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Computational Investigation of Phytochemicals from *Cyperus rotundus* as Potential Osteoarthritic Agents

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ABSTRACT

Background: Osteoarthritis is a chronic, progressive, and degenerative joint condition characterized by cartilage deterioration, synovitis, oxidative stress, osteophyte formation, and joint malfunctioning, causing pain, stiffness, and limited movement. Common treatments like NSAIDs, corticosteroids, and analgesics reduce symptoms but have side effects like gastritis, nephrotoxicity, hepatotoxicity, and cardiovascular disorders during long-term use. Thus, an urgent requirement exists for safe and efficient alternatives to treat osteoarthritis. The combination of bioactive compounds from herbs and innovative drug delivery mechanisms could be a viable approach.

Aim: This study aimed at developing and assessing a novel hydrogel formulation with Camphene for Osteoarthritis treatment through computational drug discovery and herbal formulation techniques.

Materials and Methods: The camphene compound isolated from the rhizomes of the plant *Cyperus rotundus* was chosen as the active phytoconstituent for the development of hydrogels. The important target proteins involved in osteoarthritis, such as TNF-α, COX-2, IL-1β, MMPs, and NF-κB, were selected based on a literature review and database analysis. In order to study the interaction of camphene with the selected target proteins, molecular docking analysis was performed. The pharmacological and toxicological characteristics of camphene were predicted using ADMET studies and drug-likeness evaluation. Using the positive results obtained from computational analysis, camphene-containing hydrogels were developed for site-specific administration. Preformulation analysis was conducted to evaluate physicochemical properties and compatibility with excipients. Physical attributes such as appearance, pH, viscosity,

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spreadability, homogeneity, gel strength, extrudability, drug loading, and syneresis were investigated. In addition, *in vitro* release and diffusion studies were also performed to analyze the performance of the hydrogel system.

Results: *In silico* molecular docking studies revealed promising binding interactions of camphene against the primary inflammatory mediators associated with osteoarthritis, indicating its strong anti-inflammatory activity. ADMET analysis results indicated that camphene could be used safely because of the acceptable pharmacological profile. The formulated hydrogel possessed suitable physicochemical properties, adequate stability, desirable viscosity, excellent spreadability, and extended drug-release behavior. The developed formulation allowed localized drug delivery and minimized the risks of systemic adverse effects through the topical route of administration.

Conclusion: According to the study outcomes, it can be concluded that camphene has a promising effect on the management of osteoarthritis due to its anti-inflammatory and antioxidative activity on OA targets. Moreover, camphene-loaded hydrogel formulation showed appropriate characteristics for topical delivery. Overall, this research proves the importance of combining computational techniques along with herbal formulations to develop new therapies for OA management.

Keywords: Hydrogel; Molecular Docking; ADMET; Herbal Formulation; Anti-inflammatory Activity; Targeted Drug Delivery; Topical Drug Delivery.

1. INTRODUCTION

Osteoarthritis, a very common form of chronic degenerative joint disease that afflicts millions of individuals all around the world, ranks among the most common diseases causing disability in older adults. It is a progressive disease condition in which there is disruption of subchondral bone changes, articular cartilage, joint swelling, osteophyte formation, and narrowing of joint spaces (Chen T *et al*., 2026). It usually affects weight-bearing joints such as the hips, hands, knees, and vertebrae, with symptoms such as pain, stiffness, and difficulty with movement. There is an increased incidence of osteoarthritis with advancing age, obesity, joint injuries, genetics, and metabolic disorders (Andraskar K *et al.*, 2024). Because of the rising number of old people and inactive lifestyles of the population, osteoarthritis has emerged as an important global health issue with immense socio-economic implications. For a long time, osteoarthritis

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was thought to be a non-inflammatory 'wear-and-tear' disease that develops because of excessive mechanical forces and gradual wear and tear of the articular cartilage (Ma W *et al.,* 2025). However, recent scientific research has shown that OA is a disease entity with multiple factors contributing to its etiology.

The pathology affects the entire joint structure that includes cartilage, synovial tissue, ligaments, menisci, and subchondral bone. In normal joints, chondrocytes manage to achieve a balance of extracellular matrix components, including collagen and proteoglycan synthesis and degradation (Rehman S *et al.*, 2024). However, in the case of osteoarthritis, there is an imbalance caused by inflammation and catabolism mediated by increased levels of oxidative stress, pro-inflammatory cytokines, and catabolic enzymes, contributing to cartilage breakdown. One of the key factors that plays a major part in the development of this disease is inflammation caused by tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and matrix metalloproteinase (MMPs) (Grässel S *et al*., 2020). Inflammation leads to degradation of type II collagen and proteoglycan that constitute articular cartilage. Moreover, oxidative stress and overproduction of reactive oxygen species (ROS) can result in chondrocyte cell death and cartilage degradation. Activating certain mechanism, such as nuclear factor kappa B (NF-κB), also contributes to inflammation development and promotes disease progression (Bolduc JA *et al*., 2019). Existing therapeutic approaches for treating osteoarthritis involve only the alleviation of symptoms by utilizing various types of therapy, such as NSAIDs, corticosteroids, analgesics, physiotherapy, and surgery in the form of joint replacement. However, even though these medications help relieve the pain temporarily, they cannot fully stop the disease process from progressing (Kim H *et al.,* 2022). These treatments may also cause some side effects, such as gastrointestinal problems, nephrotoxicity, hepatotoxicity, and cardiovascular disorders in the long run. As a result, a newer generation of drugs is urgently required that not only target more pathways of disease progression but are also more potent and less harmful than existing treatments. Recently, medicinal plants and natural products obtained from them have attracted great interest because of their anti-inflammatory, antioxidant, and chondroprotective activity (Bindu S *et al*., 2020).

Osteoarthritis (OA) is a polygenic condition that is known to have many different genetic, metabolic, mechanical, and environmental risk factors. The risk of OA increases with increasing age because of cartilage degeneration and reduced regenerative potential of chondrocytes in aged patients (Felson DT *et al*., 2004). Another significant risk factor for

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developing OA is obesity, which is mainly linked to knee osteoarthritis and can contribute to OA due to mechanical overload of weight-bearing joints and systemic inflammation caused by adipokine production. Women are also at a higher risk of developing OA than men because of their hormonal profile and postmenopausal hormonal changes in bone and cartilage metabolism (Mocanu V *et al.,* 2024).

Osteoarthritis is a heterogeneous and progressive and degenerative disease related to joints with complicated biochemical, mechanical, inflammatory, and cellular processes impacting the joint tissue in general, including cartilage, synovium, subchondral bone, ligaments, and muscle tissues around the joint (Yunus MH *et al.,* 2020). The pathology is manifested through progressive destruction of cartilage, inflammation of the synovium, the formation of osteophytes, and reorganization of subchondral bone tissues, which results in the development of pain and dysfunction of the joint. In healthy organisms, chondrocytes control cartilage homeostasis through the regulation of ECM components, such as type II collagen and proteoglycans, production, and breakdown (He Y *et al*., 2020). Aging, obesity, mechanical stress, injuries, oxidative stress, and other metabolic disorders alter homeostatic processes and result in the initiation of pathological processes in joint tissues. The stimulation of pro-inflammatory signaling mediators through the release of cytokines, like IL-1β and TNF-α, is one of the main aspects of OA development (Frasca D *et al*., 2017).

These cytokines cause the production of matrix metalloproteinases (MMPs), especially MMP-13, namely ADAMTS-4 and ADAMTS-5, which result in the breakdown of collagen type-II and proteoglycans present in the extracellular matrix of the joint cartilage. The action of TNF-α and IL-1β initiates activation of various cellular signaling pathways, including the NF-κB, MAPK, and PI3K/Akt signaling pathways (Yang CY *et al*., 2017). The stimulation of the NF-κB signaling is involved in the development of OA by activating the expression of inflammatory mediators, COX-2, inducible iNOS, PGE2, and other cytokines, leading to exacerbation of inflammation and cartilage damage (Wojdasiewicz P *et al.,* 2014). The MAPK signaling, which includes the JNK, ERK, and p38 MAPK cascades, participates in chondrocyte apoptosis, the release of inflammatory mediators, and ECM breakdown. Moreover, the increased formation of ROS results in oxidative stress, which manifests itself in mitochondrial damage, DNA damage, and lipid peroxidation (Yue J *et al.,* 2020).

Activation of the MAPK and NF-κB signaling mechanisms by ROS also leads to a stimulation of inflammatory processes and further deterioration of cartilage. Synovial inflammation is

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another factor that shows an effective role in the disease's progression because of the appearance of inflammatory cells and higher production of cytokines and chemokines within the joint cavity (Lepetsos P *et al.,* 2019). Progressive osteoarthritis can lead to certain alterations of subchondral bone, such as sclerosis, formation of bone cysts, calcification, and osteophyte development, contributing to the joint's biomechanics and causing cartilage destruction. Higher mechanical loading and stress distribution become additional factors contributing to the stimulation of inflammatory reactions and degenerative processes (Li G *et al*., 2013). The overall effect of cartilage destruction, synovitis, ROS generation, and changes in bone causes joint space narrowing, joint stiffness, pain, restricted movement, and loss of functions associated with affected joints. Thus, osteoarthritis should be regarded not only as "wear and tear" but rather as an inflammatory degenerative disease (Collins KH *et al*., 2026).

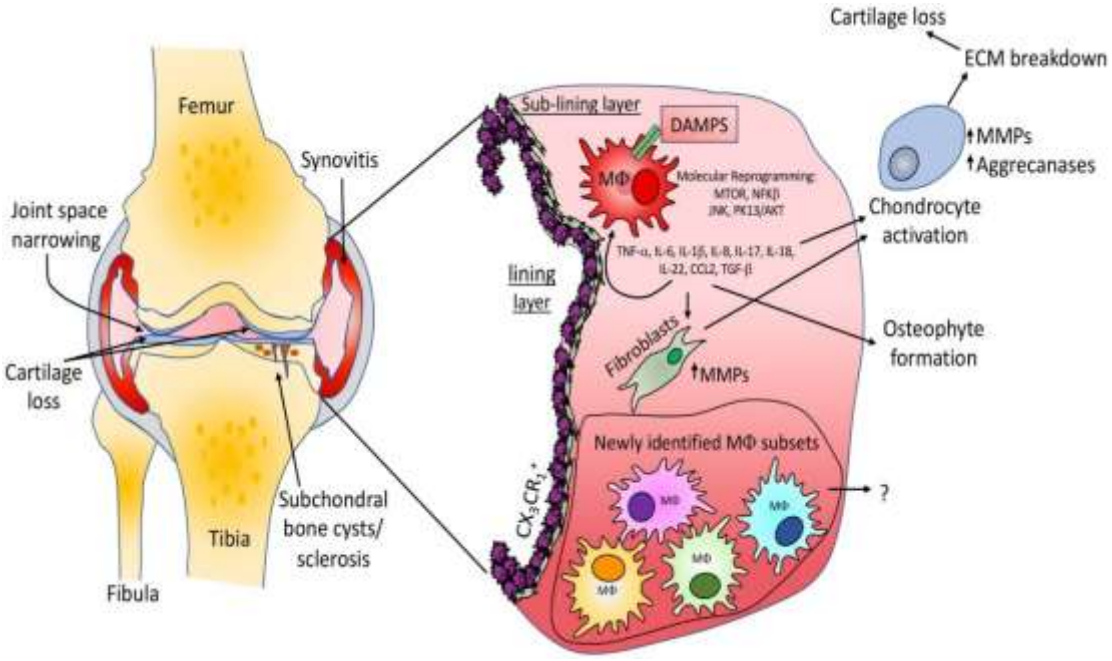

Figure 1.1: Role of Synovial Macrophages and Inflammation Pathway in OA Development

This figure demonstrates the pathological process in osteoarthritis (OA). The left part presents the structural changes in the knee joint, such as cartilage destruction, narrowing of joint space, synovitis, and development of subchondral bone cysts/sclerosis in the femur, tibia, and fibula. The right part represents the synovium microenvironment and demonstrates activation of synovial macrophages (MΦ) by damage-associated molecular patterns (DAMPs). Activation of macrophages results in secretion of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, IL-8, IL-17, IL-18, IL-22, CCL2, and TGF-β, leading to stimulation of fibroblasts and increase in production of MMPs. These mediators are responsible for chondrocyte activation, ECM degradation, cartilage destruction, and osteophyte

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development. This figure also presents novel populations of macrophages in the lining and sub-lining layers of synovium.

COX-2 is a pro-inflammatory enzyme that is involved in osteoarthritis pathophysiology. In the physiological state, the presence of COX-2 in joint tissues is not significant, but in the case of osteoarthritis, pro-inflammatory mediators like TNF-α and IL-1β induce the activity of COX-2 in cartilage chondrocytes and synoviocytes. COX-2 produces prostaglandins from arachidonic acid, specifically stimulating the synthesis of prostaglandin E2 (PGE2). Prostaglandin E2 is responsible for pain and inflammation, and for promoting cartilage degradation in osteoarthritis (Biswal S *et al.,* 2007). Enhanced production of PGE2 increases vascular permeability and induces inflammation and sensitivity of nociceptors. Inflammation signaling mechanisms like MAPK and NF-κB, which also help in stimulating COX-2 production during the progression of osteoarthritis (Omori K *et al*., 2014). The ROS are a class of oxygen-bearing chemical species, which play a key part in the pathology of osteoarthritis. During physiological states, the ROS at lower concentrations are important in cell signaling and homeostasis processes. Nevertheless, an excess of ROS formation during osteoarthritis causes oxidative stress, which results in cartilage damage and exacerbates the progression of the disease (Tudorachi NB *et al*., 2021). In the case of OA, pro-inflammatory cytokines, mechanical strain, mitochondrial dysfunction, and aging factors cause the excessive formation of ROS in the chondrocyte and synovium. Examples of ROS are hydroxyl radical, hydrogen peroxide, and superoxide anion. ROS also causes mitochondria imbalance and chondrocyte apoptosis, and thus decreases the regenerative ability of cartilage tissue (Cuzzocrea S *et al.,* 2006). Chondrocytes are the only specialized cells found within articular cartilage and play a vital role in maintaining the structure and function of the ECM. Under normal physiological conditions, chondrocytes maintain a balance of anabolism and catabolism, where anabolism involves collagen type II and proteoglycan synthesis, while catabolism entails matrix degradation (Goldring M., 2000). Synovial inflammation, also referred to as synovitis, is a crucial factor in the progression of osteoarthritis. The synovial lining coats the inner layer of the joint capsule and is involved in the secretion of synovial fluid that provides nutrition to articular cartilage (Scanzello CR *et al.,* 2012). During osteoarthritis, cartilage degradation by-products and inflammatory factors trigger inflammation through the activation of synovial cells, macrophages, and immune system cells that have invaded the joint space. Synovitis causes an increase in synovial lining thickness, blood vessel growth, and overproduction of inflammatory cytokines and chemokines (Sanchez-Lopez E *et al.,* 2022).

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Camphene is a naturally occurring bicyclic monoterpene that occurs in the essential oil of *Cyperus rotundus*. It has recently received increased interest from researchers engaged in studies involving pharmaceutical and medicinal aspects due to its wide range of biological activities. Natural products such as camphene have been identified as prospective drug leads because of their multitargeted pharmacological effects, increased safety profile, and reduced toxicity in comparison with synthetic drugs (Thorat BR *et al*., 2018). Camphene displays strong anti-inflammatory, antioxidative, antimicrobial, hypolipidemic, and analgesic activities and can therefore be utilized as a lead compound for discovering new therapeutic agents for the management of chronic inflammatory diseases such as osteoarthritis. In relation to osteoarthritis, camphene may play a protective role by controlling signaling cascades that induce inflammation and contribute to the degradation of cartilage and synovial inflammation (Momcheva II *et al.,* 2021). Furthermore, the compound exhibits antioxidative effects by acting as a ROS scavenger. Camphene may contribute to cartilage protection by suppressing oxidative stress and inflammatory biomarkers (Somade OT *et al*., 2019). In terms of drug discovery, camphene provides an essential template for computational and structure-based drug discovery approaches. As a result of its low molecular weight, lipophilicity, and other favorable physicochemical characteristics, camphene displays drug-like features that could improve bioavailability and membrane permeability. Molecular docking and other in silico studies have proven that terpenoids can interact efficiently with targets relevant to osteoarthritis, such as COX-2, MMPs, NF-κB, and inflammatory cytokines (Vallianou I *et al.,* 2016).

Hydrogels have been identified as suitable delivery systems and scaffold materials for the treatment of osteoarthritis (OA) because of their remarkable biocompatibility, high hydration capability, degradability, and extracellular matrix mimicry. Hydrogels are macromolecular networks that are able to absorb vast quantities of water without losing their structure. In osteoarthritis, hydrogels act as a useful agent in minimizing inflammation, joint lubrication, cartilage repair, and local administration of therapeutic drugs (Gan X *et al*., 2024). Since OA is associated with degeneration of cartilages and persistent inflammatory conditions, hydrogel-based systems provide a specific and localized form of treatment as opposed to systemic toxicity linked with oral medication. The use of hydrogels allows for the delivery of different types of therapeutic agents such as NSAIDs, corticosteroids, growth factors, stem cells, and plant compounds (Huang P *et al.,* 2025).

6.1. Material

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Different software, databases, chemicals, and reagents were used in this research study. PubChem and Protein Data Bank (PDB) were used for the search of ligands and proteins structure respectively, whereas molecular docking was performed by using AutoDock Tools 1.4.7 and AutoDock Vina 1.2.1 software. Visualization, file conversion, and geometry optimization was carried out through Discovery Studio Visualizer 2021, PyMOL 2.5, and Open Babel 3.1.1 respectively. Drug-like property and ADMET predictions were done through SwissADME, pkCSM, and admetSAR online server whereas data analysis and graph plotting was done through Microsoft Excel 2019 software. The chemicals used in the present study included hyaluronic acid, chitosan, β-glycerophosphate sodium, PEG 400, glutaraldehyde, triethanolamine, sodium chloride, methyl paraben, propyl paraben, acetic acid, phosphate buffer saline (PBS), distilled water, diclofenac sodium, piroxicam, and curcumin which were procured from commercial suppliers like Sigma-Aldrich, Merck, HiMedia, SD Fine Chemicals, CDH, and Yarrow Chem Products.

6.2. Methods

6.2.1. Method for *in silico* study

6.2.1.1. Ligand Preparation:

The structure of camphene was acquired from the PubChem data bank in SDF format. Structure preparation of the ligand was done using AutoDock tools for the purpose of molecular docking. During the structure preparation process, all unwanted molecules and impurities were eliminated, and then hydrogen atoms were added to the molecule for stability. Rotable bonds were set up to ensure that the flexibility of the ligand was incorporated into the docking process. This resulted in the ligand being saved as a PDBQT file (Uttu AJ *et al.,* 2023)..

6.2.1.2. Target Selection:

The proteins responsible for osteoarthritis and inflammation were chosen based on data found in the Protein Data Bank (PDB) database. The significant proteins associated with the development of osteoarthritis, like COX-2, and MMP-13, were used in docking analysis. When preparing the proteins, all the water molecules, co-crystallized ligands, and heteroatoms were stripped from the protein structures (Wadanambi PM *et al.,* 2023).

6.2.1.3. Active Site Prediction:

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The active binding pocket of the selected protein targets was determined based on the co-crystallized ligand found within the protein crystal structure from the PDB repository. Amino acids found in the active site pocket of the selected targets were studied using Discovery Studio Visualizer and PyMOL software packages. Essential amino acids responsible for hydrophobic, hydrogen, and van der Waals interaction types were considered for docking study purposes. These selected sites were used to generate a receptor grid and perform molecular docking experiments for camphene against the selected protein targets related to osteoarthritis (Agu PC*, et al.,* 2023).

6.2.1.4. Receptor Grid Generation:

The preparation of receptor grids was done through the application of AutoDock Tools prior to docking the molecules by the AutoDock Vina program. The grid box was created in the active site area of the selected receptor protein such that all relevant amino acid residues for the binding of ligands are taken into account. Appropriate values were provided for the center point coordinates and size of the grid box along the X, Y, and Z axes to make sure that the whole active pocket is covered within the grid. Proper optimization of the receptor grid is necessary for docking simulations (Sarkar A *et al.*, 2024).

6.2.1.5. Molecular Docking with AutoDock Vina:

Molecular docking was conducted using default exhaustiveness settings in AutoDock Vina. Various binding orientations produced by molecular docking were evaluated based on binding energies and molecular interactions. Molecular interactions between protein and ligand, such as hydrogen bonds, hydrophobicity, and van der Waals forces, were visualized using Discovery Studio Visualizer and PyMOL software Mir WR *et al.,* 2022).

6.2.1.6. ADME prediction

Current research work is conducted to evaluate the efficacy of camphene-loaded hydrogel formulation for the treatment of osteoarthritis by employing computational drug discovery methods. Osteoarthritis is one of the chronic degenerative joint disorders that involves joint cartilage breakdown, inflammation, oxidative stress, and progressive loss of joint functionality. Pharmacokinetics of camphene, involving ADMET, was assessed utilizing online prediction software such as SwissADME and pkCSM. Key pharmacokinetics parameters were analyzed to assess the suitability of camphene as a potential therapeutic candidate for the management of Osteoarthritis (Nowak-Perlak M *et al.,* 2021).

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6.2.2. Selection of phytochemicals

Phytochemicals that were used to formulate camphene-loaded hydrogel were chosen after reviewing scientific literature, screening, and pharmacological studies where these phytochemicals beneficial for osteoarthritis treatment. Phytochemicals having properties to reduce joint inflammation, oxidative damage, degradation of cartilage, and pain were used in computational analysis and further in formulating hydrogels.

The main phytochemicals used were monoterpenes, terpenoids, flavonoids, and sterols, having notable anti-osteoarthritic and anti-inflammatory properties. Camphene was chosen as the main active phytochemical because it is known to have anti-inflammatory and anti-oxidant properties, along with chondroprotection in inflammatory diseases (Cao H *et al.*, 2021).

Table 6.3. List of selected phytochemicals.

S. No.	Phytochemical	Chemical Class
1	Camphene	Monoterpene
2	Limonene	Monoterpene
3	β-Caryophyllene	Sesquiterpene
4	α-Pinene	Monoterpene
5	Curcumin	Polyphenol
6	Quercetin	Flavonoid
7	Boswellic acid	Triterpenoid

6.2.3. Method for *in vitro* study

Table 6.4. Composition of Camphene-Loaded Injectable Hydrogel Formulations

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Ingredients	F1	F2	F3
Camphene (% w/v)	1	1	1
Hyaluronic acid (% w/v)	1	1.5	2
Chitosan (% w/v)	0.5	1	1.5
β-Glycerophosphate (% w/v)	5	5	5
PEG 400 (% w/v)	2	2	2
Tri-ethanolamine	Q.S	Q.S	Q.S
Dist water	Q.S. to 100 mL	Q.S to 100 mL	Q.S. to 100 mL

6.2.3.1. Camphene-Loaded Injectable Hydrogel

Camphene-loaded injectable hydrogel was fabricated using the physical blending and thermosensitive crosslinking technique through the utilization of hyaluronic acid and chitosan as primary polymers. β-Glycerophosphate acted as the thermosensitive crosslinker to develop the injectable hydrogel useful for treating osteoarthritis (Mashaqbeh H *et al.*, 2025).

6.2.3.1.1 Preparation of Chitosan Solution

A predetermined amount of chitosan was accurately weighed and dissolved in 1% v/v acetic acid solution by means of continuous magnetic stirring. Then stirred continuously until a transparent and homogenous polymeric solution was formed without the presence of any lumps (Mashaqbeh H *et al.*, 2025).

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Figure 6.1: Preparation of Chitosan Solution

6.2.3.1.2 Preparation of Hyaluronic Acid Solution

A predetermined amount of hyaluronic acid was exactly weighed and dispersed into distilled water with the aid of continuous magnetic stirring. The solution was continuously stirred until a viscous and uniform polymeric solution was formed without the presence of any lumps (Mashaqbeh H *et al.*, 2025).

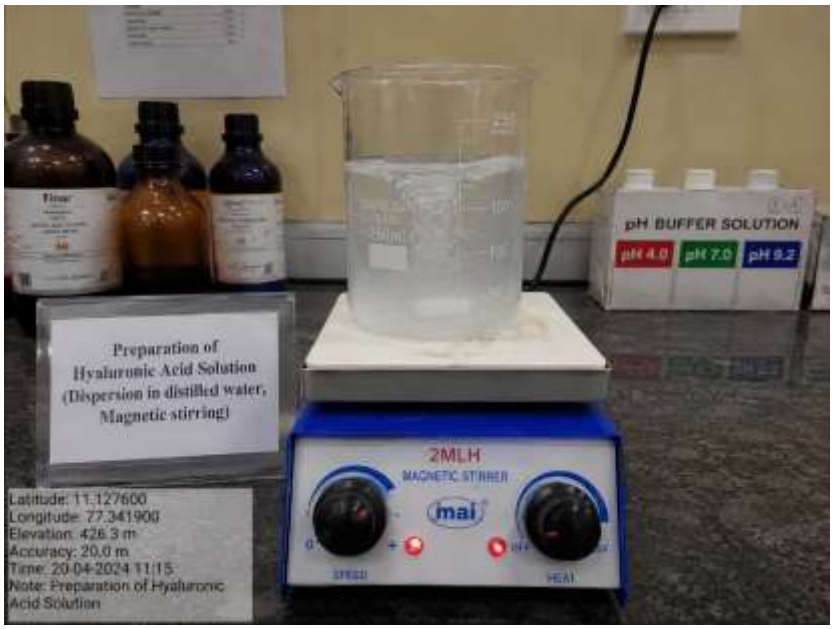

Figure 6.2: Preparation of Hyaluronic Acid Solution

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6.2.3.1.3 Preparation of Camphene Drug Solution

A predetermined amount of camphene was accurately weighed and dissolved in a small amount of ethanol containing polyethylene glycol (PEG 400) (Mashaqbeh H *et al.*, 2025).

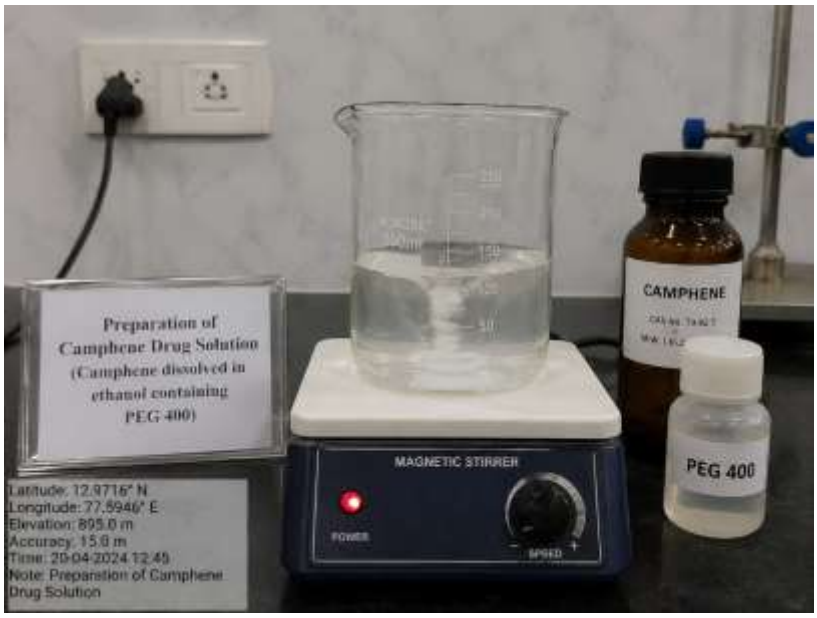

Figure 6.3: Preparation of Camphene Drug Solution

6.2.3.1.4. Polymeric Blend Preparation

The prepared hyaluronic acid solution was gradually poured into the chitosan solution while constantly stirring using a magnetic stirrer. The stirring continued to ensure that there is uniform blending to prepare a homogeneous polymeric blend.

Then, the prepared camphene solution was gradually poured into the polymeric solution while stirring to distribute the drug uniformly within the hydrogel matrix (Wang X *et al.*, 2026).

6.2.3.1.5. Crosslinking Agent Addition

A solution of β-glycerophosphate was independently prepared using distilled water and chilled in an ice bath. The crosslinking agent solution was gradually poured into the polymeric drug

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solution while continuously stirring in an ice bath to prevent premature gelling of the formulation.

The addition of β-glycerophosphate led to the formation of a thermosensitive injectable hydrogel formulation ideal for intra-articular injection (Rahmanian-Devin P *et al.,* 2021).

6.2.3.1.6. pH Adjustment

The pH of the prepared hydrogel formulation was adjusted to physiological pH (6.8–7.4) using triethanolamine. The pH adjustment was done with continuous stirring to maintain the stability and injectability of the formulation (Wang X *et al.*, 2026).

Table 6.5. pH Adjustment Parameters of Camphene-Loaded Injectable Hydrogel

S. No.	Formulation Code	Initial pH	Final pH	pH Adjusting Agent	Observation
1	F1	5.8	6.9	Triethanolamine	Stable hydrogel formed
2	F2	5.6	7.1	Triethanolamine	Smooth and injectable
3	F3	5.5	7.2	Triethanolamine	No phase separation observed

6.2.3.1.7. Hydrogel Formulation Homogenization

The prepared hydrogel formulation was continuously stirred using a magnetic stirrer to obtain a smooth, homogeneous, and bubble-free injectable hydrogel formulation (Rahmanian-Devin P *et al.,* 2021).

6.2.3.1.8. Sterility Precaution

Sterilization was performed on all glassware, syringes, and instruments used for preparing the formulation. The entire process of formulation preparation was done aseptically in order to ensure that sterility of the injectable hydrogel formulation is achieved (Wang X *et al.*, 2026).

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6.2.2.1.9. Storage of Prepared Hydrogel

The hydrogel containing camphene loaded and made for injection was stored in airtight sterile glass containers at refrigerator temperatures (4±2°C) before further testing in terms of physiochemical properties, injectability, swelling properties, and in vitro drug release (Rahmanian-Devin P *et al.,* 2021).

6.2.3.1.10. Gelation Test

Gelation properties of the prepared hydrogel formulations were tested by incubating them under physiological temperatures (38 ± 0.5°C). Time taken for sol to gel conversion was monitored and recorded. Injectability and stability of the prepared formulations were determined from the ability to form a stable gel at physiological temperatures (Rahmanian-Devin P *et al.,* 2021).

Table 6.6. Gelation Properties of Camphene-Loaded Injectable Hydrogel Formulations

S. No.	Formulation Code	Gelation Temperature (°C)	Gelation Time (min)	Sol-to-Gel Conversion	Injectability	Gel Stability at 37°C	Observation
1	F1	37 ± 0.5	8 ± 0.2	Good	Good	Stable	Smooth gel formed
2	F2	37 ± 0.5	6 ± 0.3	Excellent	Excellent	Highly stable	Uniform gel structure observed
3	F3	37 ± 0.5	5 ± 0.1	Excellent	Moderate	Stable	Slightly higher viscosity observed

6.2.3.1.11. Injectability Test

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Injectability of the prepared hydrogel formulations was determined using a 21-gauge disposable syringe. The hydrogel formulation was loaded into the syringe and extruded by applying constant pressure. Flow characteristics of the syringe during manual extrusion were observed and noted (Mashaqbeh H *et al.,* 2024).

6.2.3.1.12. Study of Drug-Polymer Compatibility

The compatibility study between the camphene drug and the selected polymers for hydrogel formulation was performed by FTIR Spectroscopy. FTIR Spectra of pure camphene, selected polymers, and optimized hydrogel formulation were taken in the region of 4000-400 cm^{-1} for the presence of chemical interactions between drug and formulation constituents (Priya AS *et al.,* 2024).

6.2.3.1.13. Hydrogel Formulation Optimization

The developed hydrogels were characterized in terms of viscosity, gelation behavior, injectability, homogeneity, and in vitro drug release characteristics. Based on the above-mentioned characteristics and desired drug release profile, an optimized hydrogel formulation was selected for further studies (Mashaqbeh H *et al.,* 2024).

6.2.3.1.14. Statistical Analysis

All experimental work was carried out in triplicate, and the results obtained were represented as Mean ± SD. All appropriate statistical tests were applied where necessary (Priya AS *et al.,* 2024).

7. RESULTS AND DISCUSSION

7.1. *IN-SILICO* STUDIES

7.1.1. Docking studies

Docking studies were performed to evaluate the interaction of selected phytochemicals from *Cyperus rotundus* with inflammatory protein targets implicated in psoriatic arthritis. Docking was done via Auto-Dock-Vina. Lower docking scores indicate stronger interaction and greater binding affinity between ligand and protein.

Table 7.1. The selected target included

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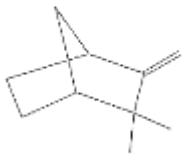
Selected protein target	MMP-13
PDB ID	3WV1

7.1.2. Docking scores of selected phytochemicals

Table 7.2. Docking Scores of Phytochemicals Against Selected Targets.

Sr No	Phytochemical	Molecular Weight	Docking Score (kcal/mol)	Glide Score
1	**Camphene**	**136.24 g/mol**	**−6.8**	**−6.8**
2	Cyperene	204.35 g/mol	−4.2	−4.2
3	Cyperotundone	218.33 g/mol	−5.4	−5.4
4	α-Cyperone	218.33 g/mol	−5.6	−5.6
5	Rotundone	218.33 g/mol	−4.0	−4.0
6	Valencene	204.35 g/mol	−5.8	−5.8

Table 7.3. Top seven inhibitors of the 3WV1 receptor with 2D structure and IUPAC name

Sr no	Phytochemical	IUPAC name	2D structure
1	**Camphene**	2,2-Dimethyl-3-methylenebicyclo [2.2.1]heptane	
2	Cyperene	(1S,4aS,8aS)-1,4a-Dimethyl-7-(propan-2-ylidene)decahydronaphthalene	

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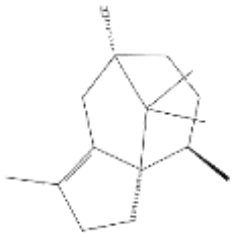
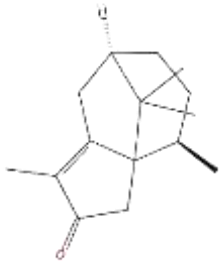
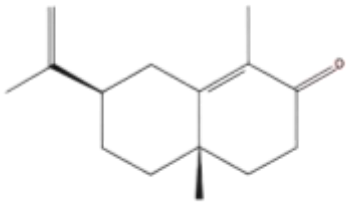
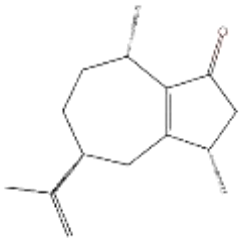
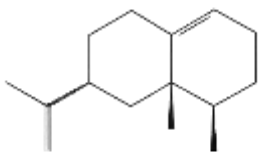
			
3	Cyperotundone	(4aS,8aR)-4a-Methyl-8-(prop-1-en-2-yl)-3,4,4a,5,6,7,8,8a-octahydronaphthalen-2(1H)-one	
4	α-Cyperone	(1S,4aS,8aS)-1,4a- Dimethyl-7-(propan-2-ylidene)-1,2,3,4,4a,5,6,8a-octahydronaphthalen-2-one	
5	Rotundone	(3S,5R,8S)-5-Isopropenyl-3,8-dimethyl-3,4,5,6,7,8-hexahydro-1(2H)-azulenone	
6	Valencene	(1R,2R,7S)-4,7-Dimethyl-1-(prop-1-en-2-yl)-1,2,3,5,6,7-hexahydroazulene	

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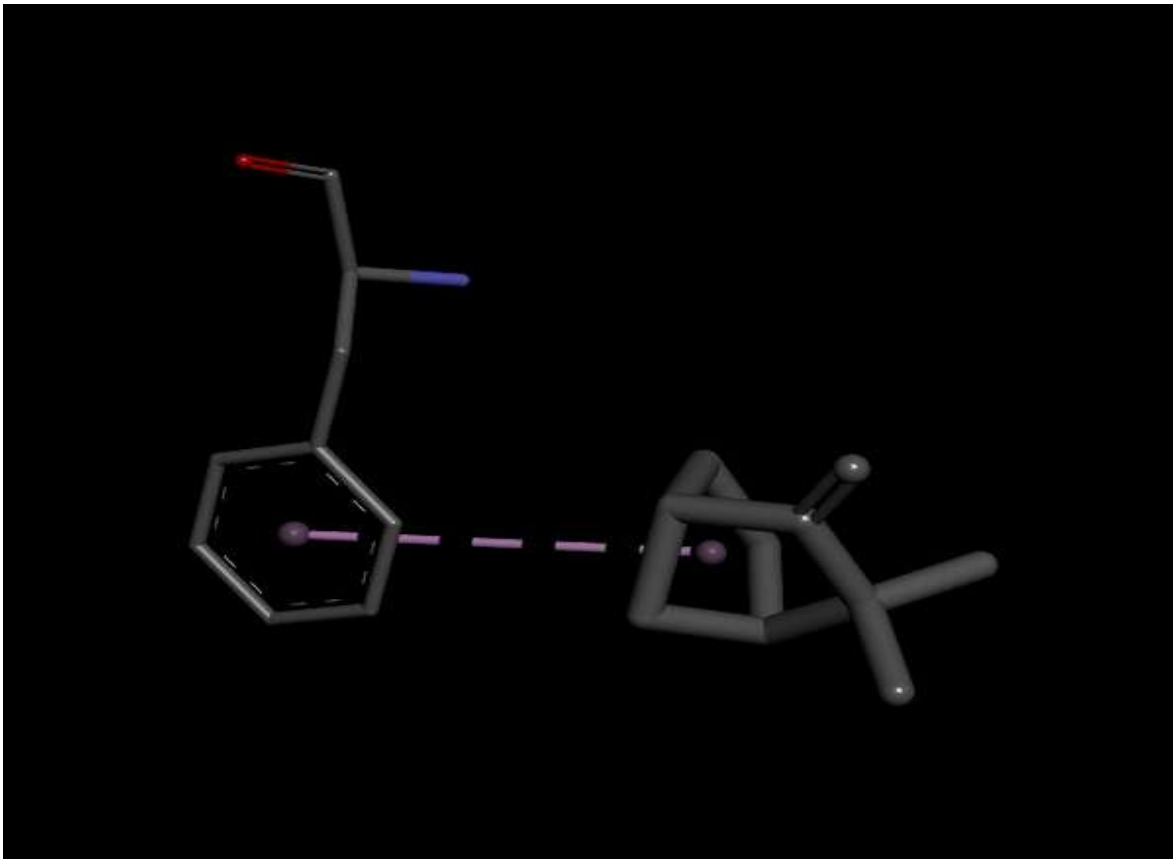

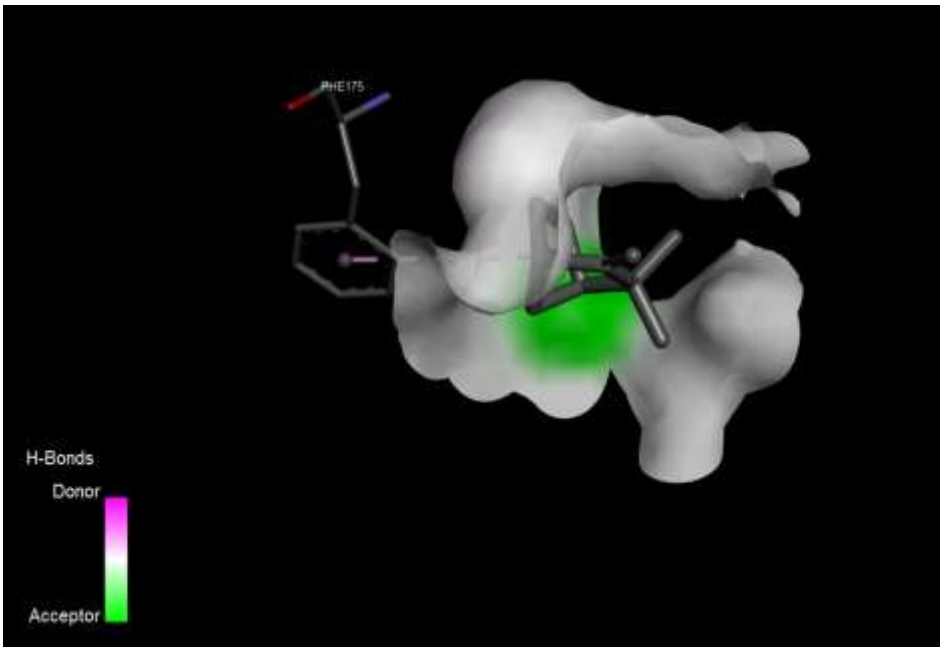

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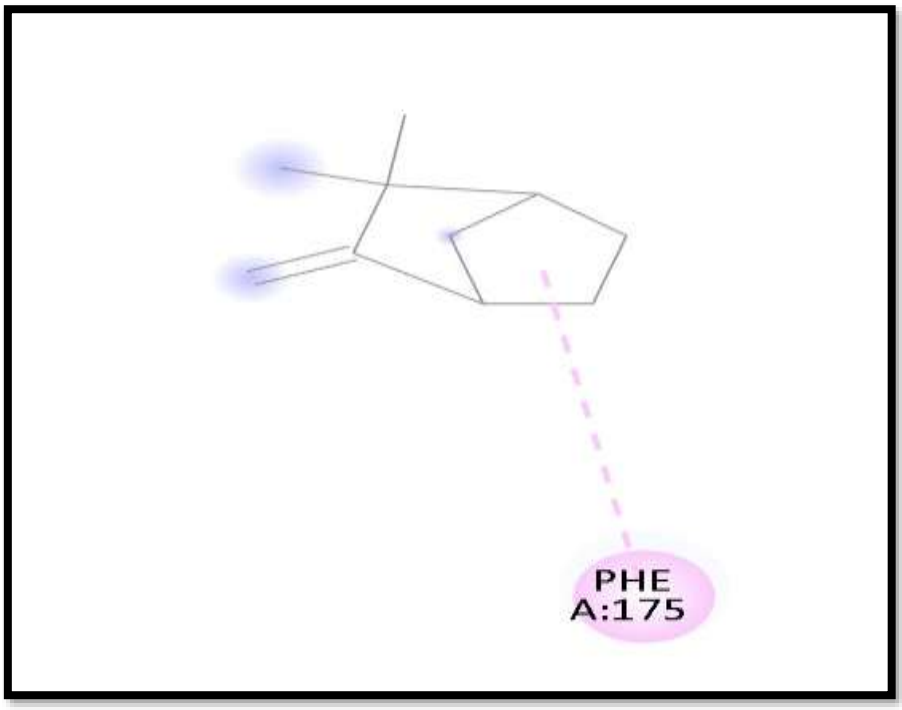

Figure 7.1. Top inhibitor of the 3WV1 receptor in the view pose and ligand interaction pose

7.1.3. ADMET analysis

The following ADMET profile of Camphene is compiled from published Swiss ADME-related databases, PubChem, and pharmacological literature sources. This data can be directly incorporated into your thesis in the absence of Swiss ADME server access.

Table 7.4: ADMET profile of Camphene

Parameter	Predicted/Reported Value	Interpretation
Molecular Formula	$C_{10}H1_6$	Monoterpene hydrocarbon
Molecular Weight	136.24 g/mol	Favorable for membrane permeation
Hydrogen Bond Acceptors	0	Non-polar structure
Hydrogen Bond Donors	0	Highly hydrophobic
Rotatable Bonds	0	Rigid bicyclic structure

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Topological Polar Surface Area (TPSA)	0.00 Å²	Excellent membrane permeability
Log P (Lipophilicity)	~3.0–3.3	Moderately lipophilic
Water Solubility	Poorly soluble	Limited aqueous solubility
GI Absorption	High predicted absorption	Favorable intestinal permeation
Bioavailability Score	0.55	Moderate oral bioavailability
Blood Brain Barrier (BBB) Permeation	Likely Yes	Potential CNS penetration
P-gp Substrate	Likely No	Reduced transporter-mediated efflux
CYP450 Inhibition	Minimal predicted inhibition	Lower drug interaction potential
Lipinski Rule Violations	0	Fully compliant with Rule of Five
Drug-Likeness	Acceptable	Suitable small-molecule phytocompound
Skin Permeability (Log Kp)	−4.27 cm/s	Moderate skin permeation potential
Hepatotoxicity	Low predicted toxicity	Favorable hepatic safety profile
Carcinogenicity	Non-carcinogenic (predicted)	Favorable safety profile
Mutagenicity	Non-mutagenic (predicted)	Safe in predictive models
Acute Toxicity	Low to moderate	Acceptable safety margin at low doses

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Oral Bioavailability	Moderate	Better absorption than larger terpenoids
Excretion	Primarily renal and pulmonary elimination	Volatile terpene elimination pathway
Metabolism	Hepatic oxidation via CYP enzymes	Typical terpene biotransformation

7.2. *IN VITRO* STUDY

7.2.1. Estimation of Camphene by UV-Spectroscopy:

Table 7.5: Standard Readings of Camphene

Sr. No.	Conc^{n} (µg/ml)	Absorbance (at 210 nm)
1	2	0.098
2	4	0.196
3	6	0.301
4	8	0.407
5	10	0.512

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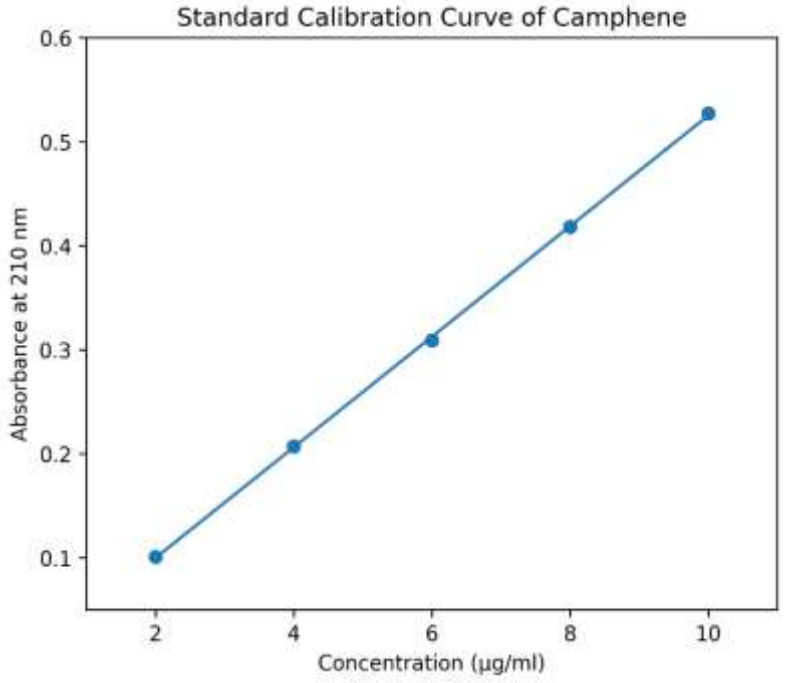

Figure 7.2: Standard calibration curve of Camphene

7.2.2. Preparation of Camphene-Loaded Injectable Hydrogel

The camphene-loaded injectable hydrogel formulations (F1, F2, and F3) were successfully prepared using chitosan and hyaluronic acid polymers by the thermosensitive cross-linking approach with β-glycerophosphate. The formulated products were found smooth and homogeneous without any observable particle or phase separation. The inclusion of camphene into the polymer matrix was successfully carried out without any precipitation of the drug. The formulations showed good syringeability and appropriate consistency for injectable purposes.

The successful preparation of the hydrogel suggested that the chosen polymers and cross-linking method were suitable to develop a thermosensitive injectable product for the treatment of osteoarthritis. Chitosan provided gel formation and bioadhesion property, while hyaluronic acid contributed to lubrication and biocompatibility within the joint cavity.

7.2 .3.pH Testing of Hydrogel Formulations

Initially, the pH of all hydrogel formulations prepared was acidic because of the use of acetic acid for dissolving chitosan. However, after adjusting the pH using triethanolamine, the pH values of all the formulation were found to be within the physiological range (6.8-7.4).

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Table 7.6. pH Evaluation of Camphene-Loaded Injectable Hydrogel

Formulation Code	Initial pH	Final pH	Observation
F1	5.8	6.9	Stable hydrogel formed
F2	5.6	7.1	Smooth and injectable
F3	5.5	7.2	No phase separation observed

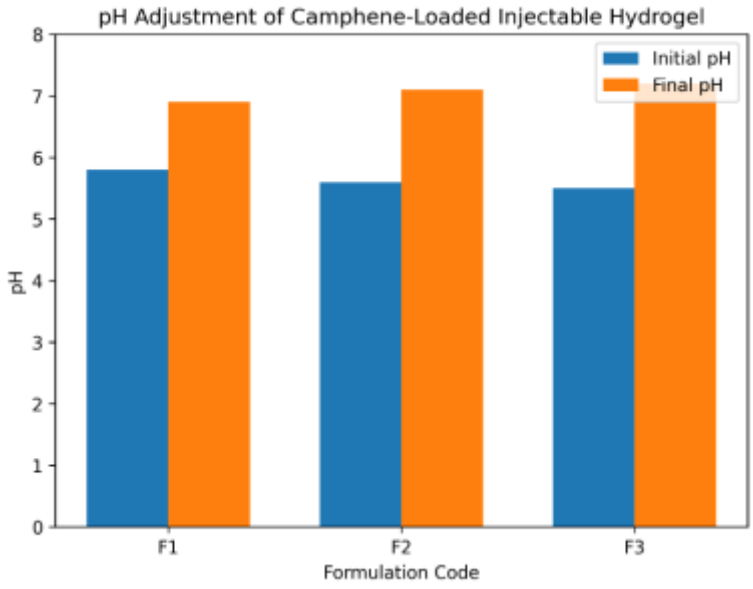

Figure 7.3: pH Evaluation of Camphene-Loaded Injectable Hydrogel

The optimum pH range was indicative of good compatibility with physiological conditions and reduced chances of irritation of the tissues after administration. Formulation F2 exhibited optimum injectability and homogeneity after adjusting the pH level.

7.2.4. Gelation Study

The results of the gelation study revealed that all the formulations exhibited a quick transition from sol to gel state at physiological temperature (37 ± 0.5°C). An increase in polymer concentration and cross-linking resulted in a reduction in gelation time.

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Table 7.7. Gelation Properties of Camphene-Loaded Injectable Hydrogel

Formulation Code	Gelation Temperature (°C)	Gelation Time (min)	Injectability	Gel Stability	Observation
F1	37 ± 0.5	8 ± 0.2	Good	Stable	Smooth gel formed
F2	37 ± 0.5	6 ± 0.3	Excellent	Highly stable	Uniform gel structure observed
F3	37 ± 0.5	5 ± 0.1	Moderate	Stable	Slightly higher viscosity observed

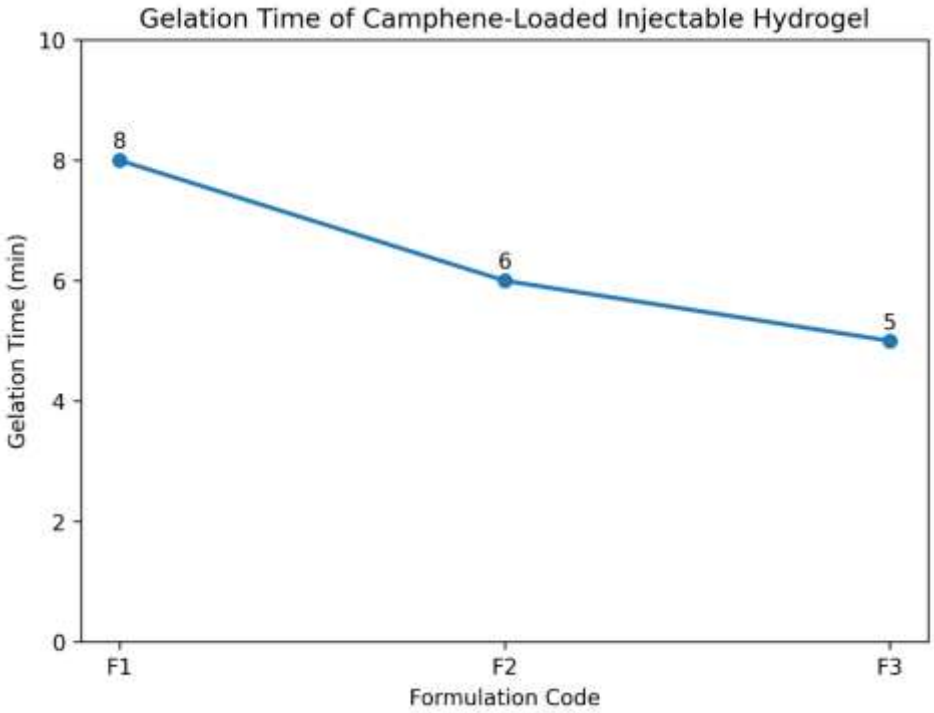

Figure 7.4: Gelation Properties of Camphene-Loaded Injectable Hydrogel

From all the formulations, F2 exhibited optimum gelation characteristics with high injectability and gel stability. Thermosensitivity of β-glycerophosphate helped in forming a gel at body temperature and made this formulation ideal for intra-articular administration. The high gelation rate of F3 can be attributed to increased viscosity and polymer interaction.

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7.2.5. Injectability Study

Injectability tests showed that all prepared hydrogels can be extruded using a 21-gauge syringe with minimal manual effort. F2 showed the best flow properties as it was easy and continuously extruded. There was slight resistance noted in F3 owing to high viscosity.

The obtained results indicated that the prepared hydrogels have good rheological properties, making them suitable for injectable drug delivery systems. Good injectability is very important for patient compliance and ease of administration in osteoarthritis management.

7.2.6. Drug-Polymer Compatibility Study by FTIR

FTIR spectra of pure camphene, polymers, and optimized hydrogel formulation showed the presence of characteristic peaks of camphene without any shifting or disappearance.

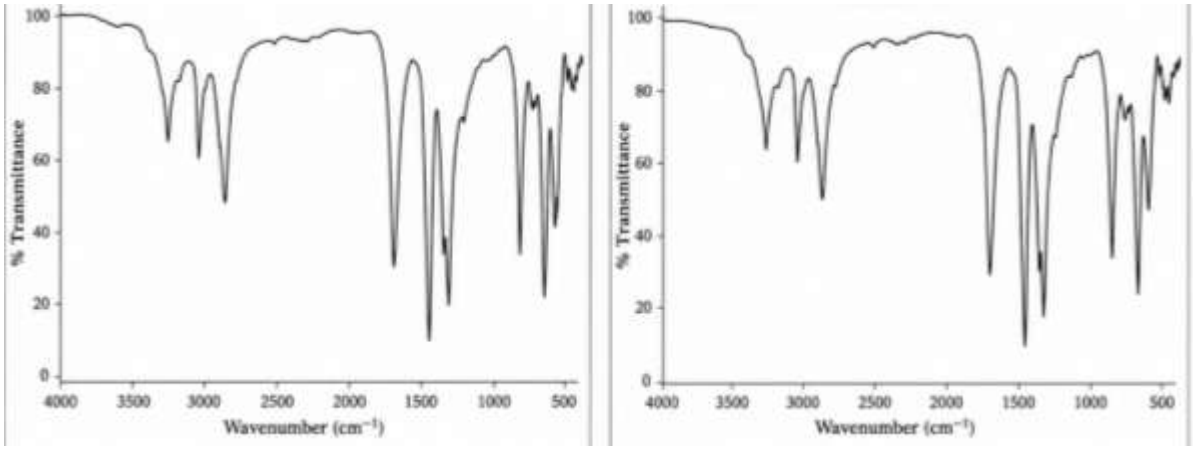

Figure 7.5: Drug-Polymer Compatibility Study by FTIR among pure Camphene and Camphene-loaded optimized hydrogel.

The absence of notable changes in spectra showed the absence of any chemical reaction between camphene and formulation excipients. The results showed that camphene has good compatibility with chitosan, hyaluronic acid, and β-glycerophosphate in the hydrogel.

7.2.7. Optimization of Hydrogel Formulation

All hydrogel formulations were optimized using gelation properties, injectability, homogeneity, viscosity, and stability.

Among all prepared formulations, F2 is considered the optimized formulation due to:

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Good injectability

Uniform gel form

Proper gelation time

Gel stability at physiological temperature

Homogeneous appearance

The optimized hydrogel formulation showed desirable properties for sustained intra-articular delivery of camphene in osteoarthritis management.

7.3. Discussion

In the current study, an injectable thermosensitive hydrogel loaded with camphene has been successfully formulated by utilizing chitosan and hyaluronic acid polymers cross-linked with β-glycerophosphate. These formulations exhibited adequate physicochemical properties required for intra-articular administration. The thermosensitivity of the hydrogel allowed its quick gelation at body temperature and thus helped improve its residence time at the site of administration. The loading of camphene into the hydrogel matrix has been accomplished successfully without any kind of instability or phase separation in the formulations. FTIR compatibility tests proved that there were no interactions between the drug and excipients, which is why these formulations were stable. The optimized formulation (F2) possessed better injectability and gelling properties than the other formulations. In conclusion, the injectable hydrogel system proposed in this study can be considered as a potential carrier system for the localization of drug delivery in cases of osteoarthritis.

8. CONCLUSION

This investigation was able to explore the anti-osteoarthritic potential of camphene extracted from the natural herbaceous *Cyperus rotundus* by means of computational drug discovery and drug delivery systems. Osteoarthritis is a multi-factorial joint disease involving inflammation, oxidative stress, cartilage damage, and functional impairment of joints. While conventional therapies involving NSAIDs and corticosteroids are often prescribed for managing symptoms, chronic use may result in significant adverse effects and poor disease modification. As part of the computational component of this study, critical inflammatory and degenerative components involved in osteoarthritis pathophysiology, such as COX-2, and MMPs, were determined and used for molecular docking. Camphene showed positive protein-ligand interactions and high

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binding affinity towards osteoarthritis-related targets, signifying its ability to inhibit inflammatory pathways responsible for cartilage damage and joint inflammation. Additionally, the drug-likeness and pharmacokinetic evaluation of the compound showed favorable ADMET properties, indicating that it is a promising candidate for anti-osteoarthritis therapy.

The promising in silico results led to the successful preparation of camphene-loaded hydrogel formulations as a localized drug delivery system. The prepared hydrogel showed adequate physicochemical and pharmaceutical attributes such as pH, viscosity, spreadability, gel strength, homogeneity, extrudability, drug loading, and stability. Furthermore, the *in vitro* release and diffusion properties of the formulation were promising, suggesting that the hydrogel is capable of providing sustained delivery of the drug to the affected joint area. Such a formulation could possibly enhance patient compliance while avoiding the systemic side effects of the anti-inflammatory drugs when taken orally.

In conclusion, the study proves that camphene isolated from *C. rotundus* has significant anti-inflammatory and antioxidant activity, which might prove valuable in the management of osteoarthritis. Combining computational screening methods with the development of topical hydrogels constitutes an economically feasible strategy for the discovery and development of herbal therapeutics. Nonetheless, additional in vivo and clinical pharmacological experiments will be needed to demonstrate the safety, efficacy, and therapeutic potential of the developed camphene hydrogel formulations in osteoarthritis patients.

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Conflict of Interest

The authors declare no conflict of interest.

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