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Original article

Fabrication and characterization of hydrogel films blended with *Chromolaena odorata* leaf extract and their antioxidant properties

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Abstract

Background: Hydrogels derived from natural polymers are widely studied for biomedical applications. Durian rind, an agricultural waste, serves as a valuable cellulose source for producing Carboxymethyl Cellulose (CMC) based hydrogels. Additionally, *Chromolaena odorata* (*C.odorata*) extract enhances the antioxidant properties of the hydrogel, aiding in the reduction of oxidative stress. These properties are essential for promoting wound healing and biomedical applications. Ensuring non-cytotoxicity is crucial for safety and effectiveness, making them promising for wound dressings and drug delivery systems.

Objective: To develop antioxidant-active hydrogel films from durian rind-derived CMC incorporating *C.odorata* extract with biocompatible and non-cytotoxic properties for potential biomedical applications.

Methods: We extraced compounds from *C.odorata* leaves and evaluated their antioxidant, cytotoxicity, and inflammatory properties. In the synthesis of CMC from durian peel, it is utilized as a polymer to be molded into a hydrogel. The formation of hydrogel from synthetic CMC contained wih *C.odorata* leaf extract was optimized. Subsequently, its structure and surface were characterized by FTIR and SEM, respectively. The biocompatibility with cell lines and antioxidant activity of hydrogel were confirmed after casting.

Results: This research showed that a hydrogel mixed with *C.odorata* leaves extract at 2X and 10X can be formed. The film was tested and demonstrated sustained antioxidant properties, and the film was non-toxic on keratinocyte, fibroblast, and macrophage cells.

Conclusion: The hydrogel derived from durian rind-based CMC contained *C.odorata* extract, preserved its structural integrity and antioxidant activity. The films exhibited biocompatibility and non-cytotoxicity, highlighting their potential for biomedical applications like wound dressings and drug delivery systems.

***Keywords**:* Antioxidant property, bioactive compound, *Chromolaena odorata* leaf extract, durian rind, hydrogel.

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Hydrogels have garnered significant attention in biomedical applications due to their high water content, biocompatibility, and ability to combine bioactive compounds.[1] In recent years, natural polymers have been extensively explored as sustainable alternatives for hydrogel fabrication, with carboxymethyl cellulose (CMC) emerging as a promising candidate. Durian (*Durio zibethinus*) rind, an abundant agricultural waste, is a rich source of cellulose, which can be modified into CMC for hydrogel film development.[2] Utilizing durian rind not only adds value to waste materials but also promotes eco-friendly biomaterial production. Several durian hydrogel-containing substances have been reported such as PVA-Durian hull gum wound dressing containing Centella asiatica extract (ECA233).[3] In addition, a hydrogel was developed using CMC and Carbopol, incorporating Rambutan Leaf Extract (RLE) to enhance its antibacterial properties. RLE was evaluated for its potential to improve the hydrogel's effectiveness as an antibacterial agent.[4] However, development and produced novel would dressing or hydrogel and enhance the functionality of hydrogel films. Moreover, *Chromolaena odorata* (Siam weed) leaf extract has been report about it incorporated due to its well-documented antioxidant and wound-healing properties.[5] Antioxidants play a crucial role in neutralizing free radicals, which are associated with oxidative stress and delayed wound healing. However, for biomedical applications, these hydrogel films should exhibit both antioxidant activity and non-cytotoxicity to ensure their safety and efficacy.

This research aims to develop a hydrogel from durian rind-derived CMC loaded with *Chromolaena odorata* extract and to evaluate their physicochemical properties, antioxidant potential, and cytotoxicity. The findings could contribute to the development of sustainable, bioactive hydrogel materials for applications in wound healing and drug delivery systems.

Materials and methods

Chromolaena odorata leaf *extraction* and biological properties assay

The *C. odorata* leaves were washed and dried. Crude *C. odorata* leaves were extracted with double-distilled water to get an active compound. Then, dry it using the freeze-drying technique. After that, the antioxidant activity, toxicity and anti-inflammatory activity were tested to be used as an important active ingredient in the further formation of hydrogels.

Durian rind preparation and CMC synthesis

The extraction and CMC synthesis process were modification method follow by.[6] Dried durian peels were crushed cellulose extracted using NaOH in alkaline conditions, and lignin was removed with H_2O_2 treatment. Then, carboxymethyl cellulose or CMC was synthesized by using monochloroacetic acid (MCA) to be used in further film-forming.

Hydrogel casting and characterization

CMC from durian peel fruit obtained from synthesis was molded into hydrogel, and the ratio between CMC, PVA, Citric and glycerol suitable for molding was studied, as well as the appropriate ratio of *C. odorata* leaf extract added to the hydrogel. Hydrogel was prepared using a film casting method, as follows. A PVA solution 6% (w/v) was prepared by dissolving PVA in distilled water and heating at 80 °C until dissolved complety. CMC synthese at concemtraitom of 3% and 1% of citric acid were added to the solution. After a solution was homogenius, *C. odorata* leaves at 2X and 10X of IC50 extracted and glycerin 10% (w/v) which was used as plasticizer, were then added. A fixed volume (25 ml) of the final polymeric solution was poured into a glass petri dish and oven dried at 50°C for 16-18 h. When the right recipe is obtained. Structural functional group and surface of hydrogel were analyzed by FTIR and SEM technique.

Antioxidant activity

The antioxidant activity was evaluated by the DPPH radical scavenging assay.[7] Briefly, 10 µL of the hydrogel film extract containing *C. odorata* leaf extract was added to 100 µL of 0.2 mM DPPH solution in ethanol. The mixture was gently mixed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a microplate reader (EnSight, PerkinElmer, USA).

Cytoxicity of hydrogel on African green monkey epitherial cells (Vero) and mouse fibroblast (3T3-L1)

The cytotoxicity of the synthesized hydrogel on animal cell lines was evaluated using the MTT assay, following the methodology described by Phosri S, et al.[8] Hydrogel films were sterilized using UV irradiation for 30 minutes, then the DMEM was added and incubated in a CO, incubator at 37°C for 24 h. Vero and 3T3-L1 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated at 37°C with 5% CO, for 24 h. Subsequently, the cells were

treated with hydrogel extract media at concentrations of 62.5, 125, 250, 500, and 1,000 µg/mL, and further incubated under the same conditions for 24 h. After incubation, the culture media was removed and replaced with MTT solution (0.5 mg/mL in DMEM). The cells were incubated for 1 h before removing the MTT solution and adding 150 µL of 100% DMSO to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (EnSight, PerkinElmer, USA). Cell viability was calculated and expressed as a percentage of the untreated control group, which was set as 100%.

Anti-inflammaory activity of hydrogel

The anti-inflammatory activity of the hydrogel films was evaluated in RAW 264.7 macrophage cells stimulated with lipopolysaccharide (LPS). RAW 264.7 cells were seeded in 96-well plates at a density of 1×10^5 cells/well in DMEM and incubated for 24 hours at 37°C in a 5% CO, incubator. The cells were then treated with film extracts at concentrations of 0.165, 0.313, 0.625 and 1.25 mg/mL for 24 h. During the treatment, LPS was added to each well to reach a final concentration of 100 ng/mL to stimulate nitric oxide (NO) production as a marker of inflammation. After incubation, the amount of NO produced was assessed in the culture supernatant by measuring nitrite levels using the Griess reagent (2% sulfanilamide in 4% phosphoric acid and 0.2% napthylethylenediamine) in a 1:1 ratio. The mixture was incubated at room temperature for 10 min., and the absorbance was measured at 540 nm using a microplate reader (EnSight, PerkinElmer, USA). Cell viability was also evaluated using the MTT assay.

Statistics analytical

The data were presented in mean ± standard deviation (SD) format by comparing the mean difference between the control group and the test group using one-way analysis of variance (ANOVA) statistics and testing the difference between the pairs by the Duncan's multiple range test has a $P < 0.05$ was considered statistically significant.

Results

Antioxidant activity

An active compound of *C. odorata* leaf showed antioxidative with 0.06-1.25 mg/ml for IC50 values after determined by DPPH scavenging, ABTS, FRAP, and NO inhibition assay.

Effect of C. odorata on NO production

The anti-inflammatory activity of *C. odorata* leaf extract was evaluated at concentrations ranging from 0.165 to 1.25 mg/mL by measuring nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophages. The extract significantly reduced NO production in a dose-dependent manner, with inhibition rates of 19.29%, 36.79%, 60.41%, and 67.30%, respectively (**Figure 1A**) . Additionally, cell viability assays revealed that none of the tested concentrations caused cytotoxic effects on RAW 264.7 cells (**Figure 1B**). These findings suggest that *C. odorata* leaf extract exhibits strong anti-inflammatory activity without inducing cytotoxicity.

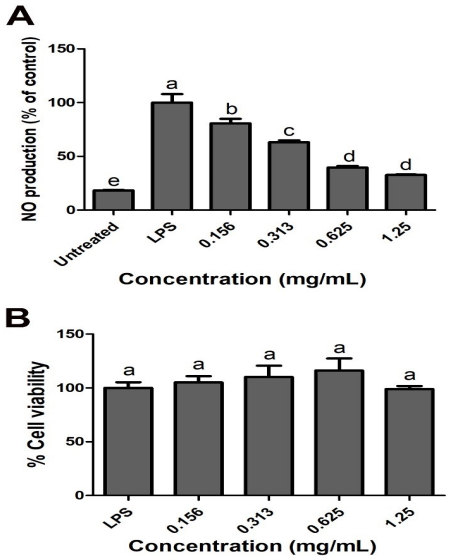

Figure 1. Effect of *C. odorata* leaf extract (0.156-1.25 mg/mL) on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells (**A**) and cell viability (**B**). Bars labeled with the same letters are not significantly different, whereas bars with different letters represent statistically significant differences ($P < 0.05$).

Hydrogel casting and structural characterization

The appropriate condition for hydrogel casting contains CMCs, polyvinyl alcohol, citric acid, and glycerol as **Figure 2**. Surface imaging using SEM revealed that the three film samples exhibited likely resulting from the inhomogeneity of the film prior to solidification. FTIR analysis of the samples revealed that the *C. odorata* leaf extracted primarily contains phenolic or aromatic groups. When the distribution of *C. odorata* leaf extract in the film was tracked using the integration value of the peak at wave number 1562-1625 cm^{-1}, which is the main peak coverage area of 1599 cm^{-1}, it was found that in the CMCs-2X and CMCs-10X samples, there was a distribution of *C. odorata* leaf extract throughout the film. In addition, it has been found that there are some areas of the film where there is a dense concentration of extracts.

Antioxidant activity hydrogel

The hydrogels containing the crude *C. odorata* extracts retained antioxidant activity, as determined by DPPH scavenging, although with reduced potential.

The effect of hydrogel film on Vero cells

Based on the cytotoxicity assessment of the films on kidney cell line (Vero) using the MTT assay at concentrations ranging from 125 to 1,000 µg/mL, it was found that the blank film, the film combined with 2× *C. odorata* leaf extract (2X), and the film with 10× extract (10X) all exhibited cell viability above 80% compared to the untreated control group. These results indicate that both the blank and *C. odorata* extract-loaded films are non-toxic to Vero cells as **Figure 3**.

The cytotoxicity of the films on mouse fibroblast (3T3-L1) cells was evaluated at concentrations ranging

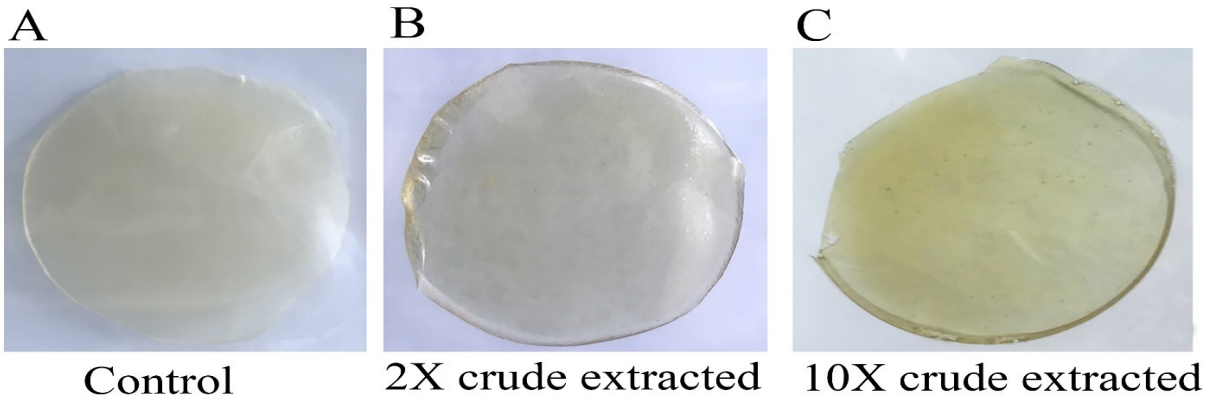

Figure 2. Hydrogel casting.

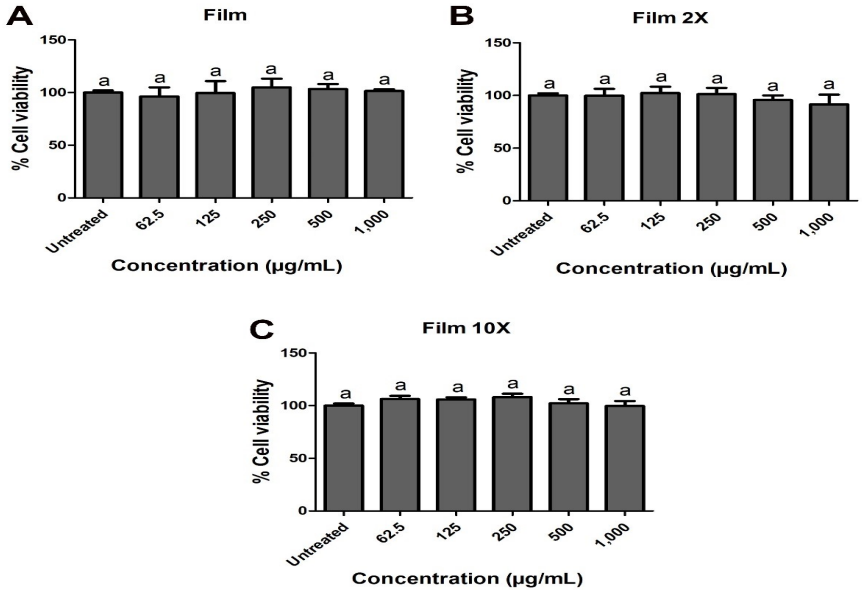

Figure 3. The effect of the hydrogel film on the viability of Vero cells was assessed at concentrations ranging from 62.5 to 1,000 µg/mL after 24 h of incubation. Cell viability was determined using the MTT assay (n = 4, mean ± SD) in **(A)** blank film **(B)** film 2X and **(C)** film 10X. Bars marked with the same letters indicate no statistically significant difference, while bars marked with different letters indicate statistically significant differences ($P < 0.05$).

from 125 to 1,000 µg/mL. The results showed that the blank film exhibited cell viability ranging from 80.71% to 81.46%. The film incorporated with 2X *C. odorata* leaf extract (2X) showed higher cell viability between 93.84% and 95.48%, while the 10X extract-loaded film (10X) demonstrated viability ranging from 87.14% to 82.90%, compared to the untreated control group. These findings indicate that all film types maintained cell viability above 80%, suggesting no cytotoxic effects on 3T3-L1 fibroblast cells as **Figure 4**.

Discussion

This research demonstrates the successful production of a biocompatible antioxidant-active hydrogel from durian rind-derived CMC incorporating *Chromolaena odorata* extract, showcasing the promising potential for biomedical applications such wound dressings or drug delivery systems.[9] This result correlated with report by Wahyuni HS, et al., which suggested that a friendly hydrogel synthesized from a natural polymer with sodium carboxymethyl cellulose (NaCMC) and co-polymerixed with PVA as similary to one used in this research.[10] From the results of previous study reported that the pulp, inner peel and seed of these durian varieties had antioxidant capacities which appropriated use to hydrogel or natural polymer for produced to would dressing.[11] The potential synergistic between durian hydrogel with bioactive compound as a *Chromolaena odorata* extract were success for combined. The study effectively utilizes agricultural waste, durian rind, as a valuable cellulose source, contributing to sustainable hydrogel production and addressing environmental concerns and promoting circular economy principles.[12] *Chromolaena odorata* extract, a natural antioxidant known for antimicrobial and wound-healing properties, enhances the hydrogel's therapeutic potential by reducing oxidative stress and inflammation.[13]

Conclusion

This study found that hydrogels containing the crude *C. odorata* extracts were developed as prototypes, which can be further optimized and utilized in the future to fully realize their therapeutic potential.

Acknowledgments

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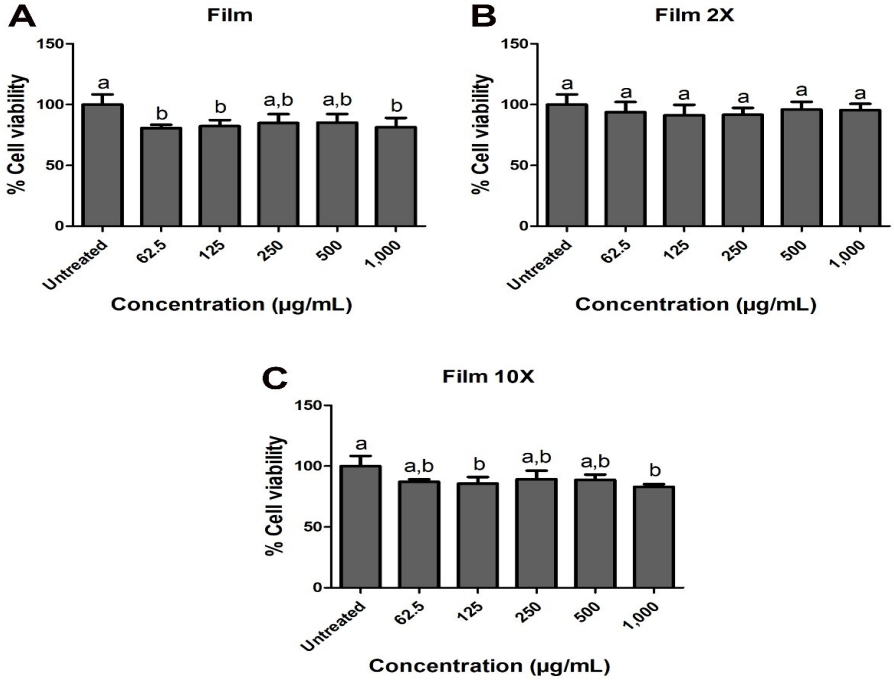

Figure 4. The effect of the hydrogel film on the viability of 3T3-L1 cells was assessed at concentrations ranging from 62.5 to 1,000 µg/mL after 24 h of incubation. Cell viability was determined using the MTT assay (n = 4, mean ± SD) in **(A)** blank film **(B)** film 2X and **(C)** film 10X. Bars marked with the same letters indicate no statistically significant difference, while bars marked with different letters indicate statistically significant differences ($P < 0.05$).

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data sharing statement

All data generated or analyzed during the present study are included in this published article. Further detail are available for noncommercial purposes from the corresponding auther on reasonable request.

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