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EVALUATION OF ANTIOXIDANT PROPERTIES OF A PLANT SEED BASED CHEMICALLY MODIFIED HYDROGEL AS A PRECURSOR FOR FUTURE DRUG DELIVERY APPLICATIONS

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Abstract

Hydrogels derived from natural polymers have emerged as promising biomaterials for biomedical and drug delivery applications owing to their excellent biocompatibility and biodegradability. In the present study, a chemically modified plant seed-based hydrogel was evaluated for its antioxidant potential using an in vitro free radical scavenging assay. The hydrogel was synthesized through chemical modification to improve its functional and biological properties. Antioxidant activity was assessed by measuring its ability to neutralize free radicals at different concentrations. The results demonstrated a concentration-dependent increase in radical scavenging activity, indicating the presence of effective antioxidant functional groups within the hydrogel network. The enhanced antioxidant performance may be attributed to the synergistic effect of the plant-derived biopolymer and the introduced chemical functionalities. Such antioxidant behavior can protect therapeutic agents from oxidative degradation while reducing oxidative stress at the target site. These findings suggest that the modified hydrogel possesses favorable biological characteristics for pharmaceutical applications. Therefore, the developed plant seed-based hydrogel represents a promising candidate for future antioxidant-assisted drug delivery systems.

1. Introduction

Oxidative stress, resulting from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system, has been implicated in the pathogenesis of numerous chronic diseases, including cancer, diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, and inflammatory conditions. Excessive ROS production damages essential biomolecules such as proteins, lipids, and nucleic acids, thereby disrupting normal cellular functions and accelerating disease progression. Consequently, there has been growing interest in the development of biomaterials possessing intrinsic antioxidant properties that can effectively scavenge free radicals and protect biological tissues from oxidative damage. Such materials are particularly attractive in controlled drug delivery systems, where oxidative stress at the target site may compromise therapeutic efficacy and tissue regeneration [1–3].

Hydrogels are three-dimensional, hydrophilic polymeric networks capable of absorbing and retaining large amounts of water without dissolving due to the presence of physical or chemical crosslinks. Their high water content, soft tissue-like characteristics, tunable mechanical properties, excellent biocompatibility, and ability to encapsulate a broad range of therapeutic agents have established hydrogels as promising carriers for drug delivery, tissue engineering, wound dressing, and regenerative medicine [4-10].

Recent advances have focused on engineering multifunctional hydrogels that not only provide sustained and stimuli-responsive drug release but also exhibit biological activities such as antioxidant, antimicrobial, and anti-inflammatory effects to enhance therapeutic outcomes [11–22].

Natural polysaccharides have attracted significant attention as hydrogel matrices owing to their biodegradability, non-toxicity, renewable origin, and excellent biological compatibility. Plant-derived polysaccharides are particularly advantageous because they possess abundant hydroxyl and carboxyl functional groups that facilitate chemical modification and crosslinking, allowing the fabrication of hydrogels with tailored physicochemical and biological properties. Moreover, the incorporation of naturally occurring phytochemicals into these polymeric systems can significantly improve their antioxidant performance, making them suitable for biomedical applications involving oxidative environments [23,24].

Plant seeds represent an abundant yet underutilized source of valuable biopolymers and bioactive compounds. Many edible and medicinal seeds contain polysaccharides, proteins, phenolic acids, flavonoids, tannins, tocopherols, and other antioxidant constituents capable of neutralizing free radicals through hydrogen atom donation, electron transfer, and metal ion chelation mechanisms. Seed-derived polysaccharides have demonstrated remarkable film-forming ability, swelling capacity, and gel-forming characteristics, making them attractive candidates for hydrogel preparation. Chemical modification of these naturally occurring polymers further enhances their stability, swelling behavior, mechanical strength, and functional performance without compromising their inherent biocompatibility [25–35].

Among various modification strategies, graft polymerization and chemical crosslinking have emerged as effective approaches for improving the physicochemical characteristics of plant-based hydrogels. Introduction of synthetic monomers containing carboxyl, hydroxyl, or amide functionalities increases the density of hydrophilic groups, resulting in enhanced water uptake, improved drug encapsulation efficiency, and controlled release behavior. Furthermore, these modifications create additional active sites capable of interacting with reactive oxygen species, thereby contributing to improved antioxidant activity. Environmentally friendly crosslinking agents, such as citric acid, have gained considerable interest because they produce non-toxic, biodegradable hydrogels while avoiding the use of hazardous chemical crosslinkers traditionally employed in biomedical materials [36-38].

The antioxidant potential of hydrogel systems is commonly evaluated using radical scavenging assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and hydroxyl radical scavenging assays. These methods provide valuable insight into the electron-donating and free radical neutralization capabilities of the prepared biomaterials. Hydrogels exhibiting high antioxidant activity are particularly advantageous for drug delivery applications because they can minimize oxidative degradation of encapsulated therapeutics, reduce inflammation at the target site, promote cellular proliferation, and accelerate tissue repair. Therefore, integrating antioxidant functionality into hydrogel-based delivery systems represents an effective strategy for improving the overall therapeutic performance of advanced biomaterials [39–41].

In the present study, a chemically modified hydrogel prepared from a plant seed-derived biopolymer was investigated for its antioxidant properties to assess its suitability as a prospective drug delivery material. The antioxidant capacity of the hydrogel was evaluated using established in vitro free radical scavenging assays and correlated with its potential biomedical applicability. The findings are expected to provide valuable insights into the development of sustainable, biocompatible, and multifunctional hydrogel systems that combine efficient drug delivery with intrinsic antioxidant protection, thereby expanding their potential applications in pharmaceutical and regenerative medicine.

2. Material and methods

2.1. Materials

Mimosa seeds were obtained from the nearby market in District Sargodha. The following reagents were used with this research work: N-N-Methylene bis acrylamide (MBA, ≥99%, Sigma-Aldrich, Germany), methacrylic acid (MA, 99%, Sigma-Aldrich, Germany), ammonium persulfate (APS, ≥98%, Sigma-Aldrich, Germany), n-hexane (≥95%, Riedel-de Haen, Germany), and ethanol (≥99.8%, Riedel-de Haen, Germany).Reagents and chemicals were used as received and were of analytical grade. Solutions/dispersions were prepared using distilled water (DW).

2.2. Formulation of hydrogel

The copolymer hydrogel was synthesized by copolymerization of Am as a monomer, following the procedure reported by peers [19].

2.3. Assay for 2,2-Diphenyl-1-picrylhydrazyl

The DPPH radical scavenging test was carried out using a slightly modified version of a previously published protocol [39]. One milliliter of 0.1 mM methanolic DPPH solution and two milliliters of methanol were added to the hydrogel suspension (100–500 µg/mL). After fully mixing the mixture, it was left to settle at room temperature for half an hour in the dark. A Shimadzu UV-1800 spectrophotometer was used to detect the absorbance at 517 nm. The standard was ascorbic acid.

2.4. FRAP

The procedure was followed as described previously [40] to estimate the FRAP of hydrogel. The ferric reducing antioxidant power assay is called FRAP. The (0.2 M, pH 6.6, 2.5 mL) sodium phosphate buffer solution was added to the various concentration range of hydrogel, that is 20-100 µg/ml. After 20 minutes incubation at 50 °C, 2.5ml of 10 percent tri-chloric acid is added and centrifuged for 10 minutes at 3000 revolution per minute. The absorbance was determined at 700nm by a spectrophotometer (specification A) from Shimadzu. The results were reported as micrograms of (AAE) per milliliter of hydrogel and in contrast to a standard for $C_6H_8O_6$ (ascorbic acid) .The standard curve approach was used to calculate the antioxidant properties.

2.5. TPC

The Folin-Ciocalteu reagent method was then used to determine the TPC (total polyphenol content) with a slightly modified method [3]. In the modifications, sample quantities about 20-100 µg/ml of hydrogel have been tested. All samples were kept for eight minutes at room temperature in the dark. Ninety-five hundred (950) µL of distilled water and seven hundred and every sample received fifty (750) µL of the 20% sodium carbonate. Thirty minutes culture period without the presence of any light for these reaction samples. Each reaction was then measured on a spectrophotometer at 765 nm. The content of total phenols was determined based on a standard curve of quercetin in µg/mL of hydrogel. The standard curve method was used for calculating the scavenging activity.

2.6. Total Flavonoid Content

The total antioxidant activity of flavonoid in hydrogel was measured by colorimetric method using Aluminum chloride ($AlCl_3$) [2]. Five hydrogel samples were made under different concentrations e.g. 20-100 µg/ml correspondingly. A 0.75 mL methanol was added to these samples. Distilled water was then added to these solutions to give a total volume of 2 mL. These solutions were supplemented with 300 microlitres (µL) of 5% sodium nitrate and 300 microlitres of 10% aluminium chloride. Every solution was made up to 5 mL with 2 mL of 1 molL-1 sodium hydroxide solution after 10 minutes. The absorbance at 510 nm was measured after 40 minutes incubation in the dark.

3. Results and discussion

3.1. DPPH Assay

To determine the ability of hydrogel to scavenge DPPH (free radicals) at 20-100 µg/mL (µg per milliliter), a DPPH experiment was employed. To determine the antioxidant activity of hydrogel, the ability of the synthesized polymer to scavenge the DPPH radical was compared with that of ascorbic acid (AA), the standard antioxidant]. The ability of the hydrogel to inhibit the formation of DPPH free radical

which are concentration-dependent. Since DPPH test shows the presence of bioactive compounds in the extract that can be involved in elimination process, it is a reliable method to evaluate the antioxidant activity of the chemical compound. The scavenging activity of hydrogel (20–100 µg/mL) on DPPH radicals was evaluated and the results are presented in figure 2a. Similarly, the percentages of the DPPH radical scavenging for hydrogel at 20-100 µg/mL correspondingly.

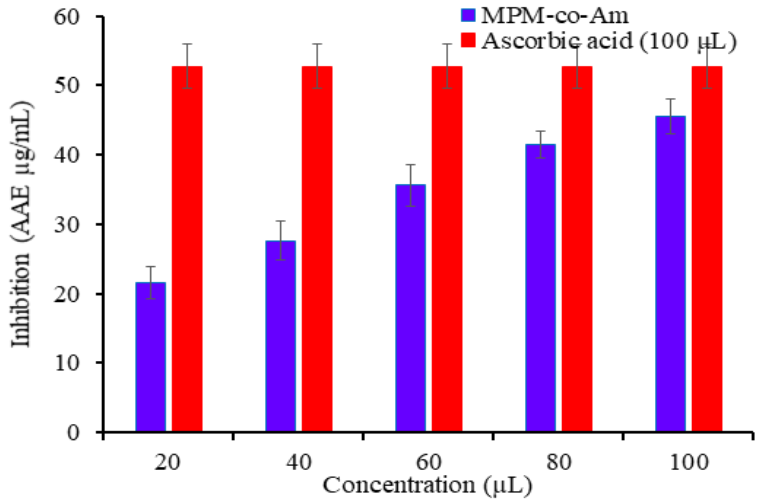

Fig. 1. DPPH test

3.2. FRAP Analysis

FRAP analysis measures the total antioxidant capacity. Ascorbic acid is a secondary antioxidant, and it is very effective at free radical neutralization and in blocking the oxidative chain reactions and hence is widely used in pharmaceutical and biological applications [23]. As a result of its high polyhydroxyl content and free hydroxyl groups, ascorbic acid is a powerful antioxidant and is able to effectively combat harmful free radicals. The trichloroacetic acid solution was used in this study to remove (K4[Fe(CN)6]) in deposited form.

Upon the addition of FeCl3, a compound is formed that turns from green to blue. This change is due to the reduction of Fe^{3+} to Fe^{2+} and allows the quantification of the green coloured antioxidant activity of hydrogel and determination of the non-oxidising strength. The radical scavenging activity of hydrogel was found in the range of 39.29 to 71.67 µg ascorbic acid equivalents per milliliter (AAE µg/mL) within the amount limit of hydrogel from 20 to 100 µg/mL, by using data obtained from an ascorbic acid standard curve for further calculations. The increase in the antioxidant activity observed for hydrogel is believed to be due to the capability of the polymer to capture free radicals by electron transfer, proton transfer and electron transfer, or by direct proton and hydrogen atom transfer.

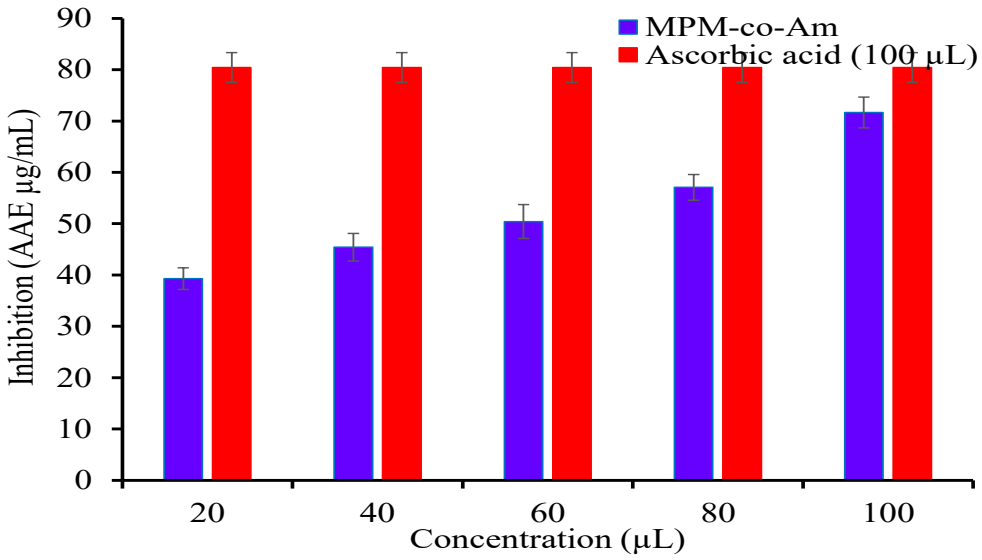

Fig. 2. FRAB test

3.3. TFC and TPC

The curve absorbance-concentration relation was used for quercetin and the curve absorbance-concentration relation was used for gallic acid to create TFC and TPC, respectively, as shown in Figures 2c and 2d, respectively, [24,25]. The study established that the concentration was directly proportional to the TFC and TPC. QCE µg/mL ranged from 27.05 to 71.17 µg, while GAE µg/mL ranged from 32 to 69.25 µg. The obtained data indicate that this product may have an antioxidant effect due to the presence of bioactive components of hydrogel. The antioxidant activity of hydrogel may be correlated with the presence of hydroxyl-rich polysaccharide chains and phenolic/flavonoid components that are able to participate in hydrogen atom donation and electron transfer reactions. As indicated by the increasing levels in DPPH, FRAP, TFC and TPC, the antioxidant potential of hydrogel increased with concentration. But since no independent regression analysis was performed no direct correlation coefficient was included.

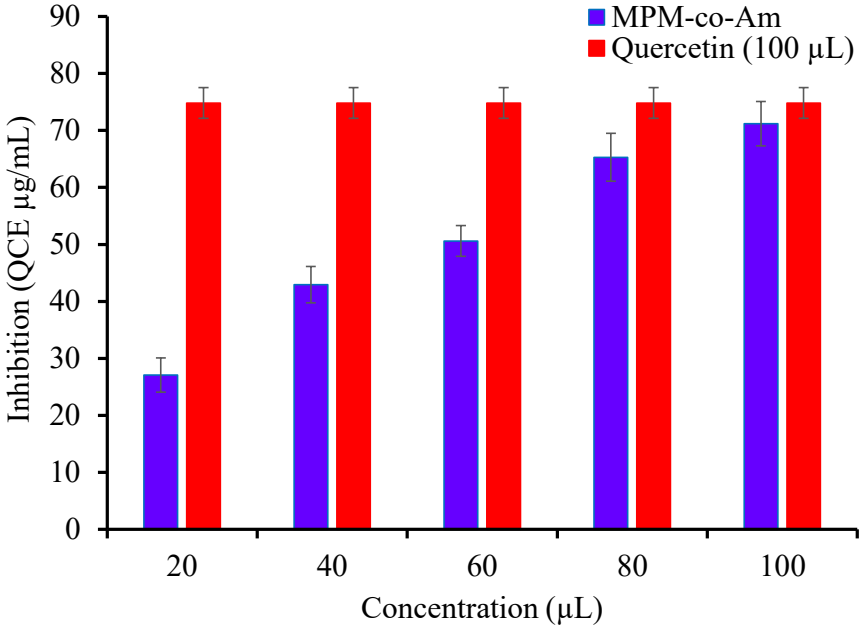

Fig. 3. TFC test

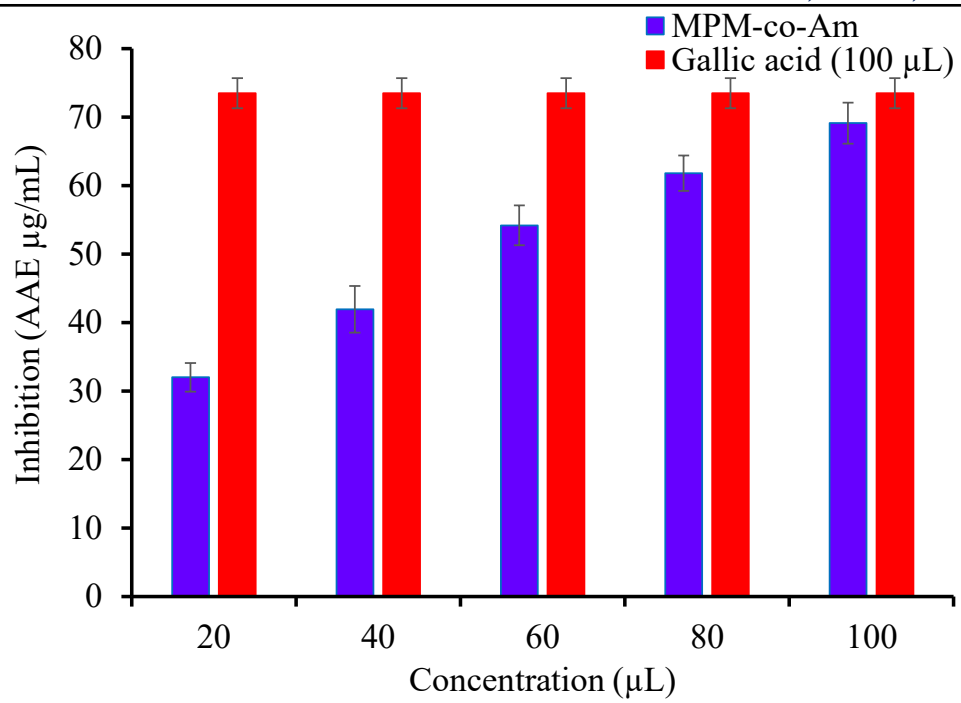

Fig. 4. TPC test

4. Conclusion

The present study demonstrated that the chemically modified plant seed-based hydrogel exhibited significant antioxidant activity, highlighting its potential as a multifunctional biomaterial. The antioxidant capacity increased with concentration, confirming the effective free radical scavenging ability of the modified hydrogel. Chemical modification successfully enhanced the functional properties of the native plant polymer without compromising its biocompatible nature. The observed antioxidant performance suggests that the hydrogel can minimize oxidative stress and improve the stability of encapsulated therapeutic agents during drug delivery. These characteristics make the material suitable for controlled and sustained pharmaceutical applications. Furthermore, the use of renewable plant-derived polymers provides an environmentally friendly and sustainable alternative to synthetic materials. Overall, the developed hydrogel shows considerable promise for advanced drug delivery systems and other biomedical applications. Future studies should focus on drug loading, release kinetics, cytocompatibility, and in vivo evaluations to further validate its clinical potential.

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