Anti-inflammatory potential of Herbal gel containing Urtica dioica extract against Arthritis

chandrakala 2153 venkatesh

chandrakala52@gmail.com

East Point College of Pharmacy https://orcid.org/0000-0001-8824-4787

Kanika Joshi

East Point College of Pharmacy

Research Article

Keywords: Urtica dioica, aerial part extract, phytosomal gel, Extended release, Anti-inflammatory effect

Posted Date: September 25th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7531782/v1

Abstract

Despite the anti-inflammatory potential of plant extracts against Arthritis, no major studies have been published about the effect of *the Urtica dioica*(UD) plant's aerial part on the disease. Arthritis, an autoimmune joint disorder, causes pain, inflammation, and cartilage and bone damage. Synthetic drugs used for pain relief often have adverse effects. This study utilized plant UD, to prepare herbal gel and compared its efficacy with marketed diclofenac gel. Novel drug technology was employed to enhance the gel's release and stability. Herbal gels were prepared by using polymers such as gum katira, carbopol and sodium alginate with a simple dispersion method. Polyherbal gel was prepared using different oils dispersed in sodium alginate gel. Phytosomes were prepared using soy lecithin and dichloromethane via thin film hydration with methanolic UD extract and then dispersed into a gel base. The gels were evaluated for various parameters and compared with marketed diclofenac 1% gel. Prepared UD gels exhibited a smooth, dark- to light-brown appearance, with 32.4 mg/TAE/g polyphenol content. DSC analysis confirmed the chemical integrity of the prepared gel. Skin irritation tests on rabbits were negative and showed inhibition of edema in a carragenan-induced hind rat paw edema model. The gels were stable when subjected to accelerated stability studies for 3 months. These findings suggest that herbal gels could serve as effective, safer alternatives to synthetic NSAIDs for chronic conditions.

1. Introduction

A systemic autoimmune condition that mostly affects the joints, arthritis is marked by discomfort, inflammation around the joints, and bone and cartilage degradation. In anti-arthritic therapy, controlling moderate-to-severe pain and inflammation is the main obstacle(Di Paola &Cuzzocrea, 2008). Joint pain, stiffness, and swelling can be caused by a variety of rheumatic diseases and other ailments together referred to as arthritis. The types include those linked to inflammation brought on by an overactive immune system (like rheumatoid arthritis) and those linked to cartilage deterioration (like osteoarthritis) (2). More than 350 million people have arthritis globally and it’s estimated by 2040, 78 million adults will have arthritis. The most often given medications for the treatment of arthritis are Nonsteroidal anti-inflammatory medicines (NSAIDs).Oral NSAIDs frequently require high dosages and have adverse effects such mild changes in plasma levels and stomach irritation that might increase the risk of overdosing(3).To overcome these hurdles topical or transdermal delivery can be utilized. Around 60–90% of people with arthritis are inclined to use botanicals since they are interested in the herbal alternatives(4).Reduced adverse effects and reduced expenses for phytochemical derived from natural resources establish new channels for maintaining health through a variety of methods and emphasize the "back to nature" era(5).

In Indian Himalayan regions, the nettle plant (Urtica dioica), a member of the Urticaceae family, is grown as a weed but is traditionally eaten as a vegetable. The intact leaf, which has tiny projections that resemble hairs, is applied to the irritated area to reduce pain and inflammation, but this procedure is painful. This plant is known for its anti-inflammatory potentials. (6).

A novel herbal medicine delivery system provides a scientific perspective to confirm the standardization of herbal drugs and offers up new avenues for delivering them at the proper time, location, and concentration (Talebi et al., 2025).Herbal extract phytosomes are frequently more accessible than simple herbal extracts because of their enhanced capacity to cross lipid-rich cell membranes. The extract is integrated into a suitable polymer that is lipophilic in nature to create phytosomes, a revolutionary nano drug delivery method(8).(9)Polyherbal formulation consists of two or more plant extracts. Polyherbal preparation is known to have better efficacy due to the synergistic effects of one another(10). Phytosomal gel was prepared utilizing phytosome technology offers a significant improvement in bioavailability, consistent tissue distribution, and clinical benefit.(11,12)

Therefore, in the present study herbal, polyherbal, phytosomal gel was prepared by using simple dispersion method utilizing an appropriate thickening agent and vehicles. Thickening agent is added after the drug has been dissolved in an aqueous medium and triturated in a mortar(13–15). The process of triturating is continued until a homogeneous gel is prepared. Therefore, the present study was designed to formulate totally herbal anti-inflammatory gel against arthritis which will be equally potent to marketed NSAIDs.

2. Material & Methods

2.1 Plant procurement and authentication

The plant was obtained from Almora Uttarakhand in spring which was authenticated by S. Noorunnisa Begum, curator, Foundation of revitalization of local health traditions Bangalore.

2.2 Chemical and reagents

Petroleum Ether, Chloroform, Methanol, Sodium Alginate, Sodium Benzoate, Calcium Gluconate, Potassium Di Hydrogen Ortho Phosphate, Di Sodium Hydrogen Ortho Phosphate, Sodium Carbonate was purchased from SD FINECHEM LTD. Soya Lecithin was purchased from Vitaegen Life sciences. Dichloromethane, Sodium Chloride, Sodium Hydroxide was purchased from MERCK. FolinCiocateu Reagent was purchased from Finar reagents. Camphor was purchased from Molychem, Clove oil, Castor oil, Eucalyptus oil, Gaultheria oil was purchased from DODDABETTA MPDA Tamilnadu. All chemicals were analytical grade and purchased from authentic suppliers.

2.3 Extraction:

Powdered aerial part was extracted using successive extraction technique. Petroleum Ether, chloroform, methanol was used as solvents. The final methanolic extract which contains polyphenols was concentrated to dryness under vacuum desiccators. The percentage yield obtained by using different solvents were calculated and reported which provides a clear picture about the method to be utilized for obtaining the polyphenols in highest percentage(16).

2.4 Phytochemical screening:

The main constituent responsible for anti-inflammatory activity are polyphenols like quercetin, kampeferol, caffeic acid etc so the following chemical test was performed for all the extracts.

a) Shinoda test – Magnesium Hydroxide reduction test –Take 1 mL of extract in a test tube, add few magnesium turnings to the test solution, then added conc. Hcl drop wise. Pink Scarlet, crimson red or occasionally green to blue color indicates the presence polyphenols.

b) Sodium Hydroxide test - To 1 mL of the extract increasing amount of sodium hydroxide solution was added which produces yellow color which disappears after adding an acid indicates the presence polyphenols.

c) Lead acetate test – Few drops of 10% lead acetate solution was added to 1 mL of extract, yellow colored precipitate develops which indicates the presence of flavonoids.

d) Ammonia test – To 1 mL of extract added ml of dilute ammonia solution and then conc. sulphuric acid was added along the sides of the test tube yellow coloration indicate the presence of flavonoids(17).

2.5 Analytical Methods:

The standard calibration curve of tannic acid equivalent was prepared to calculate the mg TAE/g of dry extract (i.e., amount of polyphenols present in extract) was obtained by Folin Ciocateu method(18).

2.6 Preparation of herbal gel:

Herbal gels were prepared by using different gelling agents by simple dispersion method. Briefly, Polymer was triturated with glycerin and other excipients. UD PE was incorporated into the gum with sodium benzoate. Extract is incorporated in the last and the consistency was adjusted upto 100mL at the end with purified water(19).

2.7 Preparation of Polyherbal gel:

Polyherbal gels were prepared by using simple dispersion method. Sodium alginate was triturated with glycerin and calcium Gluconate. The various oils were then incorporated until a smooth consistency obtained. Lastly UD PE was incorporated into the gum with sodium benzoate. Extract is incorporated in the last and the consistency was adjusted upto 100mL at the end with purified water(20).

2.7.1 Preparation of Phytosomal gel:

Phytosomal dispersion was prepared by using thin film hydration method, 20 mL of dichloromethane was mixed with 1g of soyalecithin in a round bottom flask and dissolved at 40˚C at 100 rpm for 3 minutes and this was kept overnight for complete removal of the solvent. To the Thin film obtained 20 ml of 7.4 phosphate buffer containing 1 g of extract was added and rotated at 80-160 rpm for 1 hour. Sonication was done for 3 minutes. This dispersion was subjected to various tests like microscopic

examination, SEM analysis, Zeta potential and particle size analysis. After evaluation the phytosomal dispersion was incorporated into sodium alginate gel. 7% of sodium alginate polymer was triturated with 7% glycerin, calcium Gluconate and distilled water. Phytosomal dispersion containing UD PE was incorporated into the prepared gel with 0.5%w/w sodium benzoate. Extract was incorporated lastly and the consistency was adjusted upto 100mL with purified water(21).

3. Evaluation of gels

3.1: pH:

The pH of was measured by direct immersion of the electrode of the pH meter (pH meter) in the mixture of water and gel at room temperature.

3.2: Extrudability

A collapsible tube was filled with sample gel and then pressed firmly at the crimped end. When the cap was removed, gel extruded until pressure dissipated, weight in grams required to extrude 0.5 cm ribbon of gel in 10 second was determined.

3.3: Spreadability:

The spreadability of the prepared nettle extract gel was measured by spreading of 0.5g of gel on a circle of 2cm diameter pre-marked on a glass plate and then second glass plate was employed. Half kilogram of weight was permitted to rest on the upper glass plate for 5 minutes. The increase in diameter of the circle after spreading of the gel was determined. (22)

3.4: Viscosity:

Viscosity of the gels was measured by using Brookfield viscometer using spindle number 7 determined at 25˚C and 100-250rpm. Result reported in average after triplicate experiment.

3.5: Assay:

The assay of each formulation was calculated by using Folin Coicateu method as described in calculation of tannic acid equivalent.1 g of prepared gel was diluted to 100 mL with 50 % methanol. Then 0.1 mL of this solution was diluted to 10 mL volumetric flask containing with 4 mL water, 0.5mL of FC reagent, 1mL of sodium carbonate which was kept in dark for 30 min and then diluted to 10mL with water again kept for 20 minutes after which the absorbance was checked in UV spectrophotometer.

$$Assay = \frac{Absorbance}{Slope} \times Dilution\ factor$$

3.6: In vivo drug release:

In vivo drug release was calculated using fabricated Franz diffusion cell. Sample absorption was calculated spectrophotometrically at 741 nm and % cumulative drug permeation was calculated. Detailed procedure reported in supplementary file.[28]

3.7: Kinetics of drug release:

The diffusion studies data of all formulations were fitted into different kinetic models i.e.,zero order, first order, higuchi and korsmeyer peppas equations (23).

Release Mechanisms:

Table-1-. 'n'value for determination of drug release.

'n'value	Drug Release
<0.5	Fickian
0.5<n<1	Non–Fickian
>1	Super Case II transport

3.8 Differential scanning calorimetry (DSC) analysis:

DSC thermograms of the pure extract, Polyherbal gel, phytosomal gel were recorded on DSC, TA instrument. 5-6 mg of sample was placed in aluminum pan and scanned at heating rate of 10˚C /min from 50-300˚C under the nitrogen gas stream. The temperature calibration was performed using in diumas standard. Procedure was repeated with an empty pan as a reference(Machado &Sencadas, 2017).

3.9 Stability studies:

Stability of a drug in a dosage form at different environmental conditions is important, because it determines how stable the prepared formulation is. As per ICH guidelines the test are conducted for six months but due to the shortage of time we did it for 3 months. Stability studies were conducted according by storing the gel formulation at 40˚C ± 2˚C, 75±5 %RH. The oven temperature was kept at 40˚C and KCl saturated solution was kept at the bottom of the Desiccator to maintain the RH. The samples were withdrawn at initial 1st month, 2nd month, 3rd month and analyzed suitably for the physical characteristics, drug content and cumulative drug release(25)

3.10 Animal Studies:

Lastly the gels were subjected to in vivo drug testing including skin irritation test and anti-inflammatory paw edema method by using carrageenan.

Acute dermal irritation / Skin irritation test – As per OECD guidelines no. 404, Healthy, young male rabbits weighing 2.5-3.0 kg were used for the checking skin irritation with prior approval of study protocol from East Point animal Ethical committee bearing no EPCP/IAEC/12/2022/23. The rabbits were kept fasting for 24 hours .6 cm^2 inches area of rabbit skin was shaved and the gels were applied onto this shaved region. The skin was covered after applying gel with sterile gauze and checked for appearance of erythema and edema after 3hr, 24hr, 72hr, 10^{th} day, 14^{th} day and the reports were produced(26).

Carragenan induced rat paw edema method for checking anti-inflammatory activity:

8 groups of wistar albino rats consisting of 6 rats each were selected based on their weight i.e., 180-240 g. Each group animals were marked in their head, body and tail with different colors for identification and kept on separate cages. The animals were kept on fasting for 24 hours. Edema was induced on the left paw by using carragenan. After 1-hour initial paw volume was measured then gels were applied, again after one hour percentage inhibition of paw edema was measured, noted. Same procedure was followed upto 1 week and readings were noted at 1 hour, 24hour, 48hour, 68hour, 96hour, and 120 hours. Mercury displacement method using plethysmograph was used(2).

Statistical Analysis:

GraphPad prism software using Two-way ANOVA was used to plot the graphs. All the gels were compared with control group and the marketed standard gel.

4. Result and discussion

4.1 Extract characterization:

Urtica dioica aerial part extract organoleptic properties were determined and the results were reported in supplementary file table 1. All the three extracts were semisolid, brownish with a characteristic odor.

4.2 Phytochemical screening:

Various phytochemical screening tests were performed in methanolic extract found positive. This indicates the presence of polyphenols in the methanolic UD extract. The test was performed for the petroleum ether and chloroform extract also but it did not produce positive results. We reported in supplementary file Table 2.

4.3 Percentage Yield:

The different solvents used for extraction leads to a conclusion that each solvent provides different percentages of yield. The five solvents we utilized were water, 70% ethanol, petroleum ether, chloroform, and methanol. The highest percentage of extract was obtained from Methanol as shown below in Table 2.

Table 2 - Percentage yield of extract obtained from different solvents.

Solvent	Percentage yield Extraction I	Percentage yield Extraction II	Percentage yield Extraction III
Water	0.12%	0.15%	0.20%
Ethanol 70%	0.4%	0.5%	0.45%
Petroleum ether	0.72%	0.41%	0.61%
Chloroform	2.5%	4.3%	3.04%
Methanol	5.02%	10.04%	10.09%

4.4. Analytical methods:

The standard calibration curve of tannic acid equivalent was prepared to calculate the mg of tannic acid equivalent present in extract at which was found to be 32.44mg TAE/g of drug extract. Detailed procedure we reported in supplementary file section 4.4.

4.5 Evaluation of UD Phytosomal dispersion:

4.5.1 Microscopic Studies- The dispersion was first evaluated with light microscopy and then subjected to SEM analysis. The dispersion contained particles in nanometer range which was visible as dots so we utilized the SEM analysis to confirm the shapes and sizes.

4.5.2 SEM Analysis:

The phytosomes were analyzed under Scanning electron microscope the phytosomes were found in the nanometer range as depicted in the photographs below. For the further investigations particle size analysis was done.

Table 3-Particle size and zeta potential of methanolic Urtica dioica aerial part extract phytosomes

Phytosomal Formulation	Particle Size(nm)	Poly Dispersity index (PDI)	Zeta Potential(mV)
Phytosomal dispersion	38.8nm	0.421	-29.4 mV

4.6. Evaluation of UD PE gels:

4.6.1 General appearance test for the various gels were performed. The color of the formulations was found from light brown to chocolate brown. Details we have reported in supplementary file Table 4.

4.6.2 pH, Extrudability and Spreadability of extract and various gels:

The prepared gel was examined for pH, extrudability and spreadability. The spreadability was best in case polyherbal due to the incorporation of various oils. The best physical characteristic was found phytosomal gel as the particles were in nano range. The carbopol gel, gum kaitra gel was found to have very low extrudability and spreadability.

Table 4: pH Extrudability and Spreadability of extract and various gels.

Gels	pH	Extrudability(g/cm2)	Spreadability (cm)
Extract	5.9	-	-
Gum Kaitra Gel	5.0	*	4
Carbopol Gel	4.1	*	3.3
Sodium Alginate Gel	6.2	**	5.9
Polyherbal Gel	6.1	***	5
Phytosomal Gel	5.8	***	5.5

* Satisfactory, ** good, *** excellent

NOTE - The Gum Kaitra gel was not evaluated further as the formulation was not having smooth texture, good extrudability and spreadability and also the drug was not mixing properly in the gum.

4.6.3 Viscosity:

Viscosity by using Brookfield viscometer was evaluated. High viscosity makes the flow of the gel difficult so optimum viscosity should be present. The polyherbal formulation was having the lowest viscosity because of the oils incorporated and the phytosomal gel was also having less viscosity due to the presence of nano size phytosomes which do not change the chemical integrity of the excipients used. Details we have reported in supplementary file Table 5.

4.7 Percentage drug content:

The assay of different gels was calculated using Folin ciocalteu method which was used to calculate the tannic acid equivalent also as described under method section. The highest value was obtained in

Polyherbal gel which can be because of the synergistic effects of the various oils incorporated. Details we have reported in supplementary file Table 6.

4.8- In vitro drug release profile of each formulation:

Drug release profile was studied using percentage drug release versus time shown in Fig-3. The Polyherbal gel showed the highest release in 3 hrs which shows its fast acting action so can be used in acute conditions. The faster action may be because of the synergistic effects of the various oils incorporated. The highest drug release in a sustained manner was observed in the phytosomal gel i.e. 96.89% which shows its stability over extended period of time in a controlled release manner so it can be used in chronic conditions. Phytosomes are the novel drug delivery systems and the results have proved the importance of using NDDS in herbal drug by prolonging the release of drug upto48hrs.The Polyherbal and phytosomal gel was selected as the best formulation even animal studies support the same results. Comparison table inserted in supplementary file Table 8.

4.9 Release kinetics of phytosomal gel:

Phytosomal gel Kinetic study was carried out to analyze the mechanism of drug release. The phytosomal gel follows zero order kinetics as it has highest R2 value 0.990.Hence it shows controlled release pattern of the drug. Table inserted in supplementary file Table 9.

5. DSC - Differential scanning calorimeter

DSC for extract, Polyherbal gel, phytosomal gel was performed.

In DSC the sample was introduced in the system and the different melting points of the compounds were observed. As seen in the FIG.NO-6 the extract gives the peak onset at 221.93C to 269.72 ◦C which is very near to the melting point of kampeferoli.e.276–278◦C.Polyherbal gel shows peaks at 302–302◦C which is very near to the melting point of quercetin i.e.316◦C, 269◦C which is near to the melting point of caffeic acid. Phytosomal gel shows the peak 300–302 ◦C, 117◦C, 223◦C etc which indicates the presence of flavonoids.

5.1 Stability studies:

The stability of gels was assessed using ICH guidelines. The parameter studied for stability studies of gels were in limits (except the carbopol gel) indicates that the formulations remain stable during 3 months. Details attached in supplementary file Table 10 ,11.

5.2 Animal studies:

5.2.1 Skin irritation test:

The gel was non irritating to the skin. There was no erythema and edema in rabbits in 3 hr, 72hr, 7th day,14th day post application of the gel in primary skin irritation test. Pictures incorporated in supplementary file Fig. 3.

5.2.2 Carragenan induced rat paw edema method for checking anti-inflammatory activity:

Graph Pad prism software was utilized to plot graphs of animal studies using Two-way ANOVA. Graphs obtained are shown in fig no. 7-. The P value < 0.0001 shows all the gels have significant effects. The percentage inhibition of rat paw edema was observed. Table for Percentage inhibition of carragenan induced rat paw edema incorporated in supplementary file. The phytosomal gel showed an inhibition of 54%, polyherbal showed 57% and the standard marketed diclofenac gel showed an inhibition of 66%. So it can be concluded that the herbal formulation also possess anti-inflammatory action similar to the marketed formulation so it can be used an alternative to the NSAIDs.

6. Discussion

In the present study, we have formulated herbal gels utilizing nettle extract for treatment of inflammation caused by Arthritis. *Urtica dioica* is a traditional plant. Methanolic extract was used to prepare Herbal gel, polyherbal gel, phytosomal gel was, its evaluation was done using various tests and animal studies. The present study aimed to increase the anti-inflammatory activity of the gel by preparing polyherbal gel and to increase the stability and release by formulating phytosomal gel. The various natural polymers like Gum Kaitra, Carbopol 934, and Sodium Alginate were utilized to prepare gel out of which the best formulation was found prepared with Sodium Alginate.

The highest percentage yield was obtained with methanol and the reason could be the solubility of polyphenols. The various phytochemical screening tests also showed the presence of polyphenols. The tannic acid equivalent present in extract was found to be 32.44mg TAE/g of dry extract. The phytosomal dispersion prepared was subjected to SEM analysis and the shapes of phytosomes found to be oval shape with particle size ranging from 35 nm to 400 nm. The zeta size analyzer also showed the similar result of 38.8 nm with zeta potential − 29.4 mV and PDI 0.421 as shown in Fig. 2.

The prepared gel was examined for pH, extrudability and spredability. The spredability was best in case polyherbal reason could be incorporation of various oils. The best physical characteristic was found phytosomal gel as the particles were in nano range. The pH range was found to be suitable for topical preparation. The extrudability was excellent for both polyherbal and phytosomal gel. Percentage drug content was highest for Polyherbal gel which can be because of the synergistic effects of the various oils incorporated. The highest drug release in a sustained manner was observed in the phytosomal gel i.e. 96.89% and it followed zero order release which shows its stability reason for this might be the use of novel phytosome technology which provides stability to the crude nettle extract. For the comparison in a research work done in phytosomal gel of Aloevera it showed release of 56.91% in 24 hours and our results showed better controlled release upto 48hrs(27).The DSC results showed the presence of

flavonoids and polyphenols which is responsible for the anti-inflammatory effect as proven in earlier research works also.

The gels were kept for stability studies for 3 months as per ICH norms and result proved the most stable formulation was the phytosomal gel. The primary skin irritation test for rabbit were negative as shown in supplementary fie Fig. 3. In Carragenan induced rat paw edema method for checking anti-inflammatory activity, the phytosomal gel showed an inhibition of 54%, polyherbal showed 57% and the standard marketed diclofenac gel showed an inhibition of 66%. Many previous studies have examined the use of plant as anti-inflammatory, in one of such article they have used root extract gel of plant and the percentage inhibition was found to be 55.05% which is similar to our results(28). In Graph Pad Prism software the P value of < 0.0001 shows all the gels have significant effects. So these equally potent herbal gels can be used as a substitute of the synthetic drugs which causes side effects on long usage.

7. Conclusion

On the basis of the study, the data showed that among all the formulations the polyherbal gel and phytosomal gel prepared from the methanolic extract of *Urtica dioica* showed significant anti-inflammatory activity when compared with marketed diclofenac gel. As the phytochemical test showed the presence of polyphenols in methanolic extract they might be responsible for the anti-inflammatory activity. The polyherbal gel showed synergistic effect compared to other gels, the phytosomal gel showed extended release which can be used for the treatment of inflammation caused by arthritis. So, herbal gels can be a safer alternative for arthritis and other inflammatory conditions as they have no side effects like the synthetic drugs.

Declarations

8. Acknowledgement:

We thank Department of Pharmaceutics, East Point College of Pharmacy, Bengaluru, India for providing chemical and lab to conduct the experiments.

9. CRediT authorship contribution Statement:

Kanika Joshi: Concept, Design, Methodology, Formal Analysis, Validation, and Writing Origina-Manuscript. V.Chandrakala: Formal Analysis, Data Curation, Supervision, Review and Editing.

Conflict of Interest- This study reports no conflict of interest.

10. Ethical Declaration:

The ethical clearance was taken from the East Point animal Ethical Committee bearing no EPCP/IAEC/12/2022/23. All the animal related experiment was conducted as per OECD guideline no. 404.

References

1. Di Paola R, Cuzzocrea S. Predictivity and sensitivity of animal models of arthritis. Vol. 8, Autoimmunity Reviews. 2008. p. 73–5.
2. Liao JC, Wei ZX, Ma ZP, Zhao C, Cai DZ. Evaluation of a root extract gel from Urtica dioica (Urticaceae) as analgesic and anti-inflammatory therapy in rheumatoid arthritis in mice. Tropical Journal of Pharmaceutical Research. 2016 Apr 1;15(4):781–5.
3. Al-Azayzih A, Al-Azzam SI, Alzoubi KH, Jarab AS, Kharaba Z, Al-Rifai RH, et al. Nonsteroidal Anti-inflammatory Drugs Utilization Patterns and Risk of Adverse Events due to Drug-Drug Interactions among Elderly Patients: A Study from Jordan. Saudi Pharmaceutical Journal [Internet]. 2020 Apr 1 [cited 2025 May 23];28(4):504–8. Available from: https://www.sciencedirect.com/science/article/pii/S1319016420300566
4. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Vol. 4 JAN, Frontiers in Neurology. 2014.
5. Yuan L, Chen Y, Guan Y, Fu X, Tan L. Current Perspectives on the Potential Role of Medicinal Plants in Anti-aging, Skin-related Disorders and Stem Cell Proliferation. Pharmacogn Mag [Internet]. 2025 May 20; Available from: https://journals.sagepub.com/doi/10.1177/09731296251341498
6. Bhokare SG, Dongaonkar CC, Lahane S V, Salunke PB, Sawale VS, Thombare MS. HERBAL NOVEL DRUG DELIVERY-A REVIEW. WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES SJIF Impact Factor 6 [Internet]. 041(8):593–611. Available from: www.wjpps.com
7. Talebi M, Shahbazi K, Dakkali MS, Akbari M, Almasi Ghale R, Hashemi S, et al. Phytosomes: A promising nanocarrier system for enhanced bioavailability and therapeutic efficacy of herbal products. Phytomedicine Plus [Internet]. 2025 May 1 [cited 2025 May 23];5(2):100779. Available from: https://www.sciencedirect.com/science/article/pii/S2667031325000521
8. Talebi M, Shahbazi K, Dakkali MS, Akbari M, Almasi Ghale R, Hashemi S, et al. Phytosomes: A promising nanocarrier system for enhanced bioavailability and therapeutic efficacy of herbal products. Phytomedicine Plus [Internet]. 2025 May 1 [cited 2025 May 23];5(2):100779. Available from: https://www.sciencedirect.com/science/article/pii/S2667031325000521
9. Monica G, Naik VV. HERBOSOMES: A POTENTIAL CARRIERS FOR THE BIOAVAILABILITY ENHANCEMENT OF HERBAL EXTRACTS [Internet]. Available from: www.wjpps.com
10. Lawal IO, Olajuyigbe SO, Akinwumi IA, Olugbami D. Assessment of Plants Used in Polyherbal Formulations for Traditional Treatment of Skin Infections in Ibadan Metropolis. J Herb Med [Internet]. 2024 Sep 1 [cited 2025 May 24];47:100919. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2210803324000769
11. S RG. PHYTOSOMES: AN ADVANCED HERBAL DRUG DELIVERY SYSTEM [Internet]. Available from: https://www.researchgate.net/publication/315117615
12. Bhosale AP, Patil A, Swami M. HERBOSOMES AS A NOVEL DRUG DELIVERY SYSTEM FOR ABSORPTION ENHANCEMENT [Internet]. Vol. 5, Bhosale et al. World Journal of Pharmacy and

Pharmaceutical Sciences. 2016. Available from: www.wjpps.com

13. Lekshmi S, Jose T, Mathews MM, R B. Formulation and evaluation of topical gels incorporated with solid dispersions of an antiinflammatory drug. Journal of Innovations in Applied Pharmaceutical Science (JIAPS). 2023 Nov 30;114–9.
14. Doshi G, Bhanuben S. Novel Techniques in Herbal Drug Delivery Systems Article in [Internet]. Vol. 28, International Journal of Pharmaceutical Sciences Review and Research. 2014. Available from: https://www.researchgate.net/publication/268520945
15. Li X, Wang M, Li K, Nie Y, Wei Y. Antirheumatoid arthritis activities and chemical compositions of phenolic compounds-rich fraction from urtica atrichocaulis, an endemic plant to China. Evidence-based Complementary and Alternative Medicine. 2012;2012.
16. Hussain G. Soxhlet Extraction principal, working & Usage [Internet]. Available from: https://www.researchgate.net/publication/372658021
17. Rao USM, Abdurrazak M, Mohd KS. Penyaringan fitokimia, jumlah asai kandungan flavonoid dan fenolik pelbagai ekstrak pelarut tepal Musa paradisiaca. Malaysian Journal of Analytical Sciences. 2016;20(5):1181–90.
18. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol [Internet]. 1999 Jan 1 [cited 2025 May 24];299:152–78. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0076687999990171
19. Fiorillo L, Romano GL. Gels in medicine and surgery: Current trends and future perspectives. Vol. 6, Gels. MDPI AG; 2020. p. 1–4.
20. Chellathurai BJ, Anburose R, Alyami MH, Sellappan M, Bayan MF, Chandrasekaran B, et al. Development of a Polyherbal Topical Gel for the Treatment of Acne. Gels. 2023 Feb 1;9(2).
21. Tafish AM, El-Sherbiny M, Al-Karmalawy AA, Soliman OAEA, Saleh NM. Carvacrol-Loaded Phytosomes for Enhanced Wound Healing: Molecular Docking, Formulation, DoE-Aided Optimization, and in vitro/in vivo Evaluation. Int J Nanomedicine. 2023;18:5749–80.
22. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian Journal of Pharmaceutical Sciences. 2016 Jul 1;52(3):493–507.
23. Ani Jose P, Mallikarjuna Setty C. RELEASE KINETICS-CONCEPTS AND APPLICATIONS [Internet]. 2018. Available from: https://www.researchgate.net/publication/369038187
24. Machado R, Sencadas V. Advanced techniques for characterizing bioinspired materials. Bioinspired Materials for Medical Applications. 2017;177–214.
25. INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE ICH HARMONISED TRIPARTITE GUIDELINE STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS Q1A(R2). 2003.
26. Test No. 404: Acute Dermal Irritation/Corrosion. 2015 Jul 28 [cited 2025 May 23]; Available from: https://www.oecd.org/en/publications/test-no-404-acute-dermal-irritation-

corrosion_9789264242678-en.html

27. Jain P, Taleuzzaman M, Kala C, Kumar Gupta D, Ali A, Aslam M. Quality by design (Qbd) assisted development of phytosomal gel of aloe vera extract for topical delivery. J Liposome Res. 2021 Oct 2;31(4):381–8.
28. Liao JC, Wei ZX, Ma ZP, Zhao C, Cai DZ. Evaluation of a root extract gel from Urtica dioica (Urticaceae) as analgesic and anti-inflammatory therapy in rheumatoid arthritis in mice. Tropical Journal of Pharmaceutical Research. 2016 Apr 1;15(4):781–5.

Figures

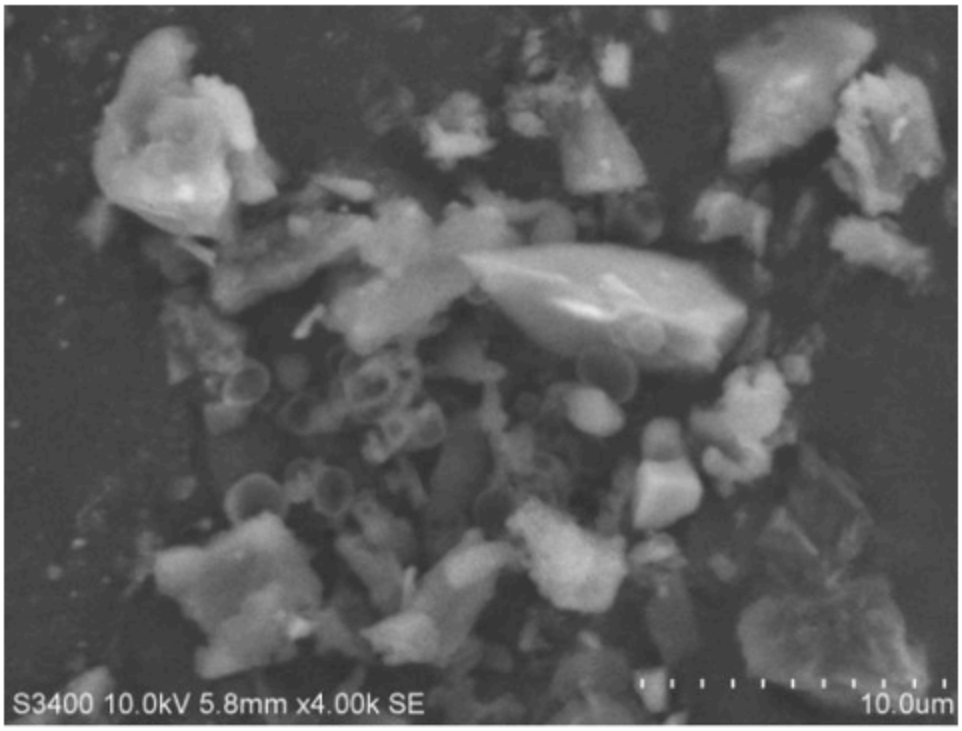

Figure 1

SEM analysis photographs depicting phytosomes.

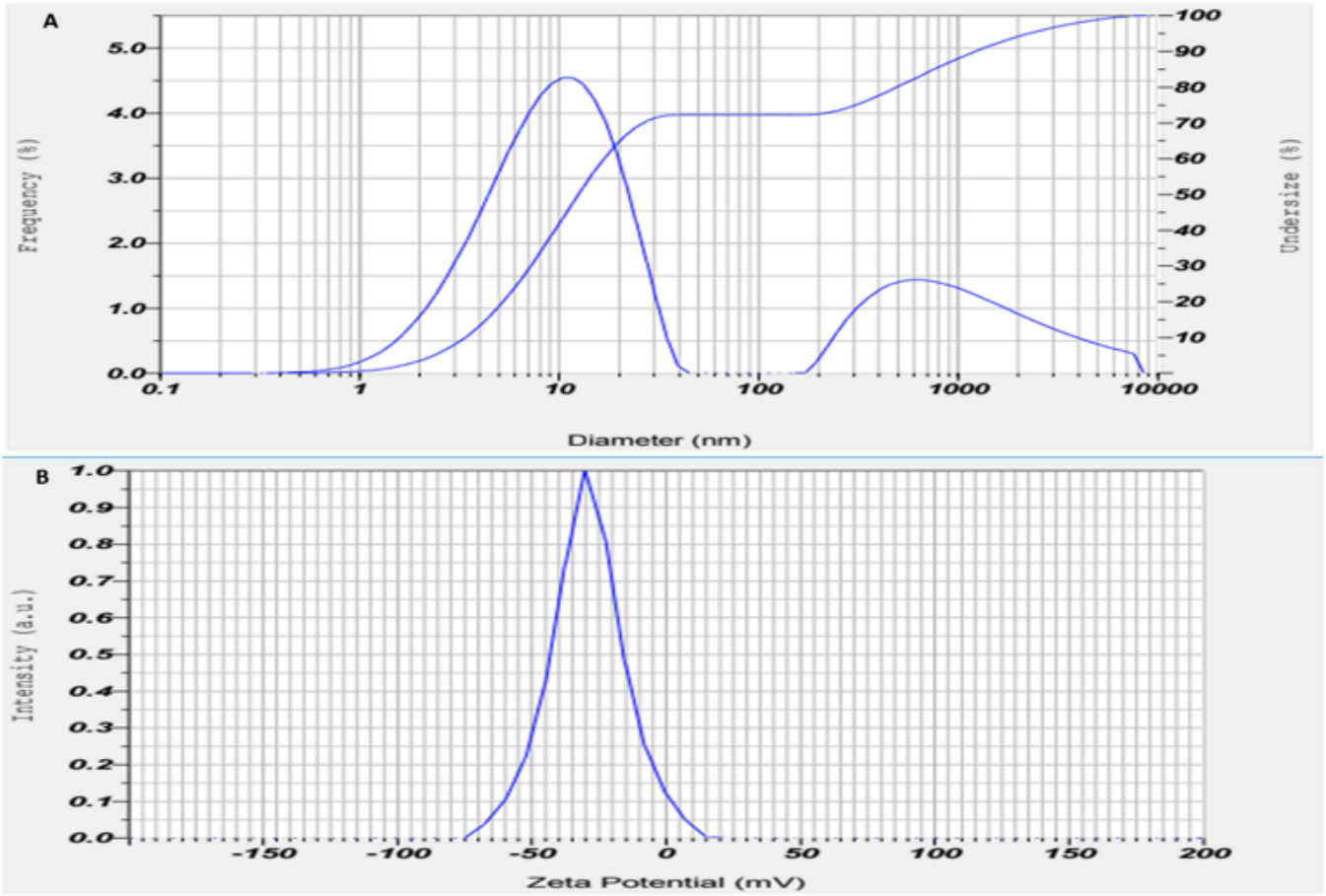

Figure 2

Particle size distribution of UD phytosomal dispersion(A) and Zeta potential of UD phytosomal dispersion(B)

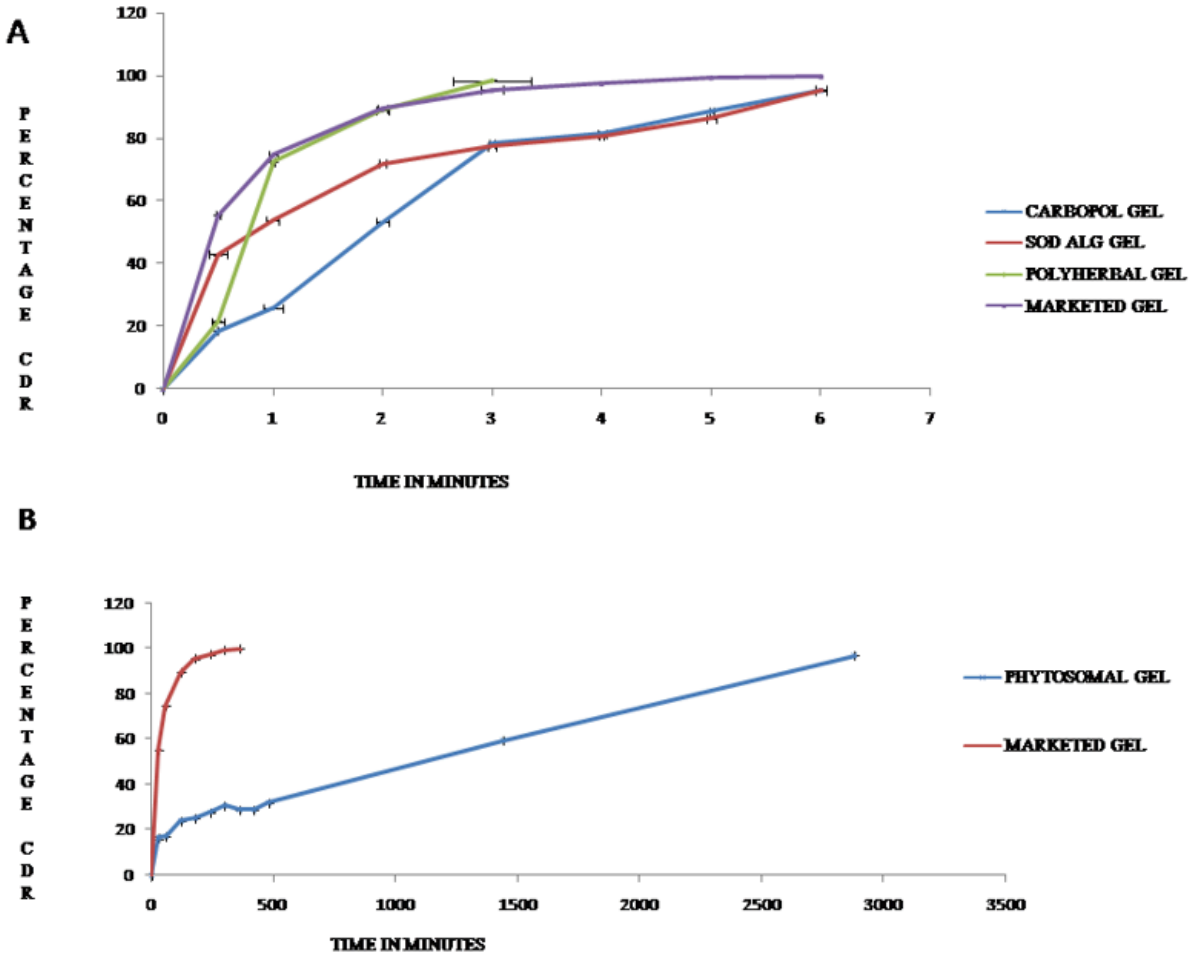

Figure 3

Cumulative percentage drug release Vs Time of carbopolgel ,sodium alginate gel, polyherbal gel and, marketed gel (A) and cumulative percentage drug release Vs Time Phytosomal gel and, marketed gel (B).

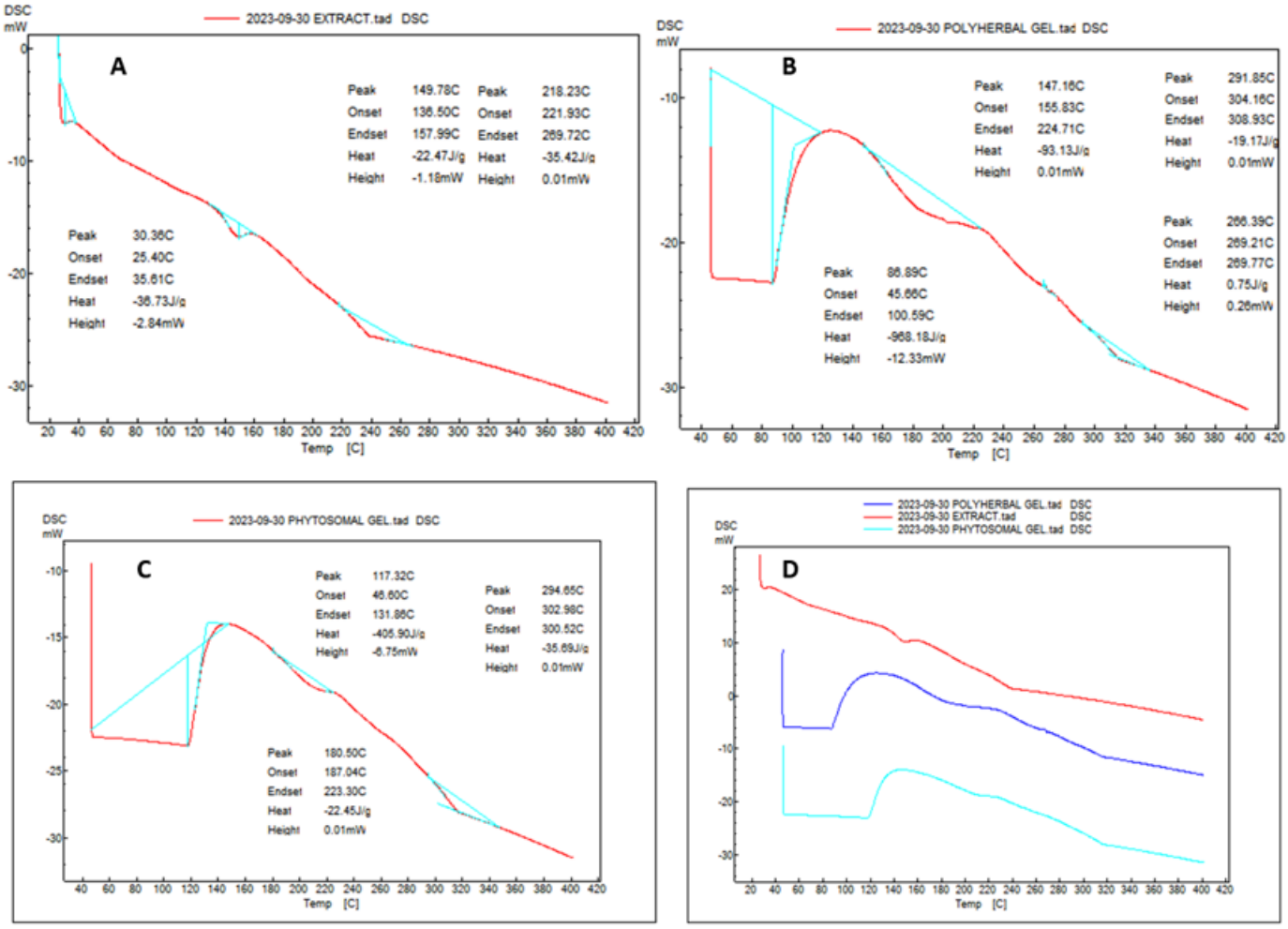

Figure 4

DSC graphs of nettle extract (A), Polyherbal gel (B), phytosomal gel(C), Extract and all gel comparison(D).

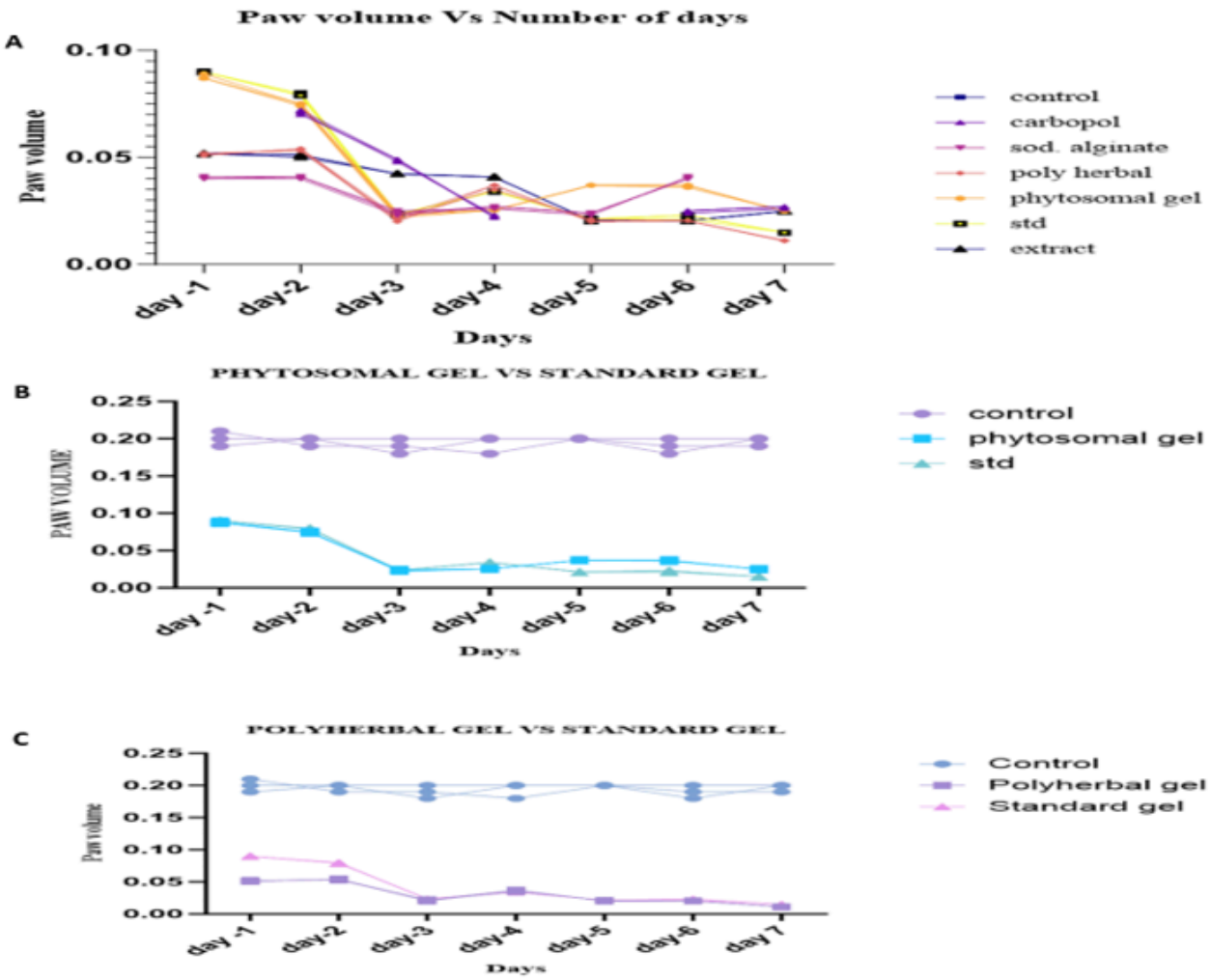

Figure 5

Percentage inhibition of edema for all formulated gels verses marketed gel(A), Percentage inhibition of edema by phytosomal gel verses marketed gel. (B) and Percentage inhibition of edema by polyherbal gel.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SUPPLYFILE.docx