after frostbite. Several biomaterials, including synthetic polymers and natural polymers such as polylactic-co-glycolic acid (PLGA), alginate, chitosan, and collagen, have been evaluated for their biocompatibility, biodegradability, and mechanical properties to determine which are suitable for the reconstruction of cold-injured tissues. (5)

Chitosan promotes the healing of frostbite-induced skin damage by enhancing cell migration and proliferation. It's biocompatible, stimulates the development of fibroblasts and keratinocytes which are necessary for new tissue formation. Its natural antibacterial characteristics assist in preventing infection, inflammation and enhancing wound healing. (6) Modified chitosan can be developed to release bioactive compounds or growth factors that promote angiogenesis and tissue regeneration. Its exceptional biocompatibility also makes it easy to incorporate into host tissues, which helps with structural repair and revascularization after frostbite wounds and decreases immunological reactivity. (7)

Genistein is a phytoestrogen isoflavone present in soybeans, exhibits significant therapeutic potential for frostbite therapy through many mechanisms. Its antioxidant properties is to remove the reactive oxygen species, which lowers the damage to cells that occurs during freezing-thawing and reperfusion injury. (8) Genistein has pro-angiogenic characteristics by augmenting the synthesis of vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS), hence promoting neovascularization and microvascular perfusion in ischemic tissues. Its anti-inflammatory effects regulate the release of cytokines, which helps to reduce swelling and damage which preventing cell death. (9) Genistein stimulates endothelial cell migration, proliferation, and fibroblast activity, it helps heal microvascular damage and produce collagen, which is necessary for tissue preservation. Its bioactive properties make it a promising therapeutic agent for promoting frostbite wound healing and tissue regeneration. (10)

Capsaicin is an alkaloid derived from chili peppers. It has considerable potential as a treatment for frostbite, as it affects multiple aspects of the nervous system and blood vessels. Capsaicin, an agonist of transient receptor potential vanilloid 1 (TRPV1) vasodilation by activating sensory nerve terminals and releasing neuropeptides. This improves blood flow to body parts that lack sufficient circulation. (11) The increased blood flow makes it easier for tissues to obtain oxygen and nutrients, both of which are essential for cell regeneration. Capsaicin acts as a pain reliever by lowering the levels of substance P in nociceptive neurons. This helps with nerve pain and your system's failure to handle the cold as you heal. (12) It reduces inflammation, changes the levels of inflammatory cytokines, and prevents capillary thrombosis. Capsaicin also improves the function of capillaries, speeds up local metabolism and makes it easier to warm up in frostbite treatment and recovery. (13)

The objective of this study is to develop a polyherbal scaffold formulation by combining the phytoconstituents such as Genistein and Capsaicin. The developed formulation will then be evaluated for its suitability for topical application.

Materials and Methods

Materials

The chitosan was purchased from HI Media in Mumbai. The acetic acid was procured from Merck. Genistein was purchased from SLV Scientifics, Bangalore and Capsaicin was purchased from Sigma Aldrich.

Methods

Determination of Wavelength for Genistein

About 10 mg of the drug was measured and placed into a 10 ml standard flask. After that, approximately 3–4 mL of methanol was added and underwent sonication for a duration of 10-15

minutes. The solution was adjusted to the mark with methanol to get a 1 mg/ml concentration (stock solution). A stock solution of 1000 µg/mL was initially serially diluted to obtain concentrations of 100 mcg/mL and 10 mcg/mL. Once the samples were diluted, the samples were undergone for analysis with UV spectrophotometry in the range of 400 to 200 nm for determining the λ max (Figure 1). (14)

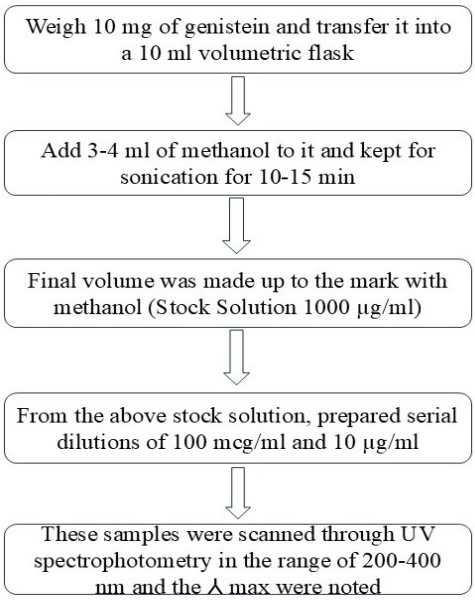

Figure 1: Procedure for wavelength determination of genistein

Determination of Wavelength for Capsaicin

About 10 mg of the drug was measured and placed into a 10 ml standard flask. After that, approximately 3–4 mL of methanol was added and underwent sonication for a duration of 10-15 minutes. The solution was adjusted to the mark with methanol to get a 1 mg/ml concentration (stock solution). A stock solution of 1000 µg/mL was initially serially diluted to obtain concentrations of 100 mcg/mL and 10 mcg/mL. Once the samples were diluted, the samples were undergone for analysis with UV spectrophotometry in the range of 400 to 200 nm for determining the λ max (Figure 2). (15)

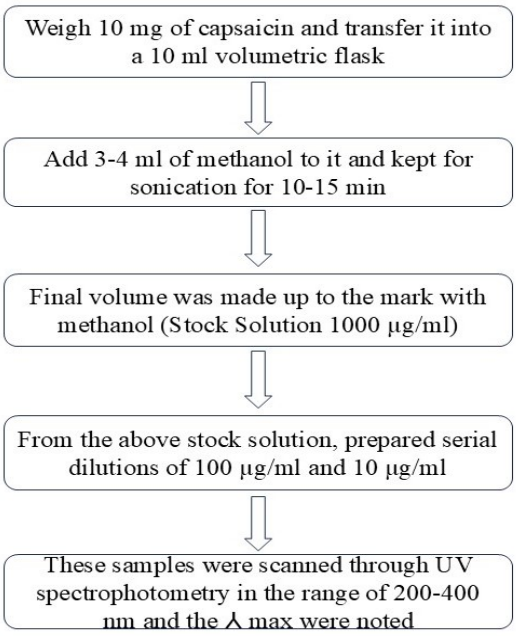

Figure 2: Procedure for wavelength determination of capsaicin

Determination of Calibration Curve (CC) for Genistein

A stock solution of genistein (100 µg/mL) was prepared by dissolving 10 mg of the drug in 3 mL of methanol, sonicating for 5 minutes and then made up to a final volume of 10 mL with methanol. Serial dilutions from 1 to 10 µg/mL were prepared by taking concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the stock solution and transferring them into 10 mL volumetric flasks and made up to the mark with methanol. A calibration curve was plotted with absorbance versus concentration and the parameters of the regression equation, slope, intercept, and correlation coefficient were determined (Figure 3). (16)

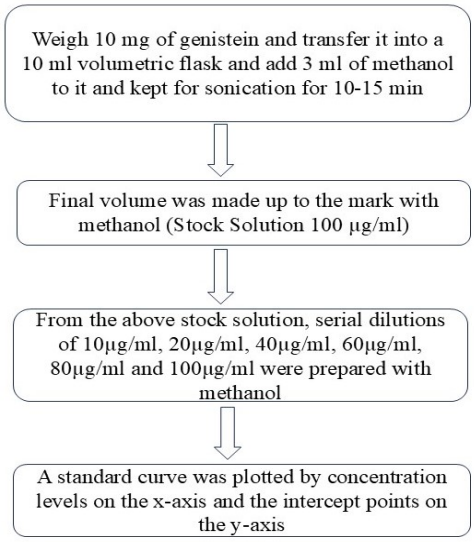

Figure 3: Procedure for CC of genistein

Determination of Calibration Curve for Capsaicin

To make the stock solution of capsaicin (100 µg/ml) was dissolved in methanol, sonicated for 5 minutes and then methanol was added up to the mark. A series of dilutions was prepared by taking volumes of 0.1 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of methanol into the stock solution. A standard curve was plotted by concentration levels on the x-axis and the intercept points on the y-axis and the parameters of the regression equation, slope, intercept, and correlation coefficient were determined (Figure 4). (17)

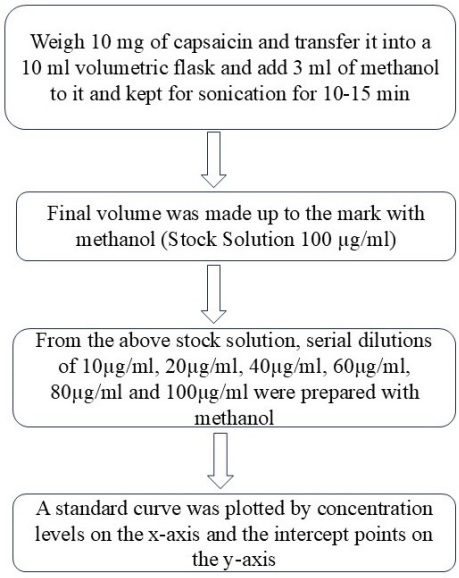

Figure 4: Procedure for CC of capsaicin

Fourier Transform Infrared (FT-IR) Spectroscopy Studies

The Fourier Transform Infrared Spectroscopy (FT-IR) was employed to interpret the substance, polymer, and formulation. The infrared spectra were acquired within the wavelength range of 4000 cm-1 to 400 cm-1. The infrared spectra of the selected substance and polymer were initially analysed and their compatibility was next evaluated through comparison. (18)

Preparation of Polyherbal Scaffold

The poly-herbal scaffold was developed using a freeze-drying technique. A 1% acetic acid solution was used to dissolve the chitosan. The solution was dissolved in 100 ml of distilled water at room temperature to obtain the required final volume. The polyherbal scaffold was developed by dissolving genistein and capsaicin in a chitosan solution.

The initial solution was placed under a magnetic stirrer until it changed, resulting in a cloudy water-based solution. The solution was sonicated for 5 minutes to remove any air bubbles present. The solutions were then transferred to Petri plates and stored in a deep freezer at a temperature of -60 °C for 24 hours. The sample was kept in a lyophilizer for a period of 72 hours to develop a polyherbal scaffold (Figure 5). (19,20)

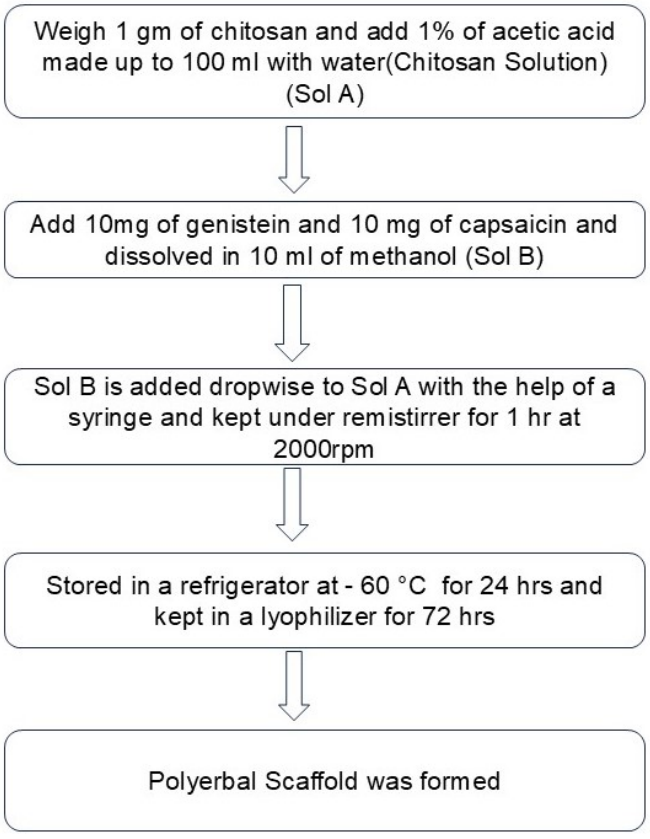

Figure 5: Preparation for polyherbal scaffold

Tensile Strength

To evaluate the compressive strength, plate samples of 3 mm x 3 mm were produced. The samples were exposed at a rate of 5 mL/min. Each group was composed of three specimens placed in parallel. Specimens with dimensions of 3mm × 3mm × 2mm were prepared for the tensile test. Each group consisted of three specimens that were parallel with one another. The elongation was determined by dividing the extensibility by the initial length (Figure 6). (21)

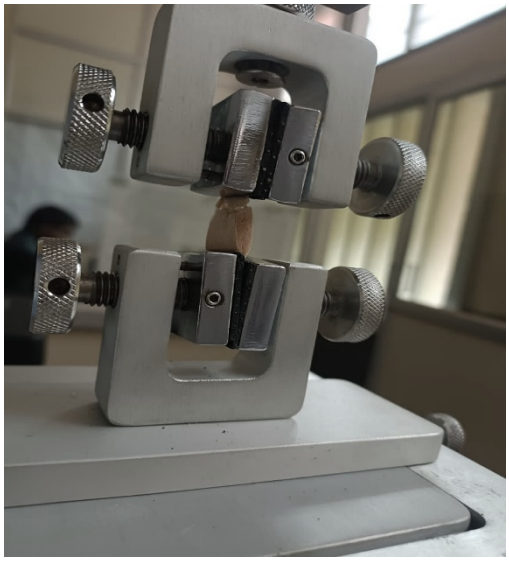

Figure 6: Tensile strength for polyherbal scaffold

Porosity

The liquid displacement method is used to determine the porosity of the scaffolds. The pore volume (Vp) and geometric volume (SV) of the scaffold are determined through the ethanol displacement method. The volumes of these discs are determined by measuring their diameter and height. To remove any air bubbles in the scaffold and along with weights (Ww) are immersed in 100% ethanol at room temperature and placed in desiccators under reduced pressure for a duration of 5 minutes. (22)

The following equation is used to determine the scaffold porosity:

Porosity (%) $=(W_w-W_d)/Sv\times100$

Water Absorption test for scaffold

The scaffolds were initially measured for their dry weight (Wo). Samples were then immersed in phosphate-buffered saline (PBS, pH 7.4) and incubated at 37°C for a duration of two hours. After incubation, any excess moisture was removed from the expanded scaffolds by blotting with filter paper prior to weighing. The samples were then subjected to lyophilization and subsequently weighed. The absorption percentage was determined using a specific formula to determine the water content. (23)

Scanning Electron Microscope (SEM)

The sample's surface morphology was analysed using a scanning electron microscope. A minimal quantity of the sample is deposited onto a carbon film-coated copper grid for the use of scanning electron microscopy analysis. The copper grip is firmly secured in the sample holder and positioned within the vacuum chamber of the scanning electron microscope, where it is examined under low vacuum conditions, and SEM images are recorded. (24)

***In-Vitro* Drug Release Studies**

The composite scaffold (3×3 cm) was immersed in 20 mL of PBS (phosphate-buffered saline) at a pH of 7.4 and a temperature of 37°C for a study on drug release for genistein and capsaicin. The supernatant was regularly removed and substituted with an equal volume of fresh PBS to maintain a consistent volume. The amounts of genistein and capsaicin released and measured through UV-visible spectroscopy at wavelengths of 262 nm and 281 nm, respectively, were calculated by referencing

standard curves developed with methanol. The drug release percentages of genistein and capsaicin were determined. (25)

Results and Discussion

Determination of wavelength for Genistein

A solution of 10µg/ml was prepared from the stock solution and then scanned using a UV-visible spectrophotometer between the range of 200-400nm and spectra were recorded through the UV-visible detector. The maximum absorbance wavelength for genistein was found to be 262 nm.

Determination of Wavelength for Capsaicin

The 10 µg/ml solution was prepared from the stock solution and analyzed using a UV-visible spectrophotometer within the 200–400 nm range, with the spectra recorded by the UV-visible detector. The maximum absorbance wavelength for capsaicin was found to be 281 nm.

Determination of Calibration Curve for Genistein

The calibration curve was analyzed by using different concentrations from 10 mcg/ml to 100 mcg/ml (10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) with □max range of 262nm (Table 1). The CC was plotted using standard solution concentration on the x-axis and peak area on the y-axis. The R^2 (correlation coefficient) was found to be 0.9984 (Figure 7).

Table 1: Calibration Curve for Genistein

S.No	Concentrations	Absorbance
1	10	1.09
2	20	1.239
3	40	1.429
4	60	1.65
5	80	1.85
6	100	2.09

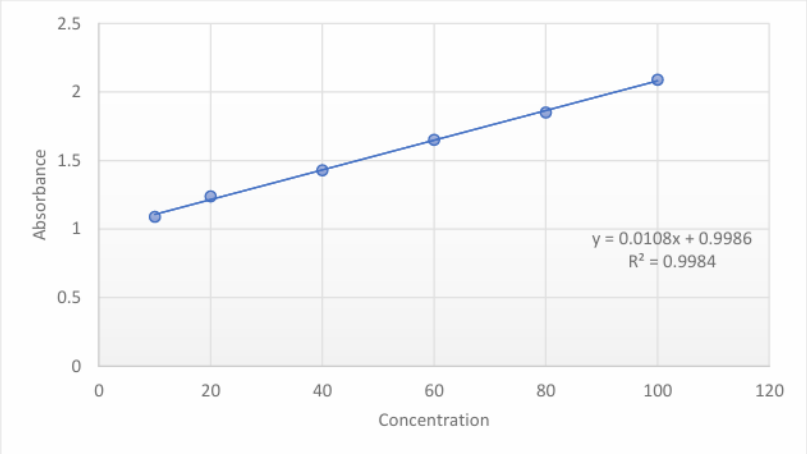

Figure 7: CC for genistein

Determination of Calibration Curve (CC) for Capsaicin

The calibration curve was analyzed by using different concentrations like 10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml with □max range of 281nm in a UV-visible spectrophotometer (Table 2). The CC was plotted using peak area on the y-axis and standard solution concentration on the x-axis. The Correlation Coefficient (R^2) was found to be 0.9912 (Figure 8).

Table 2: Calibration Curve for Capsaicin

S.No	Concentrations	Absorbance
1	10	1.31
2	20	1.43
3	40	1.62
4	60	1.805
5	80	2
6	100	2.102

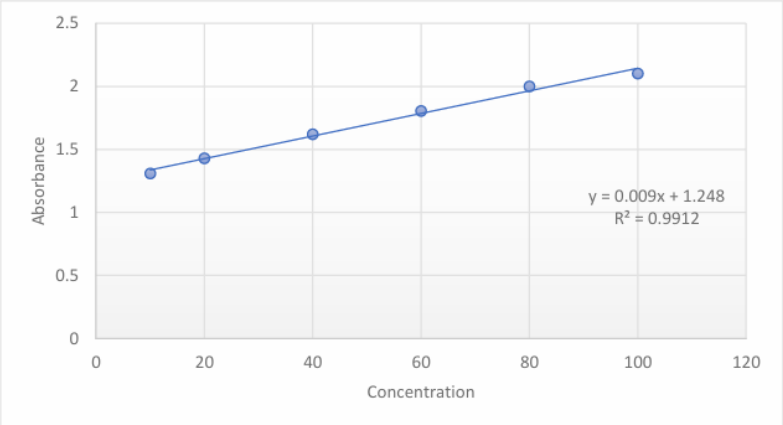

Figure 7: CC for Capsaicin

Fourier Transform Infrared (FT-IR) Spectroscopy Studies

The IR spectra of the polymer, drug and formulation were analysed with the wavelength range of 400 cm-1 to 4000 cm-1. The wave numbers of the polymer, drugs and formulation were 3367.14, 3408.59, 3657.36 and 3214.93 due to the O-H stretch, 2917.03, 2900.64 and 2924.83 due to the C–H stretch and 1060.68, 1307.10, 1279.64 and 1151.24 due to the C–O stretch. (Figure 8) FTIR analysis indicated that there were no chemical interactions between the polymer, drugs and the formulation (Table 3).

Table 3: Compatibility studies for polymer, drugs and formulation

S.No	Functional Groups	Wave Numbers	Chitosan	Genistein	Capsaicin	Formulation
1	O–H stretch	3500–3200	3367.14	3408.59	3657.36	3214.93
2	C–H stretch	3000–2850	2917.03	-	2900.64	2924.83
3	C–O stretch	1320–1000	1060.68	1307.10	1279.64	1151.24

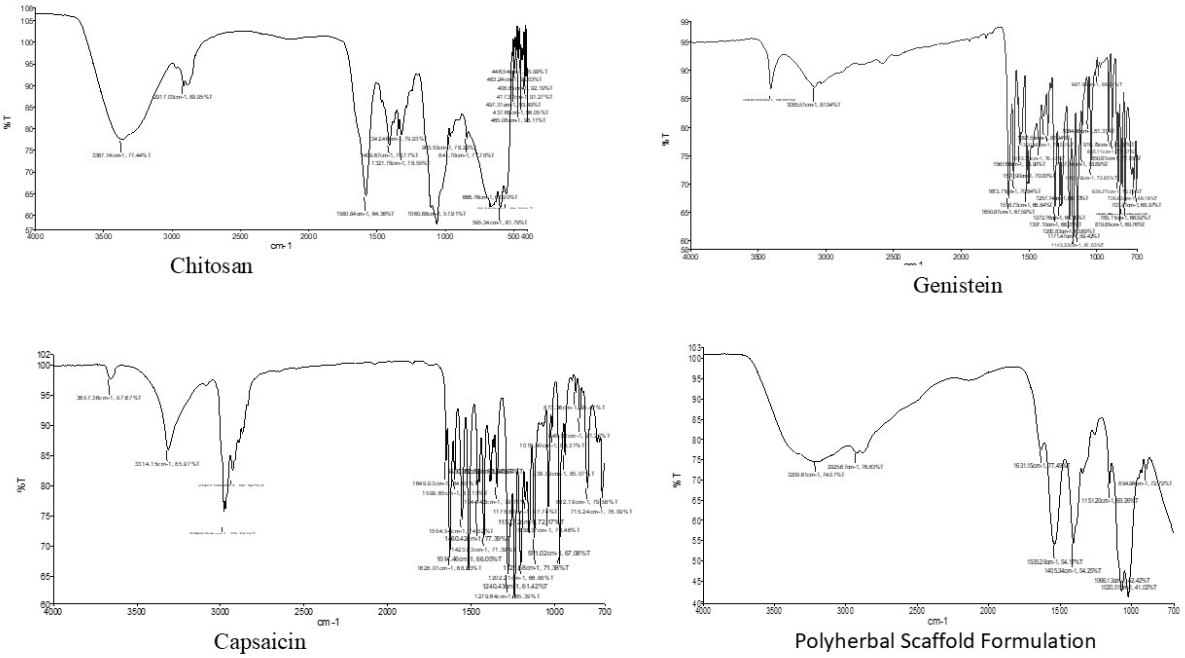

Figure 8: FT-IR spectra of chitosan, genistein, capsaicin and formulation

Preparation of Polyherbal Scaffold Formulation

The drug-loaded chitosan scaffold was developed by using freeze freeze-drying technique. The scaffold was developed by combining phytoconstituents such as genistein and capsaicin. The scaffolds had a macroscopic appearance and a three-dimensional structure similar to that of a sponge with a homogeneous and porous configuration. The composite scaffold is off-white in colour, rigid and elastic (Figure 9).

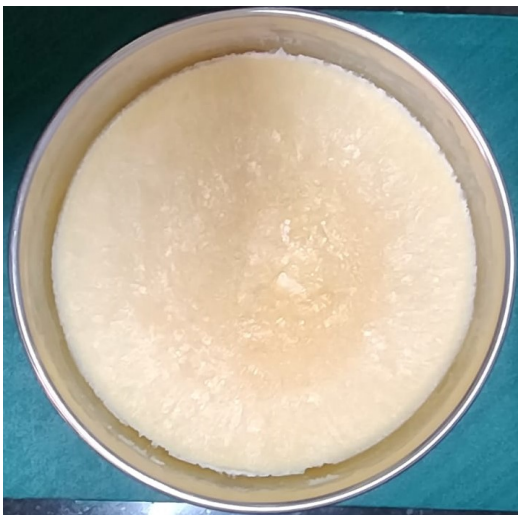

Figure 9: Polyherbal scaffold formulation

Tensile Strength

The polyherbal scaffold has exceptional strength and elasticity, significantly enhancing its reinforcing capabilities. This leads to a strong integration with the matrix and an improvement in the mechanical

strength of the materials. The average stress range is 0.015 ± 0.004 N/m2 and the average strain range is 34.7 ± 11.50. The porous structure was found to be very elastic in nature and the Young's modulus was found to be **0.004**.

Porosity

Porosity is determined by the number of open pores, which are directly linked to the permeability and surface area of the porous material. A wound with a high level of porosity develops a positive biological environment that supports the healing process. The porosity of the chitosan scaffold incorporated with polyherbal compounds such as genistein and capsaicin was found to be **78.8 %**.

Water Absorption Test

The water absorption capacity of materials has a significant impact on the structural integrity of the chitosan scaffold and the development of cells. Scaffolds developed from highly hydrophilic materials can reach saturation, potentially resulting in expansion and distortion that might prevent cell proliferation and division. An increase in chitosan concentration results in a decrease in water absorption. However, the reduction in pore diameters may unknowingly enhance water absorption by capillary action. The analysis indicated that the water absorption rate of the genistein and capsaicin-loaded chitosan scaffold was **33.04%**.

Scanning Electron Microscope (SEM)

The formulated scaffold was analyzed using analysis through scanning electron microscope (SEM). The results revealed that the formulated polyherbal scaffold is Porous. The image revealed a lack of particle aggregation (Figure 10).

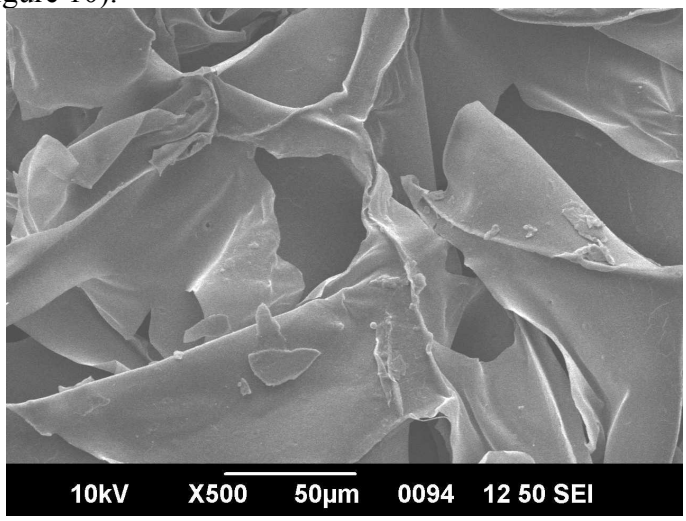

Figure 10: SEM analyses for formulated scaffold

***In-Vitro* Drug Release Studies**

The drug release from the genistein and capsaicin-loaded chitosan scaffold was studied over a period of 72 hours. Samples were collected from the tubes at various time intervals (0, 1, 2, 3, 6, 12, 24, 48 and 72 hours). Capsaicin shows a faster release compared to genistein across all time intervals (Table 4). The rate of drug release is increased because the scaffold contains a larger amount of drug (Figure 11).

Table 4: *In-vitro* drug release studies of genistein and capsaicin

Time (hr)	% Drug Release of Genistein	% Drug Release of Capsaicin
0	0	0
1	12.7	16
2	21.2	27.2
3	25.4	38.6
6	39.8	49.1
12	51.9	58.4
24	68.7	71.5
48	79.3	85.6
72	84.7	94.8

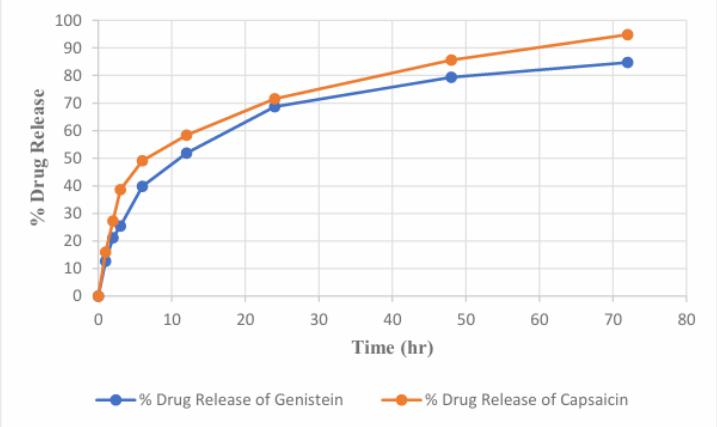

Figure 11: *In-vitro* drug release studies of genistein and capsaicin

Conclusion

The chitosan-loaded scaffold was incorporating genistein and capsaicin was successfully formulated and evaluated for the treatment of frostbite conditions. A polyherbal scaffold formulation was developed using the freeze-drying technique and evaluated for its linearity, FT-IR, tensile strength, porosity, water absorption test, SEM analysis and *In-vitro* drug release studies. FTIR analysis indicated that there were no chemical interactions between the polymer, drugs and the formulation. The tensile strength was found to be very elastic in nature, as the Young's modulus was found to be 0.0004. The porosity was found to be 78.8 % and the water absorption rate was found to be 33.04%. The SEM analyses revealed that the formulated polyherbal scaffold is Porous. The *In-vitro* drug release studies reveal that capsaicin shows a faster release compared to genistein across all time intervals. The polyherbal scaffold formulation is effective in treating frostbite by demonstrating anti-inflammatory, anti-thrombotic, and wound healing activities as indicated by the above results.

References

1. Saraoui W, Sivakumar BS, Forli A, Perruisseau-Carrier A. Frostbite to the upper limb: an updated review and management recommendations. Hand Surgery and Rehabilitation. 2025 Oct 16:102302.
2. Turner BL, van Dongen TT, Berendsen RR, de Jong FJ, Endert EL, Van Hulst RA, Hoencamp R. Frostbite: a treatment guideline for prehospital treatment in a military environment. BMJ Mil Health. 2025 Apr 1;171(2):152-4.

3. Lahari Priya M, Shanmugam R, Ramya G, Subhajit D, Priyanka Dwarampudi L, Manikandan D, Krishnaraj R. Effectiveness of Herbal and Allopathic Medicines in Treating Frostbite. Natural Product Communications. 2025 Apr;20(4):1934578X251333020.
4. Cui W, Yue Y, Liu Y, Wang J, Yang Z, Qiang Y, Gong C, Yang JL. Polydopamine Nanoparticle-Loaded Antifreezing and Photothermal Organohydrogel Based on Pullulan for Frostbite Therapy. ACS Biomaterials Science & Engineering. 2025 Mar 17;11(4):2422-34.
5. Bhushan S, Singh S, Maiti TK, Sharma C, Dutt D, Sharma S, Li C & Tag Eldin EM, Scaffold fabrication techniques of biomaterials for bone tissue engineering: a critical review. Bioeng, 9 (2022) 728.
6. El-Araby A, Janati W, Ullah R, Ercisli S, Errachidi F. Chitosan, chitosan derivatives, and chitosan-based nanocomposites: eco-friendly materials for advanced applications (a review). Frontiers in Chemistry. 2024 Jan 4;11:1327426.
7. Žigrayová D, Mikušová V, Mikuš P. Advances in chitosan derivatives: preparation, properties and applications in pharmacy and medicine. Gels. 2024 Oct 29;10(11):701.
8. Gade R, Dwarampudi LP, Swaroop AK, Reddy DP, Rao NS. Synthesis, spectral characterization and free radical scavenging activity of zinc-genistein nano complex. Indian Journal of Biochemistry and Biophysics (IJBB). 2024 Jul 26;61(8):497-503.
9. Goh YX, Jalil J, Lam KW, Husain K, Premakumar CM. Genistein: A review on its anti-inflammatory properties. Frontiers in pharmacology. 2022 Jan 24;13:820969.
10. Li F, Yang C, Li M, Liu S, Xu K, Fu X. Antifungal activity of genistein against phytopathogenic fungi Valsa mali through ROS-mediated lipid peroxidation. Plants. 2025 Jan 3;14(1):120.
11. Yadav RB, Pathak DP, Varshney R, Arora R. Elucidation of the Role of TRPV1, VEGF-A, TXA2, Redox Homeostasis, and Inflammatory Cascades in Protection against Cold Injuries by Herbosomal-Loaded PEG-Poloxamer Topical Formulation. ACS Applied Bio Materials. 2024 May 8;7(5):2836-50.
12. Regli IB, Strapazzon G, Falla M, Oberhammer R, Brugger H. Long-term sequelae of frostbite—a scoping review. International journal of environmental research and public health. 2021 Sep 14;18(18):9655.
13. Smid DJ, Klous L, Ballak SB, Catoire M, De Hoogh IM, Hoevenaars FP. Exploring the role of nutritional strategies to influence physiological and cognitive mechanisms in cold weather operations in military personnel. Frontiers in Physiology. 2025 Feb 28;16:1539615.
14. Qassim AW, Rasheed AS, Abdulrahman SK. Hydrophilic interaction chromatographic analysis of genistein in herbs and propolis. Systematic Reviews in Pharmacy. 2020 Dec 1;11(12):756-63.
15. Boonrueang N, Chaichit S, Yooin W, Okonogi S, Kiattisin K, Ampasavate C. Triple-Loaded Nanoemulsions Incorporating Coffee Extract for the Photoprotection of Curcumin and Capsaicin: Experimental and Computational Evaluation. Pharmaceutics. 2025 Jul 17;17(7):926.
16. Liu X, Liu Y, Wang H, Zhang X. Genistein solid dispersion: preparation, physical-chemical characters and anti-oxidant properties. 多酚. 2025 Sep 30;7(3):112-26.
17. Lalić M, Soldić A, Lalić A, Lalić Z, Sertić M. Development and validation of an HPLC method for simultaneous determination of capsaicinoids and camphor in over-the-counter medication for topical use. Molecules. 2022 Feb 14;27(4):1261.
18. Errico S, Moggio M, Diano N, Portaccio M & Lepore M, Different experimental approaches for Fourier□transform infrared spectroscopy applications in biology and biotechnology: A selected choice of representative results. Biotechnol. Appl. Biochem., 70 (2023) 937-61.
19. Angraini N, Syarifuddin S, Rauf N, Tahir D. Advancements in Bone Tissue Engineering: A Comprehensive Review of Biomaterial Scaffolds and Freeze□Drying Techniques From Perspective Global and Future Research. Artificial Organs. 2025.

20. Sang S, Zhao Y, Shen Z, Cao Y, Cheng R, Yan Y, Jian A, Wang J. Collagen□coated agarose–chitosan scaffold used as a skin substitute. Biotechnology and Applied Biochemistry. 2023 Jun;70(3):1206-16.
21. Mirtabaei SM, Mollajavadi MY, Ketabi MA. The pore size effect on the degradation, tensile properties and cell viability of polycaprolactone/starch scaffold: experimental study. Iranian Polymer Journal. 2025 Apr;34(4):571-81.
22. Sharma D, Nouri A, Sharma V. Strategic Control of Porosity and Post□Processing in Additive Manufacturing of Metallic Bone Scaffolds. Advanced Engineering Materials. 2025 Sep;27(17):2500749.
23. Krishani M, Chong JN, Lim WR, Jusoh N, Sambudi NS, Suhaimi H. Synthesis and Characterization of Keratin-Based Scaffold for Potential Tissue Engineering Applications. Fibers. 2025 Jul 17;13(7):97.
24. Habiburrohman MR, Jamilludin MA, Cahyati N, Herdianto N, Yusuf Y. Fabrication and in vitro cytocompatibility evaluation of porous bone scaffold based on cuttlefish bone-derived nano-carbonated hydroxyapatite reinforced with polyethylene oxide/chitosan fibrous structure. RSC advances. 2025;15(7):5135-50.
25. Dedeloudi A, Martinez-Marcos L, Quinten T, Andersen SK, Lamprou DA. 3D-printed biomaterial-based scaffolds loaded with zoledronic acid functionalised ceramic microparticles for sustained release. International Journal of Pharmaceutics. 2025 Aug 20:126101.