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EVALUATION OF ANTIOXIDANT PROPERTIES OF A CO-POLYMERIZED HYDROGEL FROM NATURAL SOURCE FOR DIFFERENT BIOMEDICAL APPLICATIONS

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Abstract

In order to introduce a material with improved antioxidant qualities, the current study focuses on the assessment of antioxidant properties of a co-polymerized hydrogel. By co-polymerizing the extracted seed mucilage of *Mimosa pudica* (MPH) with methacrylic acid (MA), a co-polymerized hydrogel (MPH-co-MA) with enhanced free radical scavenging capacity was created. The capacity of MPH-co-MA to lower oxidative stress was evaluated by measuring its antioxidant activity such as Frap, DPPH, TPC, and TFC, which makes it appropriate for use in tissue engineering, wound healing, and regulated medication delivery. FRAP values rose from 27.2–51.1 µg AAE/mL spanning 100–500 µg/mL, and the extract demonstrated dose-dependent DPPH radical scavenging activity ranging from 10.77-37.53 µg. Strong antioxidant potential was confirmed by the gradual rise in phenolic and flavonoid levels, with TPC rising from 23.2-53.2 µg (GAE µg/mL) and TFC from 25.1-69.4, µg (QCE µg/mL). These results support the traditional uses of co-polymerized hydrogel by confirming that it has significant antioxidant effects.

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1. Introduction

Hydrogels, particularly those derived from the natural polysaccharides emerged as an attractive biomaterial for a wide range of biomedical applications [1-4]. These are biocompatible and biodegradable materials, which can be chemically modified by co-polymerization and cross-linking. With these modified feature, hydrogels become more mechanically stiff as well as more swellable and responsive to environment stimuli towards more end-uses, like controlled drug delivery system, wound healing dressing material, tissue engineering, and water purification systems. By taking high-water absorption without destroying its three-dimension appearance, the hydrogel provides a proper place for cells' growth and nutrient /drug transportation. These features make hydrogels as potential candidates for drug delivery particularly, in chronic disease that requires sustained local release of drugs [5-25].

Free radical scavenger activity and antioxidant power of hydrogel with high efficiency is an important property for biomedical application. Pathologies such as chronic inflammation, cancer, cardiovascular diseases and neurodegenerative diseases, e.g., Alzheimer's and Parkinson's disease are associated to oxidative stress due to an increased production of free radicals. Specifically, free radicals such as reactive oxygen spices (ROS) and reactive nitrogen species (RNS) can induce cellular structural damage, protein damage, lipid peroxidation and DNA lesions thereby leading to tissue degeneration, development of disease, etc. In this context, bio-active hydrogels with inherent or enhanced antioxidant activity that can efficiently combat the production and accumulation of ROS as well as maintain cellular homeostasis have attracted more and more attention for their formation. It has been shown that oxidative stress is highly implicated in accelerated aging and chronic diseases, hydrogels constructed to clear free radicals will be a great promising therapeutic approach for disease prevention and healing improvement [26-29].

Among the naturally occurring sources, *Mimosa pudica*, a tropical South and Central America plant that is also reported in other continents including Asia and Africa has been examined for its bioactive potential specifically antioxidant property. M. *pudica* (sensitive plant) is the only known species that shows nastic movements in response to tactile and environmental agents such as heat, mechanical disturbance, insult or injury. This plant is a source active compound including flavonoids, alkaloids, tannins and mucilage, which proved to possess antioxidant anti-inflammatory, anti-microbial activities [30-35].

Herein, we report the synthesis of a co-polymeric hydrogel from M. *pudica* seed mucilage and methacrylic acid (MA). The hydrogel was developed to achieve improved antioxidant capabilities with the perspective of designing a material that is efficient in the treatment of diseases associated with oxidative stress. We assess the antioxidant activity of co-polymerized hydrogel using *in vitro* antioxidant assays like FRAP assay, DPPH radical scavenging assay and estimate total phenolic and flavonoid content in this study. The data show that the MPH-co-MA hydrogel possesses effective antioxidant property, suggesting potential applications in wound repair, tissue regeneration and drug release control.

2. Materials and Methods

2.1. Materials

The *M. pudica* seeds were purchased from the local market. The following highly pure chemicals and reagents were used as received without further purification: *N,N'*-methylene-bis-acrylamide (MBA, Sigma-Aldrich), methacrylic acid (MA, Sigma-Aldrich), ammonium persulfate (APS, Merck), n-hexane (Fisher Scientific) and ethanol (Merck). Deionized water (DW) was employed for all solution preparation.

2.2. Extraction of Seed Mucilage

M. pudica seeds are collected from a reliable source. They are manually cleaned, and pulverize for mucilage extraction. The seeds were soaked in DW. Stir and allow the mucilage to swell. Filter and precipitate the mucilage using an appropriate solvent (e.g., ethanol). Dry and store the mucilage for co-polymerized hydrogel synthesis. The aready reported protocol for mucilage/hydrogel (MPH) extraction with needy amendments [23].

2.3. Synthesis of Co-polymeric Hydrogel

The co-polymeric hydrogel was prepared according to the reported method with minor amendments [15].

2.4. Evaluation of Antioxidant Activity

2.4.1. Ferric reducing antioxidant power (FRAP) assay

With few modifications, the FRAP assay was carried out in accordance with the previously reported method [26]. In short, various MPH-co-MA solution concentrations ranging from 100 to 500 µL were mixed with 2.5 mL of sodium phosphate buffer (PBS) (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated for 20 min at 50°C. Following the addition of a 10% w/v trichloroacetic acid solution, the mixture was centrifuged for 10 min at 3000 rpm. 2.5 mL of DW and a 0.1% ferric chloride solution were combined with the supernatant. A spectrophotometer was then used to measure absorbance at 700 nm. To determine FRAP's antioxidant potential, the following Eq. (1) was used.

$$X = \frac{y}{0.0027}, R^2 = 0.9801 \quad (1)$$

2.4.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

According to the methodology outlined in [27], various concentrations of MPH-co-MA (ranging from 100 to 500 µL) were mixed with 2 mL of methanol and 1 mL of a methanolic solution containing DPPH free radicals (0.1 mM). The components were thoroughly mixed and incubated at ambient temperature for 30 min in the dark. Absorbance was then measured using a Shimadzu UV-1800 spectrophotometer set to 517 nm. The antioxidant potential of the samples was determined using the following Eq. (2) for the DPPH assay.

$$\text{Radical scavenging activity of DPPH (\%)} = \left[1 - \frac{A1-A2}{A3}\right] \times 100 \quad (2)$$

2.4.3. Total polyphenol content (TPC)

With a few modifications, the Folin-Ciocalteu method was employed to determine the total polyphenol content. The incubation period before adding sodium hydroxide was extended from 4 to 8 minutes, and various sample quantities were added [28]. First, 100, 200, 300, 400, and 500 µL of MP-co-MA were combined with 2 mL of DH_2O and 250 µL of 1N Folin Ciocalteu's phenol. The mixture was then left in the dark at room temperature for 8 min [29]. Thereafter, 750 µ L of a 20 % Na_2CO_3 solution and 950 µL of DH_2O were added. The solutions were stored in the dark atmosphere for 30 min, then the absorbance was recorded at 765 nm with a Shimadzu UV-1800 spectrophotometer. The obtained values were reported as µg GAE per mL of MP-co-MA, with gallic acid serving as the reference. The level of scavenging was estimated using the Eq. (3).

$$X = \frac{y}{0.0019}, R^2 = 0.9659 \quad (3)$$

2.4.4. Total flavonoid content (TFC)

Colorimetric technique utilizing aluminium chloride ($AlCl_3$) was applied to assess the content of flavonoid [29]. In brief, 0.75 mL of methanol was coupled with 100, 200, 300, 400, and 500 µL of MP-co-MA, and the entire volume was raised to 2 mL with DH_2O. Then, 300 µL of 5% sodium nitrate and 300 µL of 10% $AlCl_3$ were added to each sample and then left to settle for 10 min. Furthermore, 2 mL of 1 mol/L NaOH was added to the solution, and the total volume was increased to 5 mL with DH_2O. The test solution was incubated for 40 min at room temperature before the absorbance was measured at 510 nm using a spectrophotometer (Shimadzu UV-1800). The results were calculated with quercetin as the reference standard and expressed as mg of quercetin equivalents per µg (µg quercetin/g) of MPH-co-MA. The level of scavenging was calculated using the Eq. (4).

$$X = \frac{y}{0.0022}, R^2 = 0.9883 \quad (4)$$

3. Results and Discussion

3.1. Ferric Reducing Antioxidant Assay (FRAP)

The total antioxidant activity was determined by FRAP assay. Ascorbic acid acts as an anti-oxidant by trapping free radicals and therefore terminating chain reactions; this antioxidant activity is evidenced to be due in particular to its hydroxyl groups effectively quenching the free radicals. Trichloroacetic acid (TCA) solution was used in the present study for dissolving potassium ferrocyanide ($K_3Fe(CN)_6$) to remove it. The complex development was completed by adding $FeCl_3$ and a colour change from green to blue indicated the presence of the complex. The antioxidant potential of the as-prepared green MPH-co-MA was determined based on its superoxide radical scavenging activity reducing Fe^{3+} to Fe^{2+}. The radical scavenging activity was measured at 100–500 µL concentrations and ranged between 27.2–51.1 μg AAE/mL using the ascorbic acid standard curve. The reactive radicals were stabilized via charge transfer, proton-coupled electron transfer, proton abstraction and free radical hydrogen transfer. The hydrogel showed improved antioxidant behavior [27].

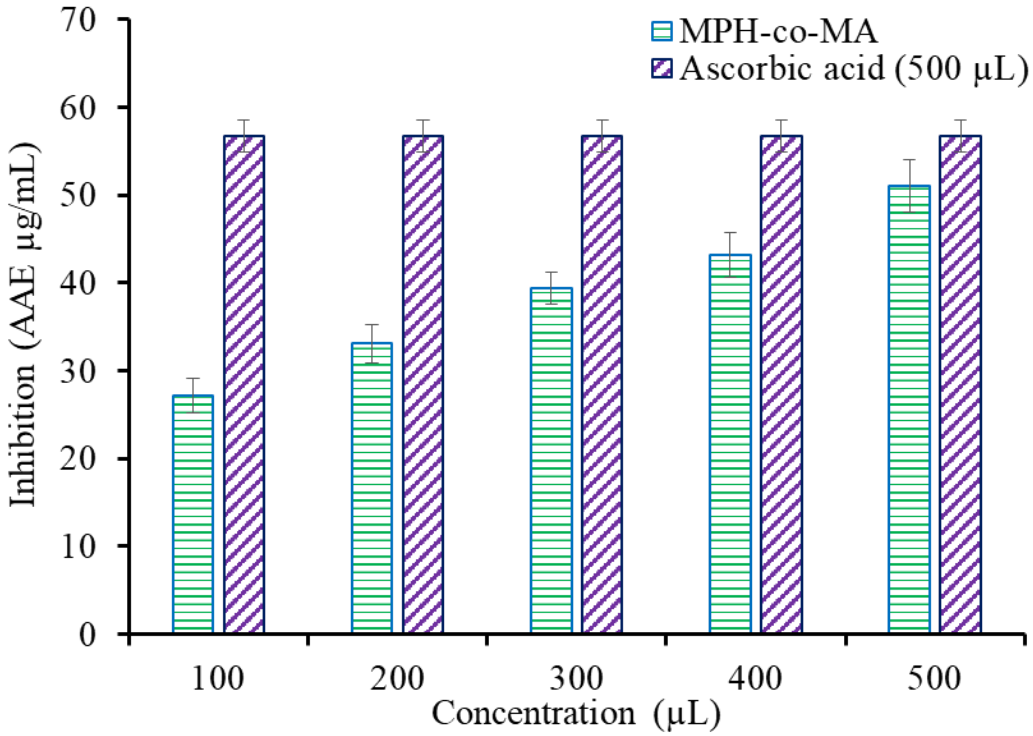

Figure 1. The antioxidant capacity of MPH-co-MA hydrogel determined via FRAP.

3.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

MP-co-MA's DPPH radical scavenging activity was evaluated using a DPPH test at various concentrations (100-500 µL). A comparison study was undertaken to assess the DPPH free radical inhibition capacity of MP-co-MA vs ascorbic acid. As the concentration of MPH-co-MA increased, so did the concentration of DPPH radical suppression. The DPPH test is a reliable way to investigate antioxidant activity. The presence of bioactive chemicals in the hydrogel may play a function in controlling this process [28]. The antioxidant capacity of MP-co-MA against DPPH was investigated range varied from (10.77-37.53 µg) (AAE µg/mL) and concentrations ranging from 100 to 500 µL.

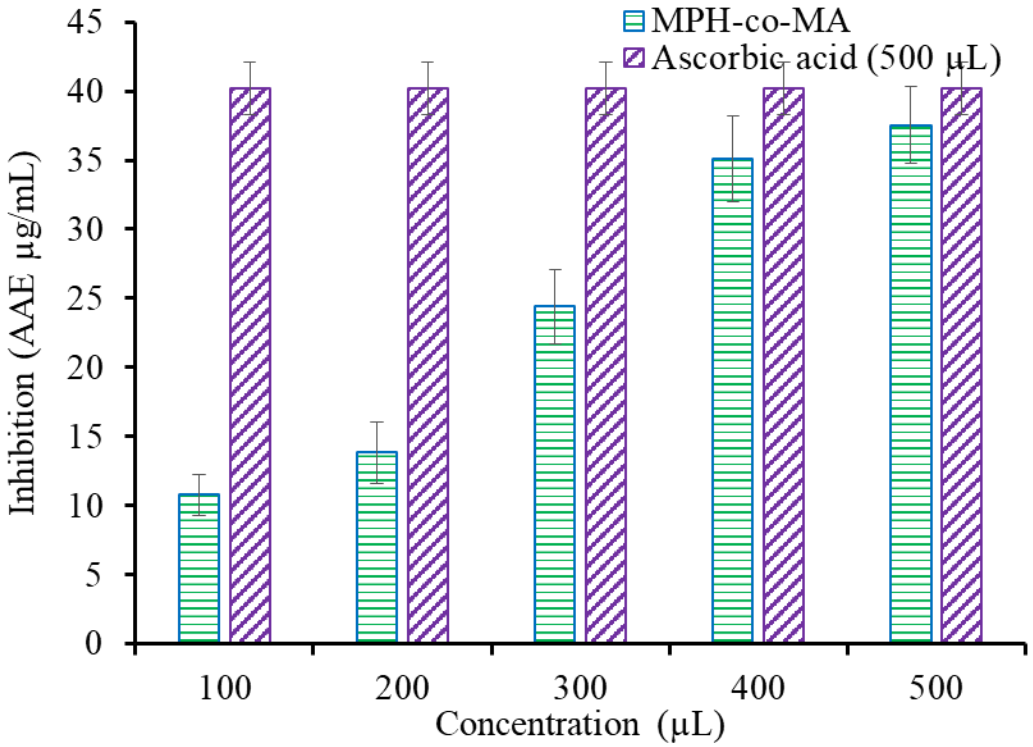

Figure 2. DPPH radical scavenging activity of MPH-co-MA hydrogel at different concentrations (100–500 µL).

3.3. Total phenolic content (TPC) and total flavonoid content (TFC)

Gallic acid (GA) and quercetin (QC) standard curves were utilized to quantify total phenolic and flavonoid contents. In the total phenolic content (TPC) and total flavonoid content (TFC) with recorded values ranging from 23.2-53.2 µg (GAE µg/mL) and (25.1-69.4, µg (QCE µg/mL) respectively, an increase in concentration-dependent was observed. The bioactive ingredients established a relationship between bioactive chemicals, metal ion reduction, and co-polymerized hydrogel facilitation [29].

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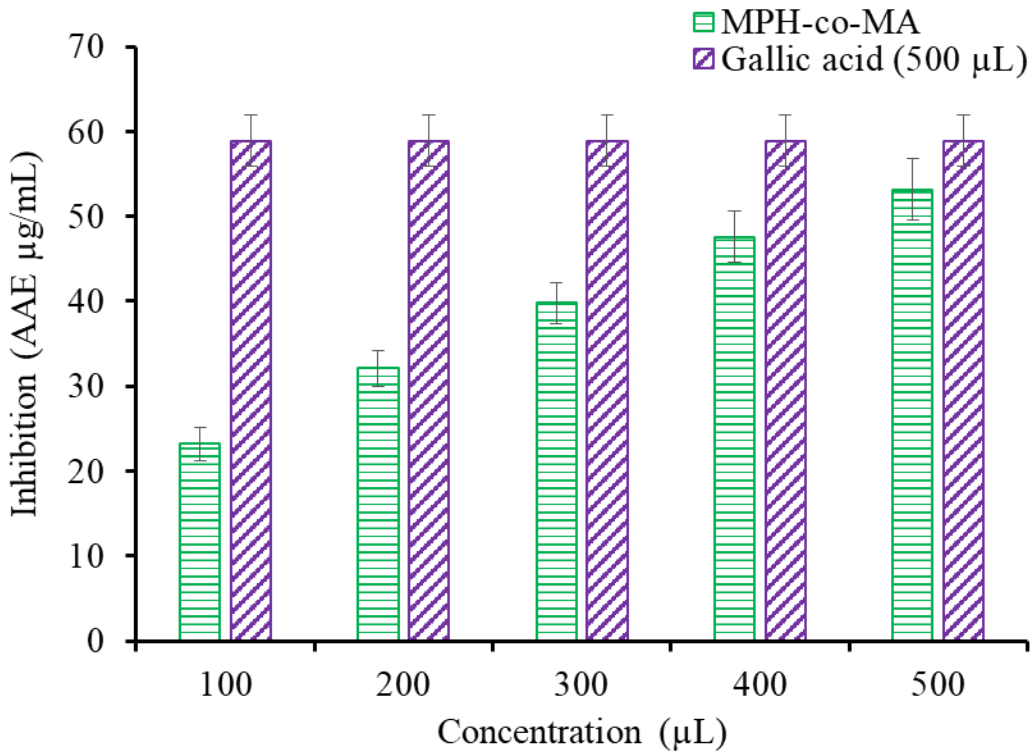

Figure 3. Phenolic content in MPH-co-MA hydrogel by Folin-Ciocalteu, µg/mL. The results are reported in gallic acid equivalent (µg GAE/mL) for the indicated concentration of hydrogel.

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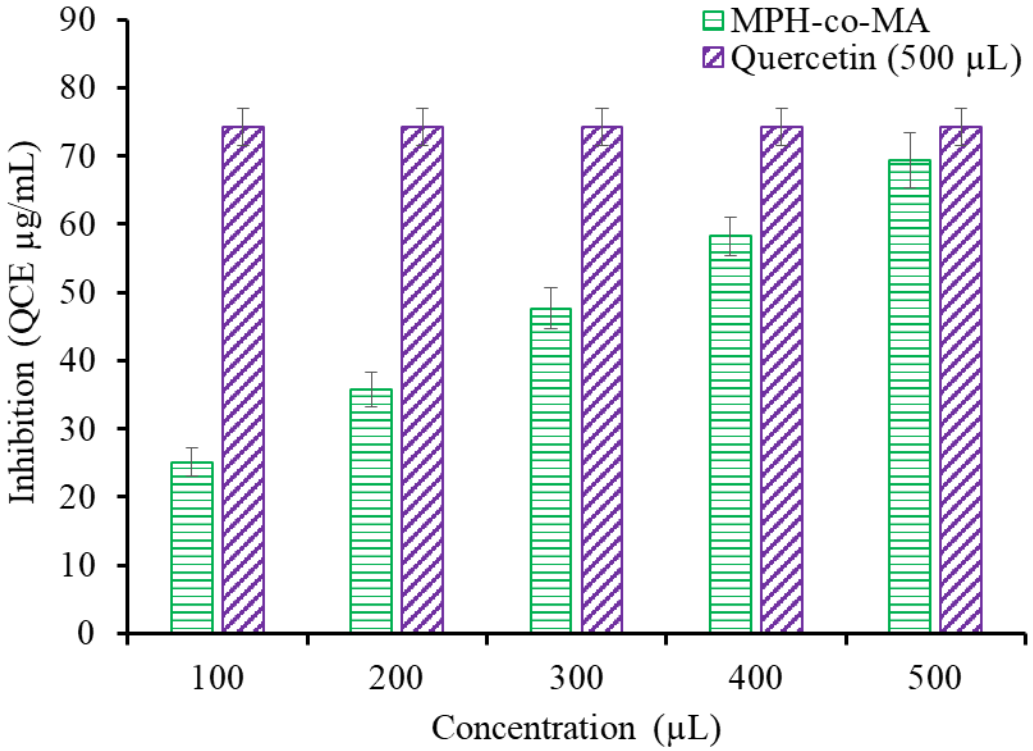

Figure 4. Total flavonoid of MPH-co-MA hydrogel analysed by aluminium chloride colourimetric method. The data is in the form of quercetin equivalents (µg QE/mL) for the various hydrogel concentrations.

4. Conclusion

In the present work, MPH-co-MA co-polymeric hydrogel was synthesized and evaluated for its antioxidant potential. The antioxidant properties of the MPH-co-MA hydrogel were examined through *in-vitro* methodologies such as FRAP, DPPH radical scavenging activity and total phenolic (TPC) and flavonoid contents (TFC). The FRAP test showed that the hydrogel was able to reduce Fe^{3+} to Fe^{2+}, showing its good antioxidant potential. The DPPH assay revealed that the hydrogel had a dose-dependent radical scavenging activity, even far better than ascorbic acid actions at higher concentrations. Moreover, TPC and TFC assays also established the existence of substantial phenolic and flavonoid content that impart antioxidant character to hydrogel. In general, this study indicates that the MPH-co-MA-based hydrogels have prominent antioxidant ability and these antioxidant abilities can be utilized for studying in tissue engineering, drug release control and wound healing, which will lead to future research for biocompatible functional materials.

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