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Formulation and Evaluation of Gel Containing *Brassica Oleracea* L.Var. *Capitata* F, *Rubra* Extract and Clinical Assessment in The Management of Inflammation in Osteoarthritis

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ABSTRACT

This research aims to investigate the formulation, evaluation, and clinical study of a topical anthocyanin gel derived from Brassica oleracea L. Var. Capitata F. rubra in managing osteoarthritis. Anthocyanin extraction was performed through maceration using 1% HCl in methanol, followed by gel formulation using chitosan, carbopol-940, and xanthan gum. The formulated gels were subjected to a series of evaluations, comprising FTIR spectroscopy, pH analysis, viscosity testing, spreadability assessment, percentage drug content determination, and In-vitro drug release profiling. Results revealed no interaction between the drug and additives (ATIR), a pH range suitable for skin (6.2-6.5), and optimal viscosity and spreadability for carbopol-940 gels. Notably, the F2 formulation (2% carbopol-940) demonstrated superior properties, with 97.25% drug content and 97.31% cumulative release at 6 hours, making it the selected formulation for clinical study. The clinical study using F2 formulation yielded significant improvements in pain, swelling, tenderness, and functional ability in osteoarthritis patients, with statistical analysis showing highly significant differences ($p < 0.001$). These findings demonstrate the potential of topical anthocyanin gel as an effective and safe treatment for osteoarthritis.

Keywords: *Anthocyanins, osteoarthritis, maceration, topical gel.*

Introduction

Osteoarthritis, a debilitating and widespread joint disorder, is the leading form of arthritis, affecting millions of people worldwide. It is characterised by bone alterations and articular cartilage deterioration, and it manifests as joint discomfort, soreness, restricted mobility, crepitus, sporadic effusion, and fluctuating inflammation. [1]. Osteoarthritis symptoms include Chronic pain, driven by increased excitatory amino acids like glutamate [2]. Joint stiffness due to deficient surface-active phospholipid. Muscle weakness, particularly in quadriceps, exacerbating Osteoarthritis progression. Bone enlargement and swelling from pathological cartilage changes [3, 4]

Systemic and local variables interact to cause osteoarthritis, which has a complex aetiology. Genetics (39–65% influence) (Spector TD et.al.,2004), age (decreased cartilage tensile property) [5], gender (higher pain/disability in women) [6] and diet (consumption of refined carbohydrates and junk foods) [7] are examples of systemic risk factors. Local risk factors comprise: joint injury/trauma (cartilage damage), obesity (knee joint overloading), occupation (heavy lifting/stressful activities), and physical activity/sports (knee injury) [8,9].

Specifically, women over 65 are more susceptible, with 68% developing OA compared to 58% of men. Genetic factors, such as osteocalcin levels, also play a significant role, especially post-menopause [10,11].

Osteoarthritis treatment options can be categorized into four main groups: non-pharmacological, pharmacological, complementary/alternative therapies, and surgical interventions. Nonpharmacologic approaches include exercise weight loss, bracing, and physical therapy modalities like ultrasound [12] Pharmacologic options start with acetaminophen, followed by NSAIDs or opioids for moderate/severe pain.Intra-articular injections of corticosteroids or hyaluronic acid offer temporary symptom relief for osteoarthritis patients, providing short-term improvement in joint pain and function [13]. Complementary/alternative therapies include acupuncture, glucosamine/chondroitin supplements, balneotherapy, capsaicin cream, and S-adenosylmethionine (SAM-e) [14]. Surgical interventions, reserved for refractory cases, include total joint replacement, with excellent outcomes for hip, knee, and shoulder replacements.

Other surgical approaches, like arthroscopic debridement, have shown limited success [15, 16].

Strong, naturally occurring pigments called anthocyanins provide a variety of fruits, vegetables, and plant species their vivid blue, purple, and red hues. According to the flavonoid B-ring structure, they are mainly glycosides with the fundamental structural form of 2-phenylchromenylium, which is divided into six primary compounds: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin [17, 18]. Because of their pH-dependent characteristics, anthocyanins appear red in acidic environments and blue in alkaline ones. As is common with flavonoids, they have a positive charge on the oxygen atom in their C-ring [19]. Anthocyanins, especially cyanidin-3-O-glucoside (C3G), which is abundant in red cabbage, have strong anti-inflammatory effects via blocking the NF-κB-regulated inflammatory pathway [20, 21]. Because of their anti-inflammatory, anti-cancer, and antioxidant properties, red cabbage anthocyanins are a useful dietary component [22, 23].

Topical drug delivery involves administering drugs through the skin to treat skin disorders, such as infections, inflammation, and wounds, offering a preferred alternative when oral or parenteral routes are unsuitable. This method provides several advantages, including targeted treatment, reduced side effects, improved drug absorption, and enhanced therapeutic outcomes. Topical formulations bypass gastrointestinal irritation and rapid liver metabolism, increasing bioavailability and ensuring direct action at the administration site. However, developing effective topical delivery systems poses a key challenge: achieving optimal penetration through the skin barrier to reach the desired site of action [24].

Materials and methods

Materials

The suppliers of methanol, Carbopol 940, and chitosan were Loba Chemie Pvt. Ltd. in Mumbai. We purchased ethanol, propylene glycol, and hydrochloric acid from SDFCL in Mumbai. Sisco Research Laboratories Pvt. Ltd., Maharashtra, India, provided the lavender oil, and Genuine Chemical Co., Bombay, provided the propyl paraben. Furthermore, NR Chem provided methyl paraben and Himedia provided xanthan gum.

Anthocyanin extraction

Red cabbage, or Brassica oleracea L. var. capitata f. rubra, was extracted using 1% v/v concentrated hydrochloric acid (HCl) in methanol in a 1:10 ratio. After covering the mixture with aluminium foil, it was left to stand at room temperature for a whole day in a dark location. Filter paper was then used to carry out the filtration process. To extract anthocyanin, the filtrate was subsequently evaporated at 40°C in a hot air oven [25]. The dried anthocyanin was gathered and kept in storage[45].

Anthocyanin identification tests

IR Spectroscopy

Infrared spectroscopy was used to compare the FT-IR spectra of the obtained drug sample with the standard FT-IR spectra of the pure drug [26].

Solubility Analysis:

As part of the preformulation research, the solubility of anthocyanin in different solvents was assessed in order to determine an appropriate solvent for drug dissolution and release investigations.

In order to test for solubility, 0.5 g of anthocyanin was dissolved in 10 mL of methanol while being constantly stirred for 10 minutes. Ethanol and phosphate buffer (pH 6.8) were then evaluated as substitute solvents. Phosphate buffer (pH 6.8) was used in drug release tests, while ethanol was chosen for anthocyanin dissolution during formulation.

Anthocyanin Confirmatory Test

Brassica oleracea l. Var. Capitata F, rubra Using a UV-visible spectrophotometer, anthocyanin extract was examined for absorbance between 200 and 800 nm. absorbance maxima were discovered to be 529 nm [27]. The extract was put through acid and basic testing to make sure anthocyanin was present. The extract showed stability and kept its red colour, which is a sign of the presence of anthocyanins, after being treated with 2 M HCl and heated to 100°C for five minutes. On the other hand observed that the extract showed a stable brown colour upon combining with 2M NaOH, further confirming the existence of anthocyanin [28].

Total anthocyanin content

By dissolving 2 grammes of gel in 100 millilitres of pH 6.8 phosphate buffer, the anthocyanin content of the gel formulation was determined. For two hours, the gel solution was agitated in a volumetric flask to guarantee the drug's full dissolution and uniform dispersion. Following filter paper filtration, the fluid was appropriately diluted. A UV-visible spectrophotometer (Shimadzu, Japan) was then used to measure the medication absorbance at λmax 529 nm, using phosphate buffer as the blank [29].

Phytochemical Tests for Extract

Extracts of Brassica oleracea L. var. capitata f. rubra were subjected to photochemical screening using the different chemical tests [30-32,42-44].

Anthocyanin Gel Preparation

Using a variety of gelling agents, such as, Chitosan, Carbopol 940and Xanthan gum, formulations of various gels were created using concentrated extract of Brassica oleracea L. var. capitata f. rubra (red cabbage) as the active ingredient (anthocyanin). Table 1 lists all of these gel formulations components in detail [33].

Accurately weigh the gelling agents, combine them with water or glacial acetic acid, and let them swell for a full day. Methylparaben and propylparaben were dissolved in half of the formulation water, and the combination was then heated to 40°C. After that, gelling components were added to the mixture and stirred for 30 minutes at 1200 rpm using a magnetic stirrer to ensure equal dispersion. To improve solubility, add a predetermined amount of anthocyanin extract and thoroughly mix with ethanol and propylene glycol. Add the anthocyanin mixture to the gel gradually, mixing until it is evenly distributed. Finally, to provide a nice scent, add a few drops of lavender oil [33,46].

Table 1: List of the ingredients used in the formulation

Weight taken in g (For 100g)	F1	F2	F3
Extract	1	1	1
Chitosan	2	-	-
Carbopol-940	-	2	-
Xanthan gum	-	-	2
Methyl paraben	0.2	0.2	0.2
Propyl paraben	0.02	0.02	0.02
Propylene glycol	5	5	5
Ethanol	5	5	5
Lavender oil	q. s	q. s	q. s
Water up to	100	100	100

Evaluation of Anthocyanin gels

Test of Extrudability

About 5 g of the gel formulation was put into a crimped end of a clean, lacquered aluminium collapsible tube, and a clamp was fastened to stop any rollback. After gently removing the cover, the gel was extruded. Extrudability was determined by measuring the weight or volume of gel that was extruded from the tip. After gathering and weighing the extruded gel, the percentage of extruded gel and grades were determined [34].
(>90% Extrudability: Excellent)
(>80% Extrudability: Good)
(>70% Extrudability: Fair)

Viscosity

The viscosity of the prepared gel was ascertained using the Brookfield viscometer. Using spindle No. 64, the gels were rotated at 50 rpm in order to assess their viscosity. The reading was taken at each speed. Three attempts were made to determine the samples' viscosity [35].

Spreadability

The gel's spreadability was evaluated using the spreadability apparatus, which consists of a glass plate block with a connected pulley system. This technique was used to measure spreadability based on the gel compositions slide and drag characteristics. An excess of around 2 grammes of gel was placed on a ground slide for examination. After that, a hook was firmly fastened to the gel, which was sandwiched between the fixed ground slide and a second glass slide with the same measurements. To eliminate air pockets and guarantee a consistent gel coating between the two slides, a 20-gram weight was placed on the constructed slides for five minutes. Carefully, the extra gel was scraped off the slide assembly's edges. The top plate was then subjected to a 20-gram tensile strain. Using a thread fastened to the hook, note the amount of time (in seconds) needed for the top slide to move 6.5 cm. A shorter period indicates optimal spreadability. The following formula was used to determine spreadability [36].

$$S = \frac{M \times L}{T}$$

where, M = Weight tied to upper slide,
L= Length moved on glass slide
T = Time taken to separate the slide completely from each other

Centrifuge Test

Following a 48-hour preparation period, formulations were put into 10-cm-long by 1-cm-wide tubes and centrifuged for 60 minutes at 2000 rpm using a centrifugal device (PR-24). The stability and sedimentation of the formulation were assessed at 5, 15, 30, and 60 minutes [37].

Temperature Change Test

Tubes were placed in temperatures of 2-8°C, 25°C, and 40-45°C to control the formulation stability in various seasons and temperature circumstances. The appearance quality of the tubes was then monitored after 48 hours, one week, two weeks, one month, and three months [38].

Drug Content:

The gel formulated drug content was evaluated by dissolving an accurately 2 gm of gel in 100ml of pH 6.8 phosphate buffer. The volumetric flask containing gel solution was shaken for 2h in order to achieve complete solubility of the drug. The solution was filtered using filter paper. After suitable dilution drug absorbance was recorded by using UV-visible spectrophotometer (UV Shimadzu, Japan) at λ max 529 nm using phosphate buffer as blank [29].

Studies on In-vitro release

Using a modified diffusion testing device, the in-vitro drug release research of anthocyanin from the produced formulations was investigated. The diffusion medium used was the recently made phosphate buffer solution (pH 6.8). After being soaked in the diffusion medium for the entire night, the gelatin sheet, which functions as a semi-permeable membrane, was attached to one end of a specially made glass cylinder with an inner diameter of 3.4 cm and open on both sides. The donor chamber was a glass cylinder into which 2 g of the gel formulation was precisely pipetted. Making sure the membrane barely contacted the medium's surface, the cylinder was delicately suspended in an acceptor chamber (beaker) filled with 50 mL of diffusion medium. A magnetic stirrer was used to maintain the acceptor chamber's temperature at 37 ± 2º C at a stirring rate of 50 rpm. At one-hour intervals, roughly 4 ml of the sample was taken out and replaced with an equivalent volume of brand-new diffusion media. A UV spectrophotometer set at 529 nm was used to analyse the aliquots [39].

Clinical Assessment of Gel

Single Arm Open Label Clinical Study

A. Study criteria

1. Inclusion Criteria
- Subjects diagnosed with osteoarthritis
- Patients of agegroup25-80years

2. Exclusion criteria
- Patients of age <25 and >80 are excluded.
- Any other systemic chronic disorders which effect the study.

B. Study Procedure

In the outpatient department of BVVS Ayurveda Medical College and Hospital, a prospective observational study was carried out over a two-month period to assess the anti-inflammatory effects of anthocyanin-rich extract gel. The Institutional Ethics Committee (IEC) for Human Subject Research already approved the project, and records of that approval are accessible. Following written consent and treatment, the 30 participants with osteoarthritis, of either sex, who were between the ages of 30 and 80, were added to the study. Predetermined study criteria were followed in the extraction of the data. The subjects were given instructions on how to apply gel three times a day. The pharmaceutical containers containing anthocyanin gel were evaluated for therapy on each study centre visit.

The investigator analysed the subjects inflammation based on characteristics such as pain, swelling, tenderness, pain after flexion, stiffness and crepitus. A clinical score was calculated by adding the four scores, which were assigned a matching score of 0–3 for each of these parameters: absence, mild, moderate, and severe. The subject's case report and progress chart provided the data for this investigation. The student paired t-test was used to statistically process the final results that were obtained [40,41,47].

Result and Discussion

Identification Tests for Anthocyanin

FTIR Studies

Compatibility studies were conducted using FTIR spectroscopy. The peaks in the anthocyanin spectrum correspond to the peaks in each sample's spectrum. The findings demonstrated that there was no chemical interaction between the anthocyanin concentrate and the excipients in the gel formulation since the distinctive peaks of the anthocyanin concentrate were retained in the physical mixture as shown in figures 1- 4.

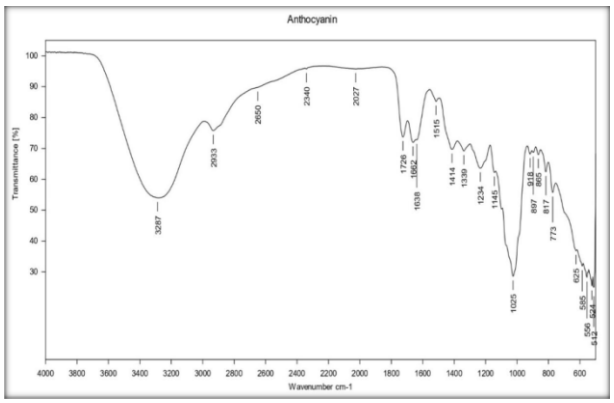


Figure 1: FTIR Spectra of Anthocyanin

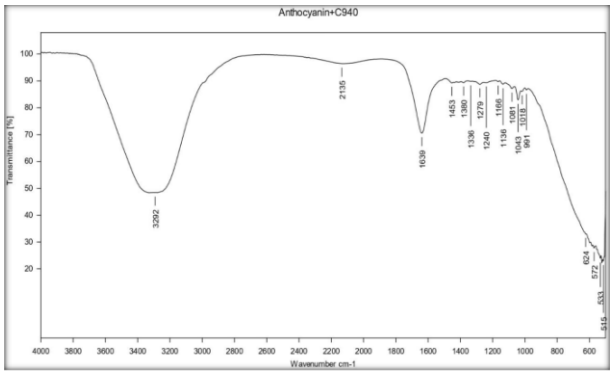


Figure 2: FTIR Spectra of C-940 +Anthocyanin

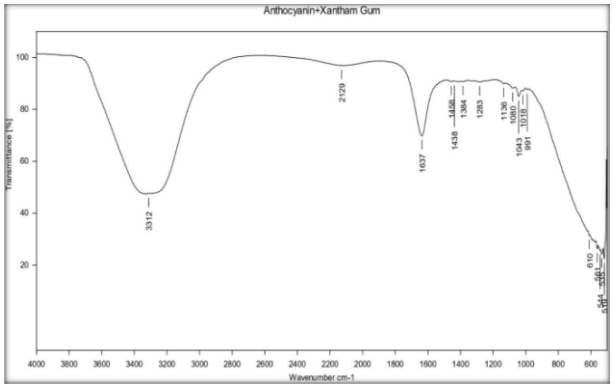


Figure 3: FTIR Spectra of Xanthan gum+ Anthocyanin

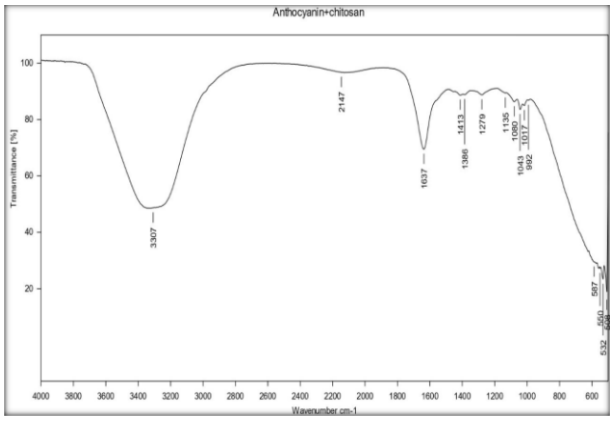


Figure 4: FTIR Spectra of Chitosan + Anthocyanin

Analysis of Solubility

The anthocyanin extract was only moderately soluble in water, but it was fully soluble in methanol and ethanol.

Anthocyanin Confirmatory Test

As seen in Figure 5, anthocyanins were verified at 529 nm using a UV-visible spectrometer. Acid and base tests were performed on the extract to verify the presence of anthocyanin. After being combined with 2M HCl and heated to 100ºC for five minutes, the extract maintained its red colour, which indicates the presence of anthocyanins. The presence of anthocyanin was further confirmed when the extract showed a stable brown colour after being mixed with 2M NaOH as illustrated in Figure 6.

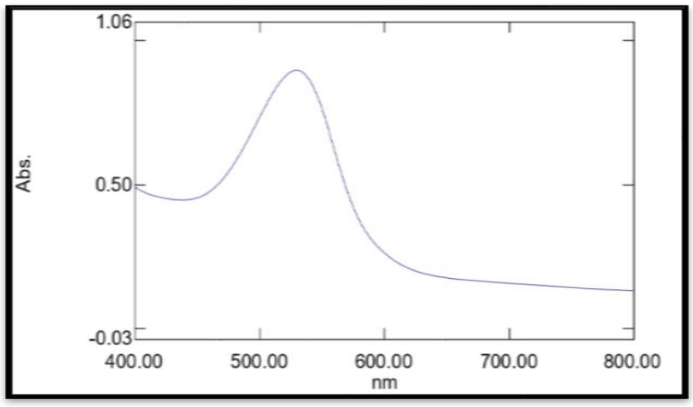


Figure 5: UV- Visible absorbance maxima for Anthocyanins

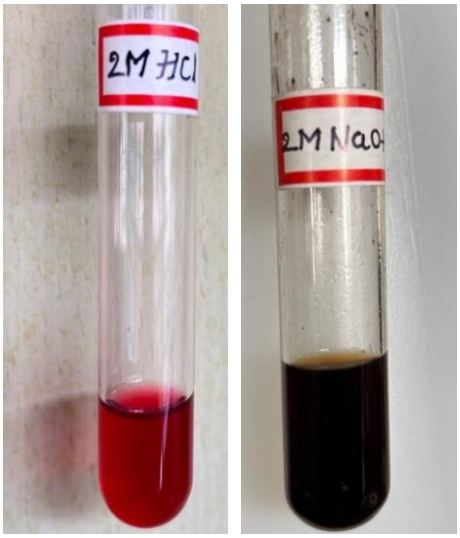


Figure 6: Anthocyanin confirmation test

Evaluation of Formulations

pH

Topically applied gel pH should match the skin pH, otherwise it may cause irritation upon application. Since the gel pH was within that of the skin, they were deemed to be non-irritating and suitable for topical usage. The pH of the prepared gels was measured over a three-month period and was quite consistent between 6.4 and 6.8 as shown in table 2.

Table 2: pH of Anthocyanin gel formulations

Time	F1 ± S.D	F2 ± S.D	F3 ± S.D
0 h	6.6 ± 0.05	6.5 ± 0.05	6.7 ± 0.05
24 h	6.5 ± 0.05	6.7 ± 0.05	6.8 ± 0.05
48 h	6.4 ± 0.05	6.5 ± 0.05	6.6 ± 0.05
1 week	6.4 ± 0.05	6.5 ± 0.05	6.7 ± 0.05
2 weeks	6.6 ± 0.05	6.4 ± 0.05	6.6 ± 0.05
1 month	6.5 ± 0.05	6.7 ± 0.05	6.6 ± 0.05
2 months	6.5 ± 0.05	6.7 ± 0.05	6.7 ± 0.05
3 months	6.5 ± 0.05	6.5 ± 0.05	6.6 ± 0.05

Homogeneity and Visual appearance

The existence of a particle, inappropriate mixing aggregation, and another residue were examined in prepared gels. The produced gels were put into sterile, transparent containers and examined visually for visible particles, homogeneity, and clarity. It was discovered that all of the formulations were homogenous and that no particle aggregates formed.

Extrudability

It's An essential factor for topical semi-solid dose forms and evaluated to ensure easy removal from packaging and smooth application. The extrudability was reported in table 3. The formulated gels demonstrated good extrudability, with Carbopol-940-based gels exhibiting excellent extrudability compared to those prepared with Chitosan and Xanthan gum.

Table 3: Extrudability of Anthocyanin gel formulations

Code	Extrudability in % ± S.D
F1	82 ± 0.10%
F2	91.6 ± 0.15%
F3	86 ± 0.05%

Viscosity

The viscosity is an important factor which has major impact on drug release, viscosity is essential to gel composition. The viscosity of all formulations was reported in table 4. While a lower viscosity gel formulation promotes faster diffusion into the medium, a higher viscosity gel formulation slows down drug release. Numerous anthocyanin gel formulations were the subject of viscosity tests; the findings demonstrated that each formulation had an appropriate viscosity, guaranteeing long-term retention at the application site.

Table 4: Viscosity of Anthocyanin gel formulations

Code	Viscosity in cps ± S.D
F1	11430 ± 9.01
F2	11470 ± 10
F3	11940 ± 9.01

Spreadability

Spreadability, essential for patient compliance and uniform skin coverage, was evaluated. This involved measuring the time for two slides to separate under controlled stress, with gel applied in between. The findings are reported in table 5, indicated that spreadability decreased as polymer concentration increased. However, all formulations exhibited easy spreadability under low shear.

Among the polymers tested, Carbopol-940-based gels showed the highest spreadability, outperforming Chitosan and Xanthan gum-based gels.

Table 5: Spreadability of Anthocyanin gel formulations

Code	Spreadability in gm.cm/sec ± S.D
F1	44.91 ± 0.05
F2	49.66 ± 0.25
F3	47.02 ± 0.05

Centrifuge test

A centrifuge test at 10,000 rpm was conducted to evaluate the uniformity and sedimentation of the gels, The results showed no visible residue or separation, indicating that the gels remained homogeneous and stable.

Temperature change test

Gel formulations should remain stable under a range of environmental conditions and temperatures without compromising their properties. Accordingly, every gel formulation that contained anthocyanin was tested across a range of temperatures but no discernible alterations in colour, texture, or appearance were noted and gel maintained its characteristics at each temperature.

Anthocyanin content of Gel Formulations

The Anthocyanin gel formulations underwent percentage drug content studies. Every gel formulation had a substantial amount of drug content; formulation F1 had 86.16%, formulation F2 had 97.25%, and formulation F3 had 94.75% as reported in table 6. Out of all of these formulations, formulation F2, which was made using carbopol-940, has the highest proportion of drug content.

Table 6: % Anthocyanin content in the gel formulations

Code	% Anthocyanin content ± S.D
F1	86.16 ± 0.0005%
F2	97.25 ± 0.0005%
F3	94.75 ± 0.0005%

In-vitro drug release studies

The cumulative percentage of drug release was assessed for each gel composition. Among these, the gel prepared with Carbopol-940 (F_2) demonstrated the most favourable release profile, with 97.31% release, outperforming formulations containing Chitosan (F_1) and Xanthan gum (F_3), which showed 86.16% and 89.03% release, respectively, at the end of 6 hours shown in fig 7.

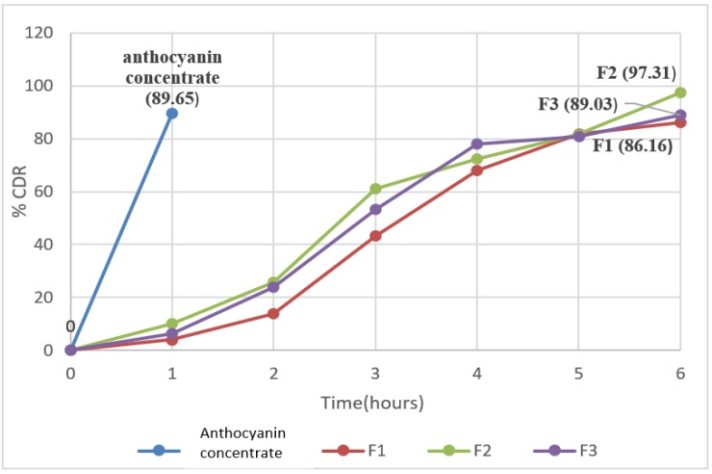


Figure 7: Comparison Release profile of Anthocyanin and formulations

Single Arm Open Labelled Clinical Study

In this study, anthocyanin extracted from *Brassica oleracea l. Var. Capitata F. rubra* was formulated into a 2% topical gel using Carbopol-940 polymer and evaluated for osteoarthritis treatment. Patients (31-80 years old) applied the gel thrice daily for 32 days, providing informed consent. Symptom severity (pain, swelling, tenderness, pain after flexion, stiffness, crepitus) was assessed, and data collected from case reports at BVVS Ayurveda Medical College & Hospital, Bagalkot, to investigate the gel's anti-inflammatory potential and results are expressed in table 7.

Table 7: Osteoarthritis Evaluation Parameters Grading Scale

Gradings	Pain	Swelling	Stiffness	Pain after flexion	Tenderness	Crepitus
0	No pain	No swelling	No stiffness	No pain	No tenderness	No crepitus
1	Mild pain	Slight swelling	Mild stiffness	Severe pain	Patient says tenderness	Palpable crepitus
2	Pain but no difficulty in walking	Moderate swelling	Moderate stiffness	Prevent complete flexion	Winching of face on touch	Audible crepitus
3	Difficulty in walking due to pain	Severe swelling	Severe difficulty due to stiffness	Does not allow passive movement	Does not allow touching the joints	Audible crepitus with pain

Pain: Anthocyanin gel treatment resulted in a significant 59.52% reduction in osteoarthritis pain, with mean scores decreasing from 2.47 to 1.00. This improvement was extremely statistically significant, indicating moderate pain alleviation.

Swelling: Anthocyanin gel significantly reduced joint swelling in osteoarthritis patients. Swelling scores (graded 0-3) decreased from 2.60 to 1.27 after treatment, showing 51.16% relief. This improvement was extremely statistically significant, indicating moderate reduction in swelling.

Tenderness: Tenderness in osteoarthritis patients occurs when the affected joint or surrounding tissues are touched or pressed, often due to inflammation, cartilage breakdown, and bone-on-bone contact. Anthocyanin gel treatment resulted in a significant 59.08% reduction in osteoarthritis-related tenderness, with mean scores decreasing from 2.37 to 0.97. This improvement was extremely statistically significant, indicating moderate tenderness alleviation.

Pain After Flexion: Pain after flexion, caused by cartilage breakdown, is a common symptom in osteoarthritis patients. Anthocyanin gel significantly reduced pain after flexion, with scores (graded 0-3) decreasing from 2.43 to 1.03 after treatment, showing 57.62% relief. This moderate improvement was extremely statistically significant.

Stiffness: Knee stiffness, characterized by restricted movement and rigidity, makes bending, straightening, and weight-bearing difficult. Anthocyanin gel treatment resulted in a 24.9% reduction in stiffness scores (graded 0-3), decreasing from 2.47 to 1.87. Although statistically significant, this improvement was modest, suggesting limited efficacy in alleviating stiffness.

Crepitus: Crepitus, or joint cracking, is characterized by grinding, grating, snapping, or clicking sounds and sensations in the joint, often accompanied by noise or vibrations. Anthocyanin gel treatment significantly reduced crepitus scores (graded 0-3) from 2.37 to 1.77, resulting in a 25.32% relief, indicating mild improvement. This reduction was extremely statistically significant.

Anthocyanin gel (F2) demonstrated significant efficacy in reducing osteoarthritis symptoms related to inflammation (pain, swelling, tenderness, and pain after flexion). However, crepitus and stiffness, linked to deeper joint structural changes, showed mild improvement, suggesting topical gel application may have limited penetration to effectively target these symptoms.

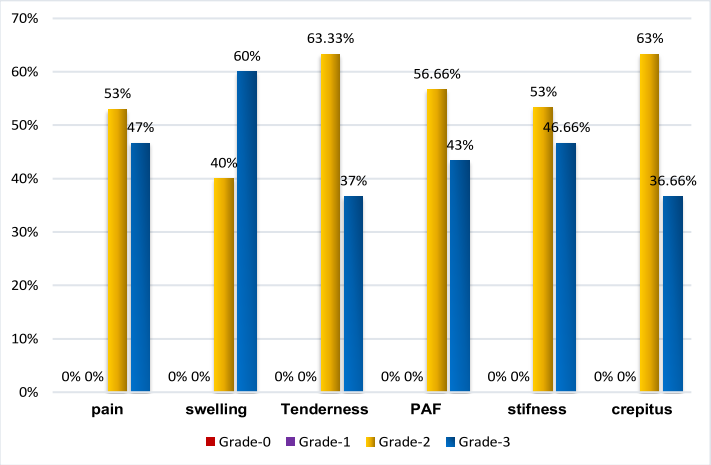


Figure 8: Graph that displays the individuals' distribution before to therapy based on pain, swelling, tenderness, pain after flexion, stiffness, and crepitus grades.

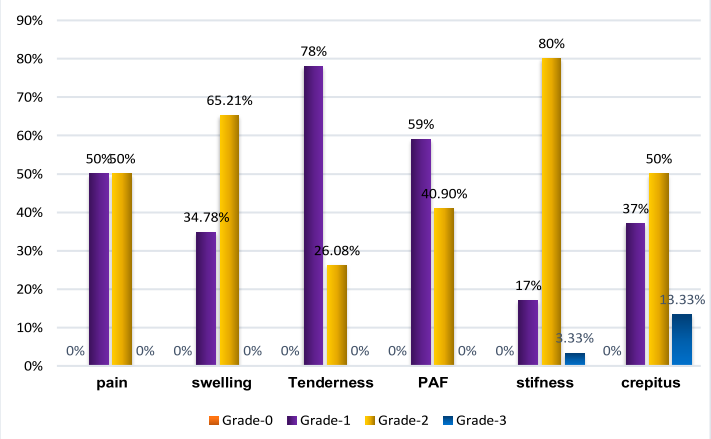
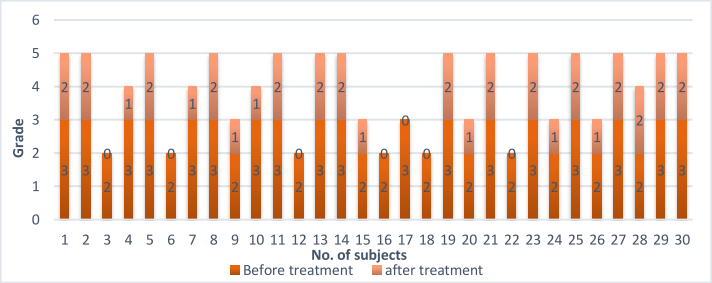


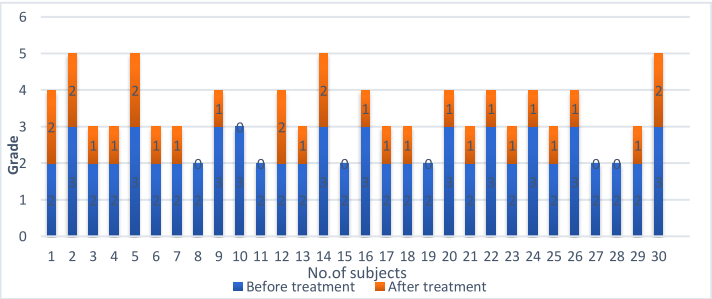
Figure 9: Graph that displays the individuals' distribution after treatment based on pain, swelling, tenderness, pain following flexion, stiffness, and crepitus grades.



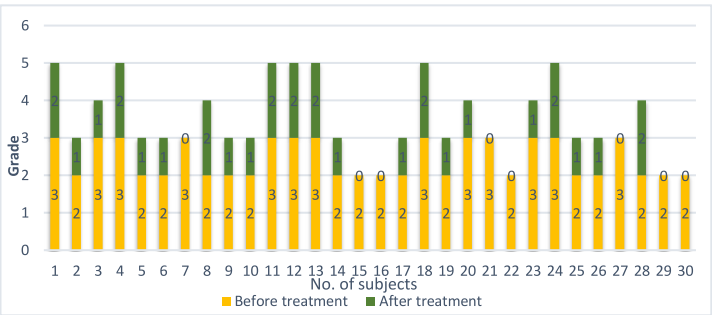
A. Pain



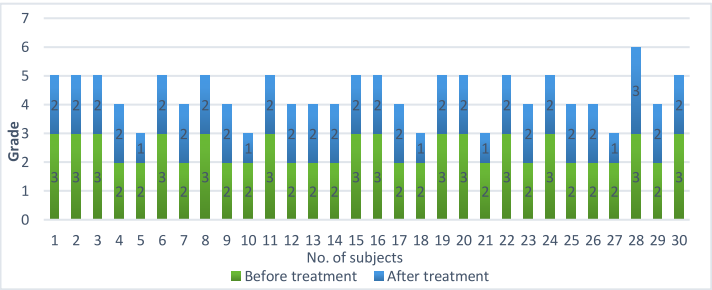
B. Pain



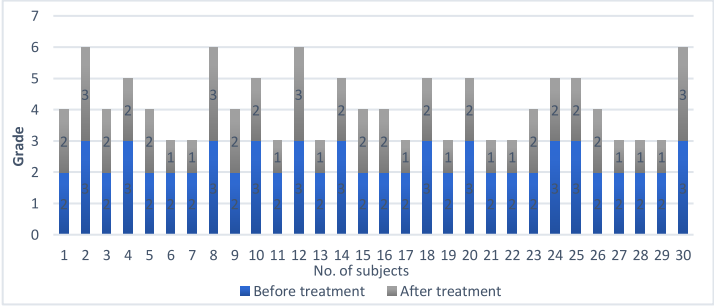
C. Pain



D. Pain



E. Pain



F. Pain

Figure 10: comparative Evaluation of Anthocyanin Gel Effectiveness: before and after Treatment Scores in Osteoarthritis Patients

Analysis of statistics: for the paired "t" test

Paired 't' was applied using SPSS software and results are reported in table 8.

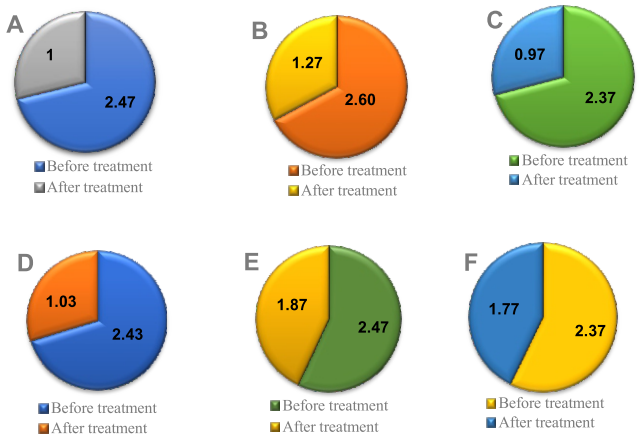


Figure 11: Improvement of Parameters Within Subjects A) Pain, B) Swelling, C) Tenderness, D) Pain after flexion, E) Stiffness, F) Crepitus

Table 8: Statistical results of Effect on pain, swelling, tenderness, pain after flexion, stiffness, crepitus.

Sl. No	Parameters	Mean		Difference in mean	Percentage of Relief	Clinical response
		B. T	A. T			
01	Pain	2.47	1.00	1.47	59.52%	Moderate improvement
02	Swelling	2.60	1.27	1.33	51.16%	Moderate improvement
03	Tenderness	2.37	0.97	1.4	59.08%	Moderate improvement
04	Pain after flexion	2.43	1.03	1.4	57.62%	Moderate improvement
05	Stiffness	2.47	1.87	0.6	24.93%	Mild improvement
06	Crepitus	2.37	1.77	0.6	25.32%	Mild improvement

CONCLUSION

Anthocyanin gel formulations were successfully prepared using Chitosan, Carbopol-940, and xanthan gum. The effective formulation (F2) containing 2% Carbopol-940 showed excellent physical and chemical properties, including pH stability, homogeneity, viscosity, spreadability and drug release. IR spectra analysis confirmed no interaction between anthocyanin and formulation additives.

A clinical study demonstrated the efficacy of formulation F_2 gel in reducing symptoms of osteoarthritis. Statistical analysis revealed significant improvements, with patients experiencing substantial reductions in pain, swelling, tenderness, and pain after flexion. Additionally, mild improvements were observed in stiffness and crepitus. Overall, the results indicate that anthocyanin gel formulation F_2 is a promising topical treatment for osteoarthritis, providing rapid and sustained relief from symptoms.

Limitations: The present study was designed as a preliminary clinical evaluation to assess the efficacy and tolerability of the newly developed formulation. Therefore, a single-arm, open-label study design was adopted to obtain initial clinical evidence regarding its therapeutic effects in the target population. This design was considered appropriate for an exploratory assessment of the formulation; however, the absence of a control group and blinding limits the ability to distinguish treatment-related effects from placebo responses, natural disease progression, or other potential confounding factors.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author Contribution

Associate Professor contributed to overall revision, guidance and approval of revised final manuscript. Assistant Professor promoted the study design, supervision during the experiment, data analysis, and manuscript revision. Students contributed to research work, data collection, formulation preparation, evaluation of results and preparation of the initial manuscript draft.

Data Availability statement

The data prepared and suitably analysed during this research study are available from the corresponding author upon request.

Ethics Approval

Since this research work was included human volunteers to perform inflammatory evaluation parameter, ethical approval was obtained from BVVS Ayurvedic College and Hospital, Bagalkote, With reference number AMUB-23-24/471.

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