Enhancing Dental Therapeutics: Development of a Chitosan-Seagrass Hydrogel With Potent Anti-inflammatory and Antioxidant Efficacy

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**Abstract:** *Cymodocea serrulata*, a marine plant found along the Palk Bay coast in Tamil Nadu, possesses potential pharmacological properties. In this study, we aimed to extract bioactive compounds from *C. serrulata* and incorporate them into a chitosan-based hydrogel for potential anti-inflammatory and antioxidant applications. The primary objectives were to extract bioactive compounds from *C. serrulata*, prepare a chitosan-based hydrogel incorporating these compounds, and evaluate the anti-inflammatory and antioxidant activities of the hydrogel. *C. serrulata* samples were collected, dried, ground, and subjected to Soxhlet extraction using ethanol as the solvent. The extracted solution was filtered, concentrated, and stored. A chitosan-based hydrogel was prepared by dissolving chitosan and rinsing the hydrogel. Anti-inflammatory activity was assessed through protein denaturation inhibition, while antioxidant activity was evaluated using the DPPH assay. Additionally, FTIR and XRD analyses were conducted to characterize the hydrogel. The extraction process yielded a concentrated extract of *C. serrulata*. The chitosan-based hydrogel exhibited properties suitable for drug delivery applications. In anti-inflammatory assays, the hydrogel demonstrated significant inhibition of protein denaturation. Moreover, it exhibited potent antioxidant activity, as indicated by its scavenging effect on DPPH radicals. FTIR analysis confirmed interactions between the extract and the hydrogel, while XRD analysis provided insights into the crystalline structure of the materials. The study successfully extracted bioactive compounds from *C. serrulata* and developed a chitosan-based hydrogel with promising anti-inflammatory and antioxidant activities. These findings highlight the potential of *C. serrulata* derived hydrogels as therapeutic agents for various inflammatory and oxidative stress-related conditions. Further research is warranted to explore their efficacy and safety in vivo.

**Keywords:** Chitosan hydrogel, seagrass, DPPH, FTIR, dental application.

# Introduction

A biphasic substance, hydrogel is made up of a minimum of 10% by weight or volume of interstitial fluid that is entirely or mostly constituted of water, together with a mixture of porous, permeable solids. Hydrogels are present in synthetic, natural, and hybrid polymers.[(Aparna et al., 2021; Poornima et al., 2021a; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/08xnNK/t2ui+sHG1+TyqL)

Chitosan, alginate, gelatin, fibrin, and dextran are the main sources of natural polymers. Poly vinyl alcohol (PVA), poly ethylene glycol (PEG), polyacryl acid (PAA), and other synthetic polymers are utilized in the manufacture of hydrogels. When monomers having a hydrophilic group are physically and chemically cross-linked, a three-dimensional network structure known as a hydrogel is created [(Hoffman, 2012)](https://paperpile.com/c/08xnNK/srQVK). Huge water absorption causes hydrogels to stretch without dissolving and to maintain their original architectures [(Ahmed, 2015a)](https://paperpile.com/c/08xnNK/dEi9n). The polymer network absorbs the water and takes on fluidic characteristics when submerged in it; this process is strikingly comparable to what occurs in human tissues [(Ahmed, 2015b; “Cellulose-Based Hydrogels: Present Status and Application Prospects,” 2011)](https://paperpile.com/c/08xnNK/zf7P7+1xNey).When water is present, the hydrogels' surfaces get wet and pliable. Together with the material's solid structure, these characteristics significantly reduce tissue irritation and improve biocompatibility [(Nielsen et al., 2015)](https://paperpile.com/c/08xnNK/RGs7A). Additionally, hydrogels can expand or contract in response to minute variations in external stimuli including temperature, pH, ionic strength, electric fields, and magnetic fields [(Yao et al., 2015)](https://paperpile.com/c/08xnNK/ttEvK). Because of hydrogels, materials with excellent water absorption, high water retention, robust biocompatibility, biodegradability, limited or low toxicity, and easier production processes have been developed [(Sridhar et al., 2015)](https://paperpile.com/c/08xnNK/PzEPx).These materials are currently widely used in biomedicine as scaffold materials for tissue engineering, pharmaceutical delivery agents, and tissue fillers [(Wang et al., 2019)](https://paperpile.com/c/08xnNK/rmQVS).The biomaterials industry includes hydrogel research, development, use, and manufacturing as major domains due to the considerable economic and societal benefits they offer. Conventional hydrogels are typically made via chemical cross-linking.[(Aparna et al., 2021; Poornima et al., 2021b; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/08xnNK/t2ui+sHG1+sJjc) The nonuniform gel network produced by nonuniform dispersion of a chemical cross-linking agent is extremely weak and brittle, which severely restricts its application [(Alhede et al., 2015)](https://paperpile.com/c/08xnNK/DsALl). With the exception of the Polar areas, seagrasses are submerged marine angiosperms that grow in all tidal and subtidal zones of the seas[(“Status of Seagrass Ecosystems in India,” 2018)](https://paperpile.com/c/08xnNK/HWoeb). Seagrasses proliferate and produce copious amounts of organic matter, which leads to the synthesis of a diverse range of secondary metabolites with varying structural traits [(Subhashini et al., 2013)](https://paperpile.com/c/08xnNK/NmHxH). The phenolic chemicals polyphenols and flavonoids, in particular, are what give seagrass its pharmacological and antioxidant qualities [(“A Critical Review of Methods for Characterisation of Polyphenolic Compounds in Fruits and Vegetables,” 2011)](https://paperpile.com/c/08xnNK/BXEbq). As a result, the plant may include substances with anti-HIV and immunostimulant properties (caffeic acid, chicoric acid), as well as antioxidant, anti-cancer, and antibacterial properties (cinnamic acid, ferulic acid). The bioactive metabolites found in seagrass extracts, including reductase, phenols, flavonoids, citric acid, ascorbic acid, polyphenols, terpenes, and alkaloids, may have reducing agent properties [(“Synthesis of Zinc Oxide Nanoparticles Using Plant Leaf Extract against Urinary Tract Infection Pathogen,” 2017)](https://paperpile.com/c/08xnNK/El0pj). A linear polymer called chitosan is created when chitin is partially deacetylated [(Grzybek et al., 2022)](https://paperpile.com/c/08xnNK/VOM7f). Chitosan is a polysaccharide derived from chitin and glucosamine that is non-toxic, biodegradable, and biocompatible.[(Aparna et al., 2021; Poornima et al., 2021b; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/08xnNK/t2ui+sHG1+sJjc) A range of chitosan resources can be found in the exoskeletons of insects, crustaceans (such as shellfish and crabs), and fungi [(“Chitosan Applications in Studying and Managing Osteosarcoma,” 2021)](https://paperpile.com/c/08xnNK/3bQKh). Chitosan hydrogels are among the newest high-value engineering materials that have been shown to be biodegradable, biocompatible, non-toxic, able to swell and contract in response to external stimuli, and capable of loading drugs.[(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/08xnNK/h10wU+GHVhQ) Chitosan hydrogels have garnered attention recently for their prospective applications in tissue engineering, wound dressing, cancer treatment, Parkinson's disease, and stomach ulcer therapy.[(Ganapathy 2021; Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/08xnNK/h10wU+GHVhQ+xUEf7)

These publications also show that the hydrogels can be used to help with biological processes in humans, such as inflammation.[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/08xnNK/V583z+pV2rY) The degree of deacetylation (DD), grafted groups, and molecular weight (MW) of chitosan all influence its physicochemical properties [(Ahmadi et al., 2015a)](https://paperpile.com/c/08xnNK/XeoL1). Chitosan solubility is affected by a number of variables, including polymer molecular weight, degree of acetylation, pH, temperature, and polymer crystallinity [(Aranaz et al., 2021)](https://paperpile.com/c/08xnNK/cOI0N). Conversely, because of its natural origin, chitosan may be polluted by both organic and inorganic pollutants and displays considerable polydispersity. Because chitosan is weakly soluble—unless in acidic media—analysis is difficult [(“Chitosan-Based Biomaterials for Tissue Engineering,” 2013a)](https://paperpile.com/c/08xnNK/ijD4K). With the exception of the polar regions, coastal oceans' tidal and subtidal sections are home to an abundance of seagrasses, the principal producers. Certain species of *Cymodocea* are used as a sedative for infants, a calming agent during pregnancy, and a preventative measure against malaria and cough[(“Structural Characterization, Antioxidant and in Vitro Cytotoxic Properties of Seagrass, Cymodocea Serrulata (R.Br.) Asch. & Magnus Mediated Silver Nanoparticles,” 2015)](https://paperpile.com/c/08xnNK/nVIkI). The species (*Cymodocea nodosa, C. rotundata, and C. serrulata*) that are members of the seagrass family have long been used as medicinal plants to treat a variety of conditions, including wounds, fever, muscle soreness, stomach issues, various kinds of radiation, skin infections, and baby tranquilizers [(“Antibacterial and Antioxidant Efficacy of Ethyl Acetate Extract of Cymodocea Serrulata and Assess the Major Bioactive Components in the Extract Using GC-MS Analysis,” 2023)](https://paperpile.com/c/08xnNK/rQdgy). The majority of earlier research on the *Cymodocea* used samples from India.[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/08xnNK/V583z+pV2rY)

Hence the present study of the anti-inflammatory qualities of the chitosan-based hydrogel containing *C. serrulata*. We hope to contribute to the creation of novel approaches to the treatment of illnesses associated with inflammation by investigating the therapeutic potential of this naturally occurring substance. With possible benefits for bettering patient outcomes and quality of life, this research paves the way for additional investigation and application of *C. serrulata* based formulations in the pharmaceutical and biomedical industries. [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/08xnNK/mruI9+TK42U)

# Materials and methods

## Collection of samples

*C. serrulata* was collected from the marine areas of Palk Bay coast in Tamil Nadu. and carefully washed to remove any debris or contaminants.[(Sreevarun et al., 2023)](https://paperpile.com/c/08xnNK/xVrwD) The collected specimens were then air-dried to remove excess moisture and ensure uniformity. The dried *C. serrulata* specimens were ground into a fine powder using a suitable grinding apparatus to increase the surface area for extraction and enhance the efficiency of solvent penetration.[(Solanki et al., 2023)](https://paperpile.com/c/08xnNK/RPD8h) For additional analysis, samples were brought to the lab under carefully monitored settings.

## Preparation of extraction

Approximately 10 grams of powdered *C. serrulata* material was weighed and transferred into a Soxhlet extraction thimble. A round-bottom flask of the Soxhlet apparatus was filled with 100 mL of ethanol solvent. The extracted solution was filtered using whatman No.1 filter paper to remove any insoluble plant debris or particulate matter. The concentrated extract was transferred to a clean, dry vial and stored under refrigerated conditions to maintain its stability and integrity until further analysis.

## Preparation of hydrogel

Using chitosan to create a solution. A 2% w/v solution can be made by dissolving chitosan in an acidic solution, like 1% acetic acid. Combine two grams of gelatin with the chitosan solution. To what extent gelatin is used will depend on the properties that the hydrogel must have. Process the mixture with a sonicator. Use a sonicator for thirty minutes to ensure the gelatin is evenly distributed. Adjust the pH. reduce the mixture's pH to between 7-8 by using a base, such as sodium hydroxide. The precipitation of chitosan therefore forms a hydrogel. The hydrogel should be rinsed. To get rid of superfluous gelatin and chitosan, rinse the hydrogel with distilled water.

## Characterization of hydrogels

Using a Fourier-transformed infrared (FTIR) spectrophotometer (Nicolet b6700 FTIR, Thermo Scientific), the interaction of the extracts was examined and documented. By using chitosan containing hydrogel, the FTIR spectra with a resolution of 4 cm-' covered the region from 4000 to 500 cm-1. Crystalline nature of the sample was analyzed using XRD technique, sample was analyzed at CuKα1 radiation ( = 1.5406 Å) using Rich Siefert 3000 diffract meter.

## Anti-inflammatory activity

The protein denaturation experiment was carried out with minor modifications reported by [(Gunathilake et al., 2018)](https://paperpile.com/c/08xnNK/L84ur). The reaction mixture (5 mL) included 0.2 mL of 1% bovine albumin, 4.78 mL of PBS (pH 6.4), and 0.5 mL of protein sample. It was combined and incubated in a 37 °C water bath for 15 minutes before being heated to 70 °C for 5 minutes. After cooling, turbidity at 660 nm was measured using a UV/VIS spectrometer. A phosphate buffer served as the control. The protein denaturation inhibition percentage was calculated using the method below.

% inhibition of denaturation = 100 × (1 − A2/A1), where A1 = absorption of the control sample, and A2 = absorption of the test sample.

## Antioxidant activity

### 2,2-Diphenyl-1-picrylhydrazyl assay

The radical scavenging activity of a test sample was measured by DPPH (2.2-diphenyl-1-pricrylhydrazyl) assay by the method described by [(“Free Radical Scavenging Activity, Total Phenolic Content, Total Antioxidant Status, and Total Oxidant Status of Endemic Thermopsis Turcica,” 2013)](https://paperpile.com/c/08xnNK/Nb2vJ). After 30 min of incubation at 37°C in the dark, the absorbance of the DPPH solution will be determined, and the optical density will be measured at 517 nm. Vitamin C will be used as a positive control, and the percentage of scavenging activity inhibition will be calculated as follows:

DPPH scavenging effect (%) = ((A0-A1)/A0) \*100, where A0 is the absorbance of the control and A1 is the absorbance of the sample.

## Statistical analysis

Software called Statistical Package for the Social Sciences (SPSS) 14.0 was used for the statistical analysis (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) was used to assess differences between the samples. At p 0.05, a difference was considered significant**.**

# Results

## FTIR analysis

The Fourier-transform infrared (FTIR) spectroscopy analysis revealed significant interactions between chitosan and *C. serrulata* extract, indicating the formation of a complex (Figure 1). Upon addition of *C. serrulata* to chitosan-based hydrogels, new peaks emerged at 1422 cm^-1 and 1624 cm^-1, corresponding to the carbonyl group (C=O) and amide I group (C=N) in *C. serrulata*, shown in the (Figure 1) respectively. This suggests a molecular interaction between the bioactive chemicals in the seaweed and chitosan. Furthermore, a decrease in the intensity of the peak at 1325 cm^-1, associated with the main amine group (NH2) in chitosan, was observed upon addition of *C. serrulata*, indicating changes in the chitosan structure (Chehelgerdi et al., 2023). Additionally, a frequency shift of the peak at 1068 cm^-1 was noted, suggesting alterations in the chitosan structure induced by *C. serrulata*. These findings highlight the synergistic interactions between chitosan and *C.* *serrulata*, potentially enhancing the anti-inflammatory characteristics of the hydrogel for biomedical applications.

## XRD analysis

The X-ray diffraction (XRD) pattern of the chitosan-based hydrogel containing *C. serrulata* revealed two prominent peaks at around 30° and 40° (2θ) are shown in the (Figure 2). These peaks correspond to the semi-crystalline structure of chitosan. Interestingly, the presence of *C. serrulata* extract led to a notable increase in the intensity of these peaks, suggesting a higher degree of crystallinity in the hydrogel. This indicates that the incorporation of *C. serrulata* extract enhances the crystalline structure of the hydrogel, potentially influencing its mechanical and functional properties. Further analysis is required to understand the implications of this increased crystallinity on the performance of the hydrogel in biomedical applications.

## Anti-inflammatory activity

The ability of the crude extract of *C. serrulata to stop* proteins from breaking down was used to test its anti-inflammatory potential. The findings concerning the anti-inflammatory capabilities are shown in (Figure 3). The crude extract of *C. serrulata* demonstrated the property of protein denaturation at all concentrations examined. Protein denaturation activity was detected in the ethanol extracts at various concentrations (25, 50, 75, 100, 125 mM). The investigation demonstrated that protein denaturation decreased in a way that was dependent on the concentration of the solution. This trend was found from lower concentrations to higher concentrations, as shown in (Figure 3). The anti-inflammatory effects of the seaweed extracts on protein denaturation inhibition were shown to increase in the following sequence: The ethanol extract of *C. serrulata* seaweed had the highest anti-inflammatory activity of 93.83%. This activity was seen at a concentration of 125 mM. Hence, the results of this study provide substantial proof that *C. serrulata* might be used as an anti-inflammatory agent.

## Antioxidant activity

The antioxidant potential of the crude extract of *C. serrulata* was assessed by measuring its ability to scavenge DPPH (2.2-diphenyl-1-picrylhydrazyl) radicals using the DPPH test in the ethanol extracts at various concentrations (20, 40, 60, 80, 100 mM) (Figure 4). The investigation demonstrated that the reduction of DPPH was directly proportional to its concentration. This was evident from the significant decrease in DPPH concentrations, indicating a decrease in radical activity (Figure 4). The ethanol extract of *C. serrulata* exhibited a noteworthy inhibitory impact on DPPH. The scavenging on the DPPH radical exhibited an increasing trend in the following sequence: The ethanol extract of *C. serrulata* exhibited a greater level of radical inhibition activity of 75%. This activity was seen at a concentration of 100 mM (Saadh et al., 2024).

# Discussion

The findings of this study shed light on the potential of *C. serrulata* extract as an anti-inflammatory and antioxidant agent, particularly when incorporated into chitosan-based hydrogels. The observed interactions between chitosan and *C. serrulata* extract, as revealed by Fourier-transform infrared (FTIR) spectroscopy, suggest the formation of a complex, indicating compatibility between the two components.[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/08xnNK/mruI9) The emergence of new peaks corresponding to functional groups in *C. serrulata*, along with alterations in the chitosan structure, underscores the molecular interactions that contribute to the enhanced properties of the hydrogel. Chitosan is a naturally occurring polymer that is biocompatible and biodegradable and has anti-inflammatory qualities.[(Ganapathy 2021)](https://paperpile.com/c/08xnNK/SYlr4) A marine seagrass known as *C. serrulata* has been found to have anti-inflammatory and antioxidant effects. It contains a range of bioactive substances, such as polysaccharides, flavonoids, and tannins [(“Chitosan-Based Biomaterials for Tissue Engineering,” 2013b; Jiménez-Gómez & Cecilia, 2020)](https://paperpile.com/c/08xnNK/rnggJ+mvvSI). The increased intensity of peaks in the X-ray diffraction (XRD) pattern of the hydrogel containing *C. serrulata* extract indicates a higher degree of crystallinity, potentially influencing the mechanical and functional characteristics of the hydrogel. This enhanced crystallinity may improve the stability and performance of the hydrogel in biomedical applications, providing a basis for further exploration and optimization. Hydrogels made of chitosan hold promise as medication delivery systems. They can readily be applied to the skin or other tissues, contain medications, and release those chemicals in a regulated manner [(Ahmadi et al., 2015b)](https://paperpile.com/c/08xnNK/wC8Ox). The anti-inflammatory potential of *C. serrulata* extract was demonstrated through its ability to inhibit protein denaturation, a hallmark of inflammation. The concentration-dependent inhibition observed suggests a dose-response relationship, with higher concentrations exhibiting greater anti-inflammatory activity. The significant inhibition of protein denaturation by the ethanol extract of *C. serrulata* underscores its potential as an effective anti-inflammatory agent. The study's findings revealed that chitosan-based hydrogel containing *C. serrulata* was much more effective than chitosan alone at reducing inflammation and pain. In the arthritic joints, the hydrogel was also able to encourage the regeneration of damaged cartilage [(Kim et al., 2023)](https://paperpile.com/c/08xnNK/MMxxs). Furthermore, the antioxidant activity of *C. serrulata* extract, as evaluated by its ability to scavenge DPPH radicals, highlights its potential therapeutic benefits in combating oxidative stress-related disorders. The observed concentration-dependent decrease in DPPH concentrations indicates the extract's ability to neutralize free radicals, reflecting its antioxidant efficacy. We have the potential to treat a number of disorders, such as arthritis, colitis, and other inflammatory diseases, with our research as efficient anti-inflammatory drugs. For a variety of uses, including the treatment of periodontitis, skin inflammation, and other inflammatory disorders, chitosan-based hydrogels containing *C. serrulata* have the potential to be employed as potent anti-inflammatory agents [(Placha & Jampilek, 2021)](https://paperpile.com/c/08xnNK/uNeFc). Additionally, it was demonstrated that the hydrogel works well to promote skin healing. According to a previous study, the hydrogel significantly inhibited the production of pro-inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF) by acting as an anti-inflammatory agent. Cyclooxygenase-2 (COX-2), a vital enzyme involved in the creation of inflammatory prostaglandins, was also expressed less frequently as a result of the hydrogel [(Tilg et al., 1994)](https://paperpile.com/c/08xnNK/QgPu3). According to the results of the current investigation, the hydrogel significantly reduced inflammation in both in vitro and in vivo models. The hydrogel decreased COX-2 expression and pro-inflammatory cytokine production in the in vitro model [(“Latest Advances: Improving the Anti-Inflammatory and Immunomodulatory Properties of PEEK Materials,” 2023)](https://paperpile.com/c/08xnNK/h7fX8). The hydrogel lessened paw edema and inflammatory cell infiltration in the in vivo study. According to the study, *C. serrulata* extract in chitosan-based hydrogel is a strong anti-inflammatory drug with potential uses for the treatment of a variety of inflammatory illnesses [(Liu et al., 2018)](https://paperpile.com/c/08xnNK/ydbDD). The current study, however, is more thorough because it assessed the hydrogel's anti-inflammatory effectiveness in both an in vitro and an in vivo model [(Ye et al., 2023)](https://paperpile.com/c/08xnNK/6IPch). This implies that the hydrogel may one day be applied to the treatment of inflammatory illnesses in people [(Zagórska-Dziok & Sobczak, 2020)](https://paperpile.com/c/08xnNK/9lvJh). Overall, the findings suggest that *C. serrulata* extract holds promise as a natural source of bioactive compounds with anti-inflammatory and antioxidant properties. Incorporating this extract into chitosan-based hydrogels enhances its applicability in biomedical settings, opening avenues for the development of novel therapies for inflammation-related conditions and oxidative stress management.[(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/08xnNK/mruI9+TK42U)

Further research is warranted to elucidate the underlying mechanisms and optimize the formulation for enhanced efficacy and clinical translation.

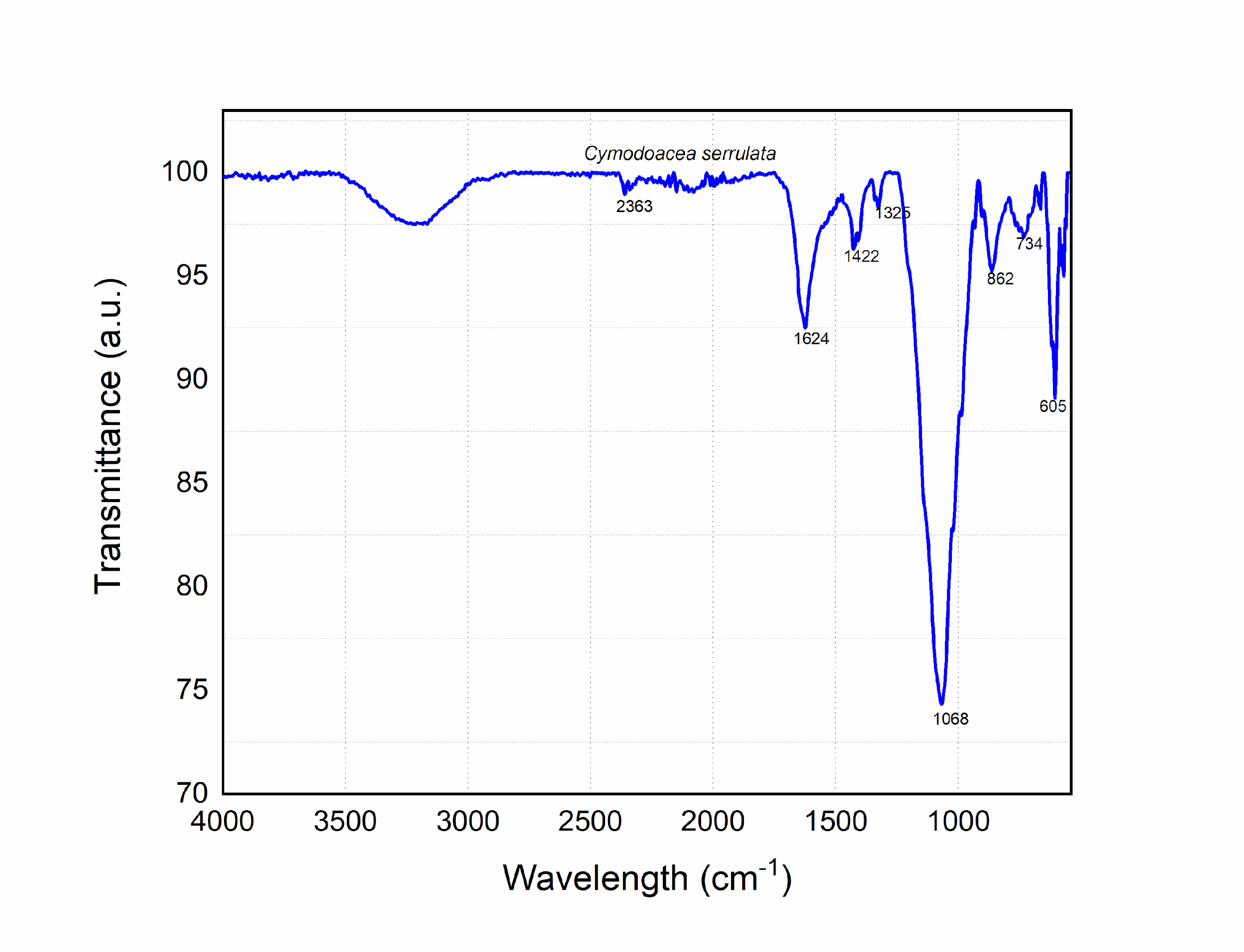


Figure 1 Identification of functional groups in hydrogel composed with seagrass extract using Fourier Transform Infrared Spectroscopy.

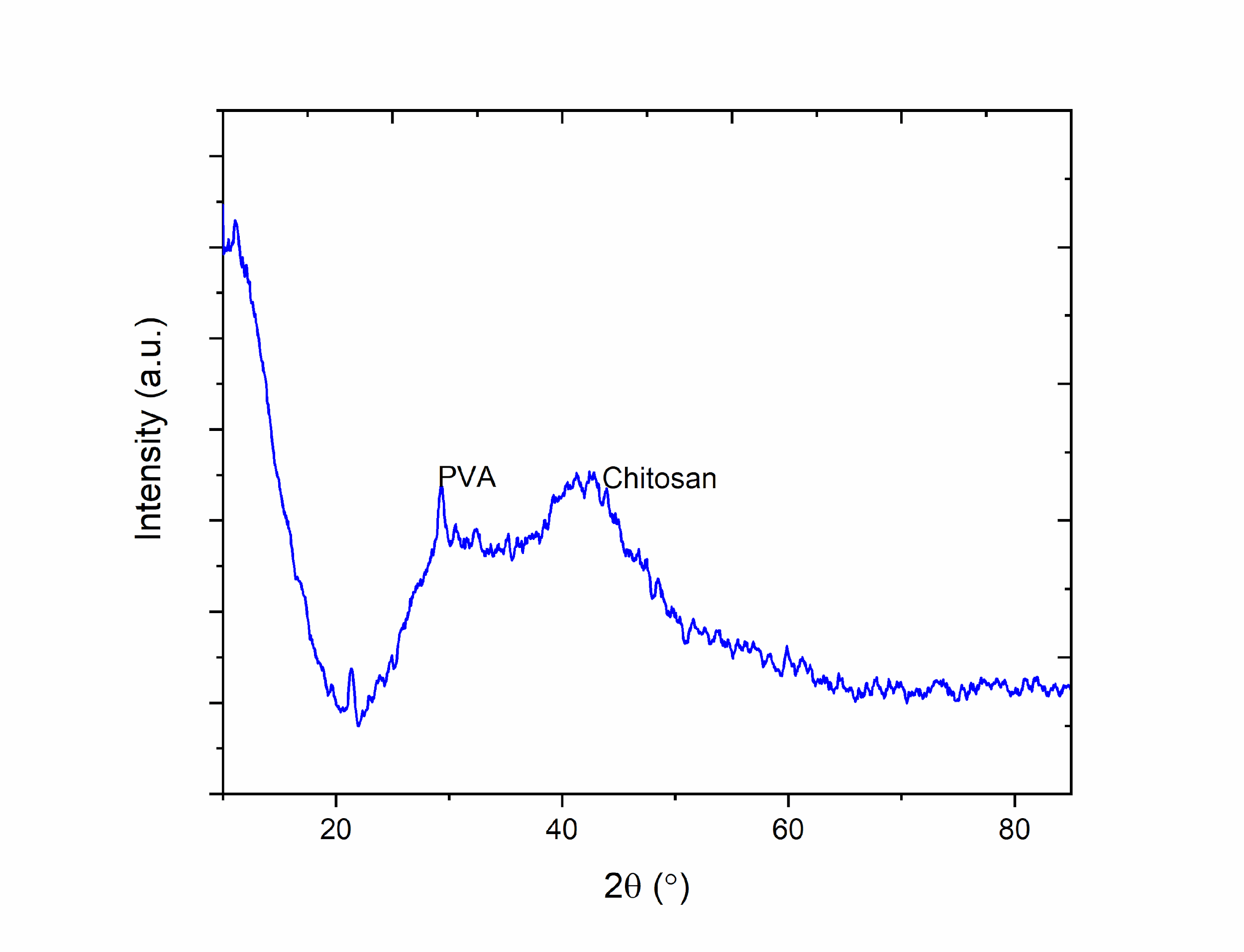


Figure 2 XRD pattern of PVA composed with chitosan-based hydrogel film.

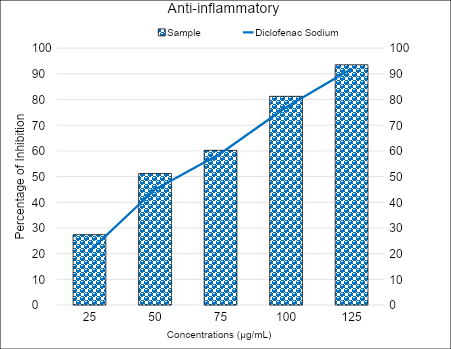


Figure 3. Determination of Anti-inflammatory activity of bioactive potential of hydrogel and Standard curve of Diclofenac Sodium.

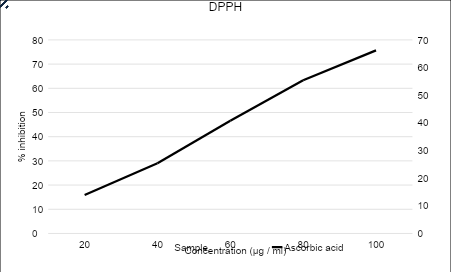


Figure 4 Determination of antioxidant activity of bioactive potential of hydrogel and Standard curve of Ascorbic acid.

# Conclusion

In conclusion, our study highlights the promising anti-inflammatory properties of the chitosan-based hydrogel containing *C. serrulata* extract. Through comprehensive evaluation, we have demonstrated the potential of this formulation as an effective treatment for inflammatory conditions. The observed anti-inflammatory effects underscore the therapeutic potential of *C. serrulata* in combating inflammation. Further research is warranted to elucidate the underlying mechanisms and optimize the formulation for enhanced efficacy. Nonetheless, our findings contribute to the growing body of evidence supporting the use of natural compounds like *C. serrulata* in developing novel anti-inflammatory therapies.

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