Synthesis of Alginate Based Hydrogel Containing Cymodoacea Rotundata and its Applications in Dentistry

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**Abstract:** The present study focuses on synthesizing and characterizing alginate-based hydrogels containing *Cymodocea rotundata* and their potential applications in dentistry. The primary objective was to systematically collect and incorporate *Cymodocea rotundata* extract into a sodium alginate hydrogel and analyze its physicochemical and bioactive properties. The hydrogel was synthesized using standardized protocols and evaluated through Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) to assess its molecular structure and crystallinity. FTIR analysis confirmed the presence of characteristic hydroxyl stretching bands at 3347 cm⁻¹, indicative of the biopolymer network. XRD results revealed the hydrogel's predominantly amorphous nature (60.3%) with a crystalline component (39.7%), contributing to its high water retention and flexibility. Antioxidant potential was assessed using DPPH and ABTS assays, with the hydrogel exhibiting 72.5% inhibition concentration (DPPH) and ABTS IC50 values ranging from 62.32% to 68.5%. The results confirm the hydrogel's biocompatibility, hydrophilic nature, and drug-carrying potential, making it highly suitable for dental applications such as wound healing, drug delivery, and tissue regeneration. This study highlights the promising role of alginate-based hydrogels in advanced dental therapies, promoting sustainable and bioactive biomaterials for clinical use.

**Keywords:**  Alginate Hydrogel; Cymodocea rotundata; Biocompatibility; Antioxidant Activity; Dental Applications.

# INTRODUCTION

Hydrogels are three-dimensional polymeric networks capable of absorbing and retaining large amounts of water while maintaining their structural integrity [(Yunus, 2012)](https://paperpile.com/c/YIJ50G/o6cj). Due to their high water content, biocompatibility, and ability to mimic natural tissues, hydrogels have gained significant attention in biomedical and dental applications.[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/YIJ50G/LBB9o+DRypa+vUasN) Among the various hydrogels, alginate-based hydrogels have been widely studied due to their natural origin, non-toxicity, biodegradability, and excellent gelling properties.[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/YIJ50G/LBB9o+DRypa+vUasN) Sodium alginate, a naturally occurring polysaccharide extracted from brown algae, forms hydrogels through ionic crosslinking with divalent cations like calcium, making it suitable for a variety of biomedical applications.[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/YIJ50G/LBB9o+DRypa+vUasN)

The incorporation of bioactive compounds into alginate hydrogels has been a growing area of research aimed at enhancing their functional properties. One such bioactive marine resource is Cymodocea rotundata, a seagrass known for its diverse phytochemical composition, including phenolics, flavonoids, and antioxidants. These bioactive compounds exhibit therapeutic properties such as antimicrobial, anti-inflammatory, and antioxidant activities, making Cymodocea rotundata a valuable additive in hydrogel formulations. In dentistry, biomaterials play a crucial role in regenerative therapies, wound healing, and drug delivery. Alginate-based hydrogels infused with Cymodocea rotundata can serve as potential materials for dental applications by promoting tissue regeneration, reducing oxidative stress, and enhancing antimicrobial resistance.

The physicochemical characterization of hydrogels is essential to determine their suitability for biomedical applications. Techniques such as Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) provide valuable insights into the molecular interactions, chemical bonding, and structural properties of hydrogels. Antioxidant assays such as DPPH and ABTS evaluate the free radical scavenging potential, which is critical for applications in tissue engineering and wound healing. This study aims to develop a novel biomaterial by integrating the benefits of sodium alginate and Cymodocea rotundata, providing a promising approach for dental and biomedical applications.

Hydrogel refers to a network of hydrophilic (water-attracting) polymer chains that are capable of holding a large amount of water within their structure. It is a versatile material with a wide range of applications in various fields, including medicine, biotechnology, and materials science. The structure of hydrogels allows them to absorb and retain significant amounts of water or biological fluids while maintaining their shape. This property makes them useful in many applications, such as wound dressings, drug delivery systems, tissue engineering, contact lenses, and even soft robotics. Hydrogels can be prepared from natural and synthetic polymers. Natural hydrogels can be derived from materials like collagen, alginate, hyaluronic acid, or chitosan, while synthetic hydrogels can be created using polymers like polyacrylamide, polyethylene glycol, or polyvinyl, this research has been conducted on a type of seagrass known as cymodocea rotundata in combination with hydrogel

Seagrass is the only marine angiosperm ( flowering plant) which can exist underwater, it can proliferate extensively in the water bodies of tropical and temperate regions, although it does not thrive in colder regions, such as the antarctic and arctic regions.

Cymodocea rotundata is commonly known as smooth ribbon sea grass, it reproduces through both sexual and asexual methods. It is found in locations subject to the effects of coastal development and strong anthropogenic activity because it frequently creates a fringing bed. dangers tend to be limited and there are no significant dangers. When broken down into homopolymeric (MM- or GG-blocks) and heteropolymeric (MG- or GM-blocks) sequences (1-4)-d-mannuronic acid (M) and (1-4)-a-l-guluronic acid (G) units create alginate, a water-soluble linear polymer derived from brown algae. Alginate is a biomaterial of particular interest for a wide range of applications, and especially as the supporting matrix or delivery system for tissue repair and regeneration, drug delivery, and wound dressing This is because of its exceptional properties in terms of biocompatibility, biodegradability, non-antigenicity, and chelating ability.[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/YIJ50G/nWeOa) Additionally, it was discovered that sodium alginate hydrogels had the highest bio adhesive property in vitro and in drug delivery.[(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/YIJ50G/A91Ax+1eS86) Blood coagulation, inflammation, proliferation, and remodeling are all components of the intricate biological process that is wound healing [(“Preparation and Controlled Degradation of Oxidized Sodium Alginate Hydrogel,” 2009)](https://paperpile.com/c/YIJ50G/G6LZ) Applications of hydrogel include its use in surgical sealants, drug delivery systems

It also has a wide variety of applications in dentistry - Treatment for periodontitis that improves periodontal regeneration by injecting a hydrogel that consists of medications with anti-inflammatory and tissue- regenerating capabilities [(Shirbhate & Bajaj, 2022)](https://paperpile.com/c/YIJ50G/FZZW) hyaluronic acid hydrogels can be used in dental pulp stem cells [(Ahmadian et al., 2019)](https://paperpile.com/c/YIJ50G/4uYL) etc. this research is being conducted to assess the wound healing properties of hydrogel in combination with cymodoacea rotundata by studying its physico Chemical properties using Fourier-transform infrared spectroscopy (FTIR), x-ray powder diffraction (XRD), and investigates their antioxidant properties using ABTS and DPPH assay.

# MATERIALS AND METHODS

## Preparation of extract

C. rotundata was collected from Palk Bay coast in Tamil Nadu, India (9o 44'05.99"N, 79o 01' 04.10"E), the seagrass species was obtained by hand and thoroughly rinsed in sea water to eradicate the debris, sand particles and other growing epiphytes. The sea grass was further transported by placing it in a casket filled with ice, it was washed thoroughly to further eliminate the excess salt present on the surface, it was placed on blotting paper to absorb the excess water, and the powdered seagrass sample ( 10 g ) was extracted using 100 % methanol and the crude extract was filtered using whatman no1 filter paper.

## Preparation of sodium alginate hydrogel

### Fourier transformed infrared spectrum

The interaction between the extract was analysed and recorded by Fourier transformed infrared ( FTIR ) ( brooker FTIR ) spectrophotometer, the FTIR spectrum ranges from 4000 to 500 cm−1, at a resolution of 4 cm−1

( Or )

​​Fourier transform infrared spectroscopy ( FTIR ) :

Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) is a powerful technique to determine any possible chemical interaction. It was used to assess the functional groups present upon addition of cymodoacea routndata. ATR-FTIR spectroscopic analysis was performed using Bruker ATR infrared spectrometer (model) which ranges between 400 – 4000 cm-1. The expected pendant functionalities of scaffolds were confirmed by the FT-IR spectrum.

### X RAY diffraction analysis

A nondestructive method known as X-ray diffraction analysis (XRD) can provide precise information on a material's crystallographic structure, chemical makeup, and physical characteristics. The dried mixture of hydrogel was collected for the determination of formation of structural characterisation by an X’Pert Pro x-ray diffractometer (PANalytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA along with Cu Kα radiation in a configuration

## Antioxidant property

### 2,2-Diphenyl-1-picrylhydrazyl assay ( DPPH )

Using the Shimada et al.18 approach, the radical scavenging activity of a test sample was measured using the DPPH (2.2-diphenyl-1-pricrylhydrazyl) assay. The optical density was measured at 517 nm after 30 min of incubation at 37 °C in the dark. The absorbance of the DPPH solution was also evaluated. The percentage of scavenging activity inhibition was evaluated using vitamin C as a positive control. It was calculated using the following equation:

DPPH scavenging effect(%)= (A0-A1/A0) ×100

Where A° stands for absorbance of control and A1 stands for absorbance of sample, and A1 is the absorbance of sample.

### ABTS ASSAY ( 2,2’-Azino-bis-3- ethylbenzothiazoline-6-sulfonic acid)

The procedure described by Giao et al. was used to carry out the 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay.

The inhibitory percentage and the positive control used for ascorbic acid were computed using the equation below:

I = Ao A1 /Ao 100

where A0 is the absorbance of the control reaction and A1 is the absorbance of the standard reaction.

# STATISTICAL ANALYSIS

Software called Statistical Package for the Social Sciences (SPSS) 16.0 (SPSS Inc., Chicago, USA) was used for the statistical analysis. Analysis of variance (ANOVA) and Duncan's multiple comparison methodology were used to assess differences between the samples.

At p 0.05, a substantial difference was considered to exist.

# RESULTS

## FTIR ANALYSIS

Figure 1. Depicts the Identification of functional groups in hydrogel composed with seagrass extract using Fourier Transform Infrared Spectroscopy. The characteristic spectrum of the Na-alginate biopolymer is composed of a broad band centered at approximately 3500 cm-1( 3347) that arises from the stretching of hydroxyl groups. The peak at 1657 depicts the C=C bond stretching vibrations, 3347 ascribes the N-H stretch, 3324 depicts the ≡C - H stretch, 2921 and 2859 ascribed the -C-H stretch, 2359 represents C≡Nstretch, 1657 and 1605 represents the C=C alkene stretch, 1453 ascribes the CH3 bend.

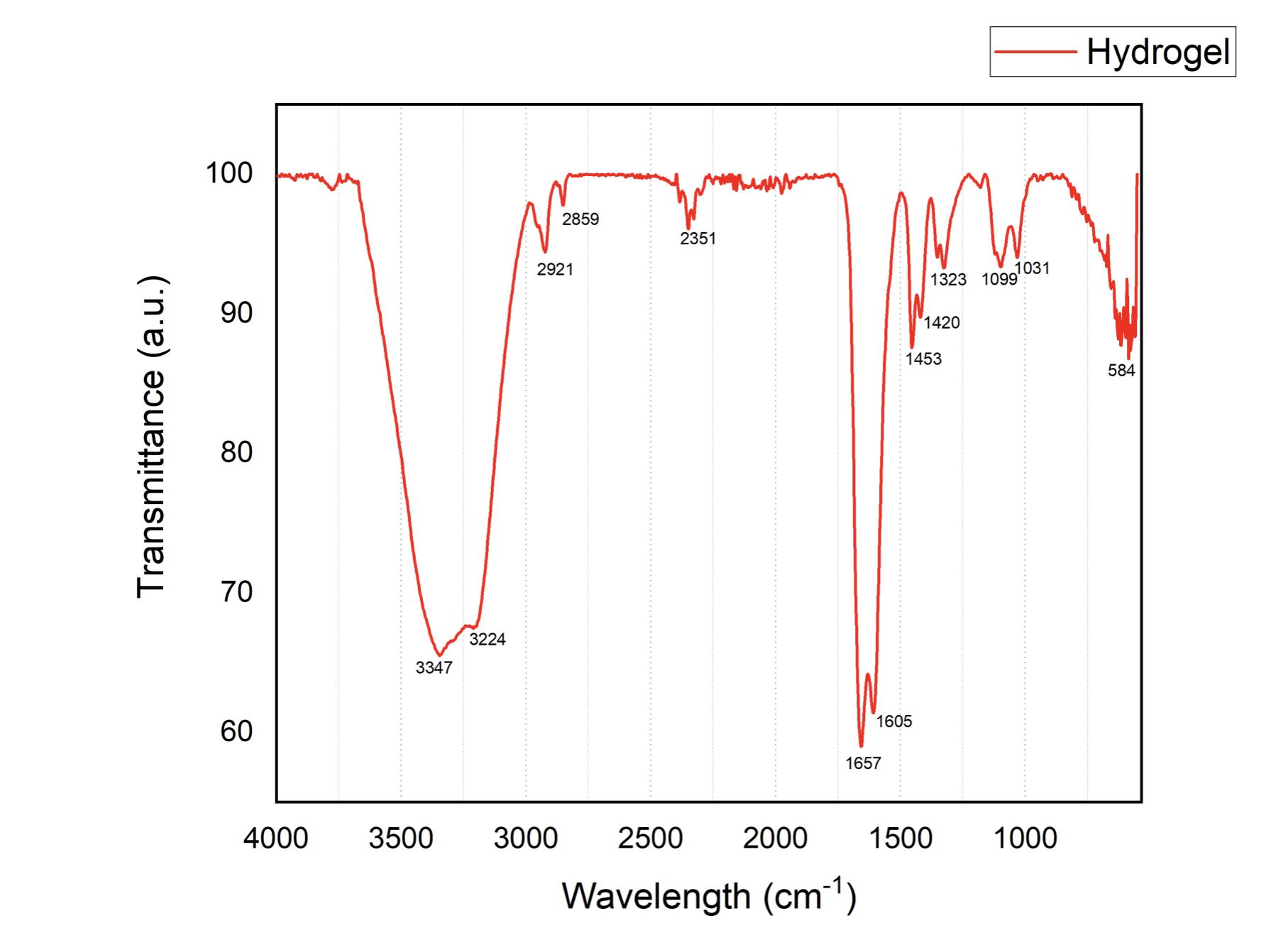
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Figure 1: wavelength vs. Transmittance

## XRD ANALYSIS

XRD analysis was performed to assess whether the hydrogel consists of sodium alginate. Reflections from the 2-theta region. It was identified to be 60.3% amorphous and 39.7% crystalline. The hydrogel is observed to have more number of peaks as it is predominantly amorphous. Additional peaks were also observed at 21° ( PVA ) and at 32° ( alginate ).

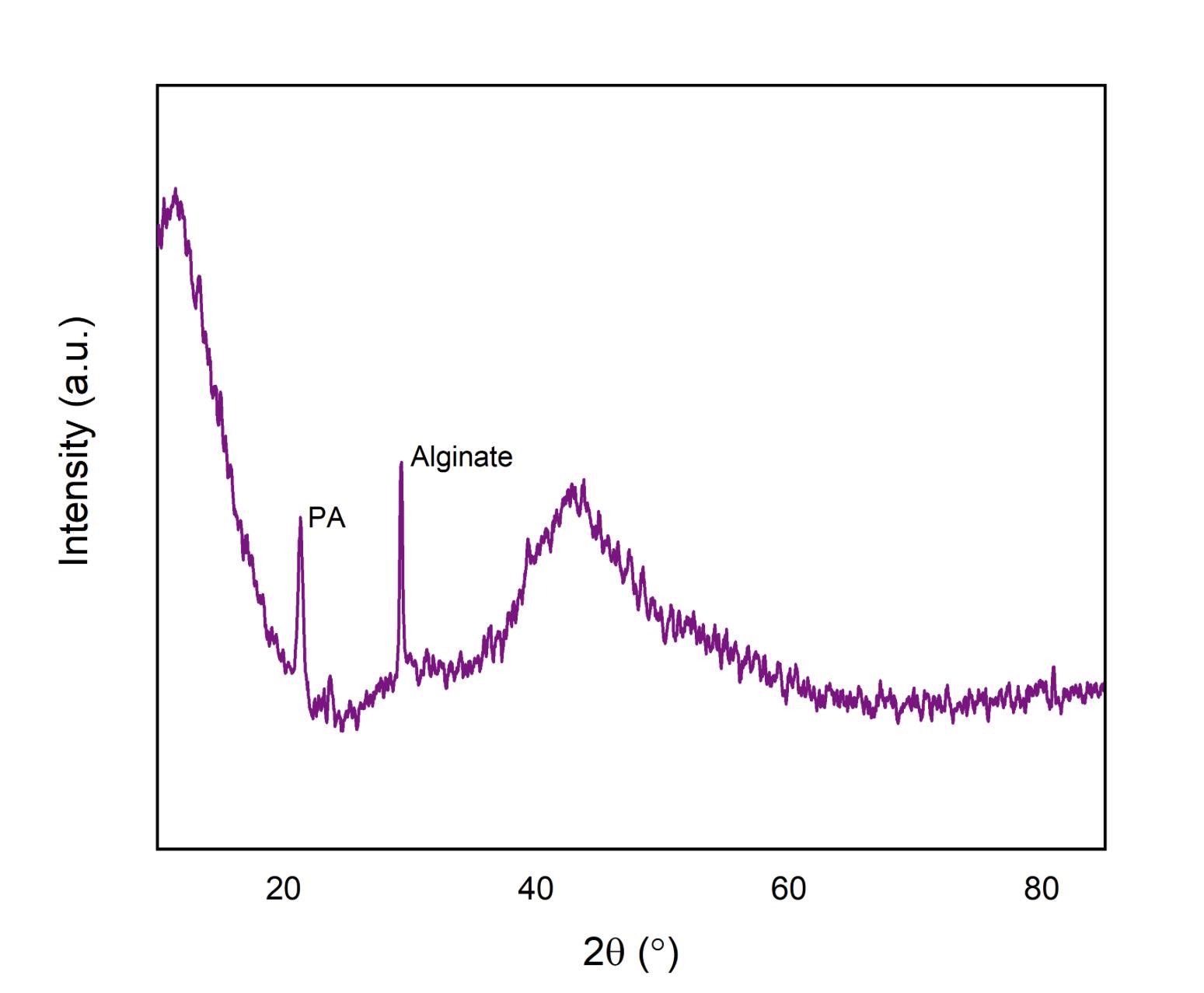


Figure 2. Represents the XRD pattern of Alginate based hydrogel film.

## ANTIOXIDANT ASSAY

## DPPH ASSAY

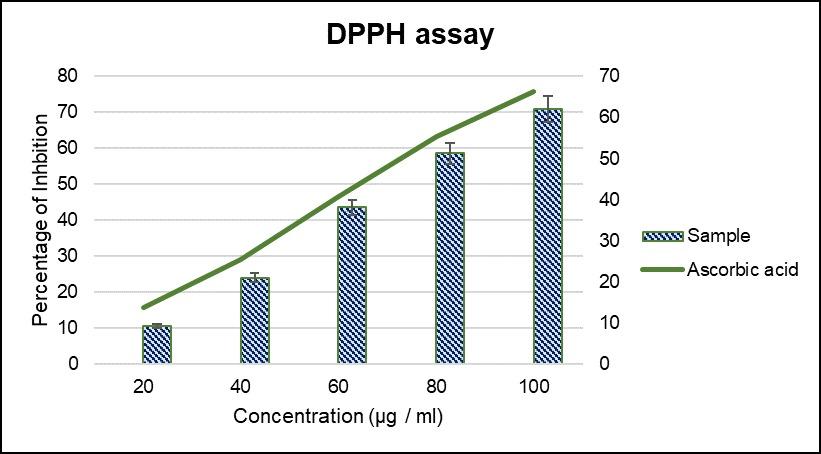
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Figure 3. Represents the DPPH assay

​​An antioxidant is a substrate that stops molecules inside of a cell from oxidizing. It is a common chemical procedure that enables the removal of hydrogen or electrons from a material. The biological oxidation process results in the production of free radicals. radical-scavenging capacity of the hydrogels was assessed using the DPPH assay. All gel formulations were able to reduce the DPPH radical, confirming their antioxidant capacity, This assay is widely used to determine [antioxidant activity](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/antioxidant-activity) of crude extracts or purified compounds from plants. There exists a linear increase in percentage of inhibition as the concentration increases. The IC50 value determines the inhibition concentration of a substance, in which 50% inhibits. The inhibition concentration of sodium alginate hydrogel was found to be 72.5%

## ABTS ASSAY

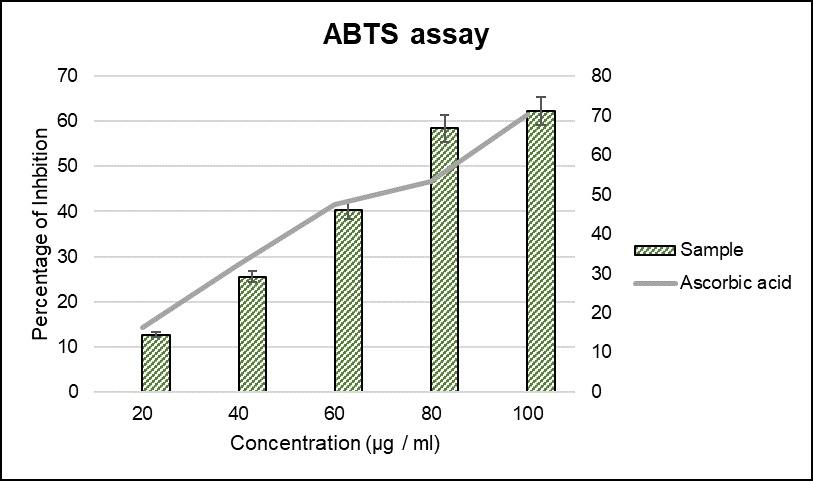
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Figure 4. Represents the ABTS assay of hydrogel sample

Antioxidant ability is dosage-dependent, and the ABTS radical scavenging assay was measured to have a minimum ( IC50) value of 68.5% and the maximum was recorded to be 62.32% . These are essentially high quantities of biologically active biomolecules, such as protein, phenols, and alkaloids, which supply hydrogen atoms to chelate the chain of free radicals and boost antioxidant action.

# DISCUSSION

A hydrogel is a network of hydrophilic polymers,it has a three dimensional (3D) structure which accounts for its various physical properties.[(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/YIJ50G/t6uhC+cWhZ7). Due to the cross-linking ability of its individual polymer chains, it can hold large amounts of water, it is structurally and dimensionally stable. [(Bustamante-Torres et al., 2021)](https://paperpile.com/c/YIJ50G/lQVs) Additionally, hydrogels are biocompatible and biodegradable (Chehelgerdi et al., 2023). They are among the most promising medicinal materials for the future and are widely used in biomedical disciplines like medication release, medical dressing, gum tissue regeneration, bone repair, etc. it has a wide variety of applications in dentistry as well. ( ( hydrogel - a versatile material in dentistry, Volume 06, Issue 01, 2019 ), In 1960, polyhydroxyethylmethacrylate (pHEMA) hydrogel, which was manufactured much later with an optimistic objective of using them in permanent contact applications with human tissues, Hydrogels are actually the first materials developed for uses inside the patient.[(Ganapathy , 2021)](https://paperpile.com/c/YIJ50G/zPpmc). Hydrogels can be classified based on many properties, such as its origin i.e whether its natural, a synthetic polymer or a combined polymer which has a mix of both.[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/YIJ50G/GqjFD+fobBs)

natural sources include hyaluronic acid, chitosan, starch, dextran, fibrin, collagen, gelatine.[(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/YIJ50G/FoGki+t6uhC) Synthetic derivatives include poly (vinyl alcohol) (PVA), poly (ethylene glycol) (PEG), poly (ethylene oxide) (PEO), poly (2-hydroxyethyl methacrylate) (PHEMA), poly (acrylic acid) (PAA), and poly (acrylamide) (PAAm), [(Bustamante-Torres et al., 2021)](https://paperpile.com/c/YIJ50G/lQVs) it can also be classified based on the type of crosslinking i.e it can either be physical i.e through physical agents such as irradiation ( gamma rays or x rays ) , sulfur vulcanization, hydrogen bonding, hydrophobic interaction, ionic interaction, crystallization, stereoselective, protein interaction and hydrogen bond.[(Wadhwani et al., 2022)](https://paperpile.com/c/YIJ50G/hYNgX) Physical crosslinking does not have permanent effects, but they can make sure the hydrogel remains insoluble in aqueous media, it produces reversible hydrogels.[(Sreevarun et al., 2023)](https://paperpile.com/c/YIJ50G/peGEY) The other type of cross linking is chemical crosslinking i.e creating chemical or permanent hydrogels by covalent crosslinking of polymers through adding different agents to alter the structure of the polymer. it can be classified based on structural configuration i.e whether it is amorphous, crystalline or semi crystalline. Cross linking can add different properties to the hydrogel such as increase in elasticity, decrease in viscosity. The hydrogels' antioxidant capacities were analyzed by using the DPPH and ABTS radical scavenging tests. Both procedures employ the degree of radical solution decolorization to gauge the antioxidants' capacity for scavenging free radicals, which is based on the idea that antioxidants are hydrogen donors (Saadh et al., 2024). When radicals are reduced by antioxidants, they lose their ability to absorb light at specific wavelengths (734 nm for ABTS and 515 nm for DPPH). [(*:: The Korean Journal of Vision Science ::*, n.d.)](https://paperpile.com/c/YIJ50G/h9O8), The inhibition concentration of sodium alginate hydrogel was found to be 72.5%, Sodium hydrogels, often based on sodium alginate or similar sodium-containing polymers, have gained attention for various biomedical applications due to their potential as drug delivery systems and tissue scaffolds.[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/YIJ50G/FoGki)

According to our findings, the sodium hydrogel has a sizable antioxidant capability. Its capacity to scavenge and neutralize these free radicals was demonstrated by the hydrogel, which showed a dose-dependent decrease in ABTS radicals. The observed antioxidant capacity of the sodium hydrogel suggests its potential applications in biomedical fields. This includes wound healing, where the antioxidant properties could aid in tissue regeneration, and drug delivery, where the hydrogel's antioxidant capabilities may protect therapeutic agents from oxidative degradation[(Tunit et al., 2022)](https://paperpile.com/c/YIJ50G/4lOA)

ABTS radical scavenging assay was measured to have a minimum value of 68.5% and the maximum was recorded to be 62.32%, according to a study conducted by muhammad zahid et al [(Zahid et al., 2021)](https://paperpile.com/c/YIJ50G/jXiy), Polymer-based hydrogels have been suggested for use in treating bruising wounds and promoting tissue regeneration. In this study, we utilized the healing capacity of Raphanus sativus L. to synthesize a wound-healing hydrogel with known antibacterial activity for the repair of cutaneous lesions, In every sample, the T-1 hydrogel showed excellent heat stability. The findings of the CAM assay showed that radish extract with hydrogels and sodium alginate together had good healing qualities. According to another study conducted by qinsheng hu et al [(“Injectable Sodium Alginate Hydrogel Loaded with Plant Polyphenol-Functionalized Silver Nanoparticles for Bacteria-Infected Wound Healing,” 2023)](https://paperpile.com/c/YIJ50G/93C0) To encourage the healing of bacterially infected wounds, a novel injectable SA hydrogel loaded with gallic acid modified silver nanoparticles was created. The resultant hydrogel has good biocompatibility and regulated silver ion release from the loaded in the hydrogel, which guarantees long-lasting antibacterial activity. According to a study conducted by katarzyna bialic - was et all [(Bialik-Wąs et al., 2021)](https://paperpile.com/c/YIJ50G/KLzX) hydrogels of sodium alginate and poly(vinyl alcohol) with 0–20% antioxidant-active Echinacea purpurea extract. The swelling ability is correlated with porosity and rises with increasing extract amounts, reaching approximately 300%. The morphology changed directly in the presence of 20% extract, becoming more porous. According to a study conducted by samir kamel et al [(*https://doi.org/10.3390/pharmaceutics15041270*, n.d.)](https://paperpile.com/c/YIJ50G/YnQ6), who found that significant free radical scavenging and antioxidant activities were demonstrated by the hydroalcoholic extract of MOE. The freeze-thawing method was successfully used to create the scaffolds, and FT-IR, XRD, SEM, and TG techniques were used to characterize them. The hydrogen bond interaction between MOE and the SA/PVA matrix was validated by the FTIR spectra.

# CONCLUSION

In conclusion, this study systematically pursued the collection of plant material for the purpose of synthesizing sodium alginate hydrogel, employing a meticulous approach to ensure the selection and gathering of specific plant components essential for the formulation. The subsequent preparation of the hydrogel adhered to precise and controlled procedures, as validated by FTIR and XRD analyses, shedding light on its molecular and structural attributes. The hydrogel, predominantly amorphous, exhibited unique characteristics such as high water content, flexibility, elasticity, and biocompatibility. Further, the comprehensive bioactivity analysis, including DPPH and ABTS assays, revealed significant antioxidant properties, suggesting potential applications in diverse biological and biomedical contexts. This research contributes valuable insights into the synthesis and properties of sodium alginate hydrogel, paving the way for its exploration in various fields.

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