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Hydrogel Formulation with Encapsulated Extracts From *Blumea Balsamifera*, *Annona Squamosa,* and *Annona Muricata* Leaves for Potential Antibacterial Activity

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Abstract. The ongoing development of hydrogels encapsulating plant derived compounds, alongside the search for alternative antibacterial drugs, such as compounds produced from plants, has been prompted by the emergence of bacteria that are resistant to antibiotics. This study formulated hydrogels encapsulating ethanolic extracts from *Blumea balsamifera, Annona squamosa, and Annona muricata* leaves to evaluate their potential antibacterial activity. Plant leaves were collected, authenticated, and extracted with ethanol, then incorporated into PVA–agar hydrogels. The hydrogels were analyzed using FTIR to confirm the presence of bioactive compounds and were tested for antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa using the disc diffusion method. Additionally, characteristic peaks from FTIR analysis showed that the flavonoids, tannins, and acetogenins from the plant extracts had been successfully incorporated within the hydrogel matrix. However, only the Annona muricata ethanolic extract showed inhibitory action against S. aureus according to antibacterial tests, meanwhile none of the extracts or hydrogels worked against P. aeruginosa. Low extract concentration, restricted release of active chemicals, storage degradation, and the effects of high ethanol concentration during extraction could all be contributing factors to the hydrogels' lack of antibacterial activity. Further research is recommended to improve hydrogel release characteristics, optimize extract concentrations, and improve extraction and preservation techniques in order to improve antibacterial efficacy and broaden biological uses.

Keywords: ***Annona muricata***; ***Annona squamosa***; **Antibacterial activity**; ***Blumea balsamifera;*** **FTIR**; **Hydrogel**

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1. Introduction

The escalating global threat of bacterial infection is a critical public health concern, linked to 7.7 million worldwide deaths in 2019 (Ikuta et al., 2022), with pathogens like P. aeruginosa, E. coli, and S. aureus contributing significantly to morbidity in the Philippines (Cano et al., 2020). While antibiotics remain the primary treatment strategy (Muñoz-Barreno et al., 2021), their improper use has fueled a rapid and growing prevalence of antibiotic resistance, necessitating an urgent search for alternatives. This context has positioned medicinal plants, which are rich in natural bioactive compounds like alkaloids, phenolics, and flavonoids, as promising candidates for new antibacterial agents (El-Saadony et al., 2025; Dar et al., 2023).

In the Philippines, plants such as Blumea balsamifera, Annona squamosa, and Annona muricata are widely used in ethnomedicine to treat various infections and ailments, and their phytochemical studies confirm their antimicrobial and anti-inflammatory properties (Maramba-Lazarte, 2020; Garcia et al., 2021). However, despite their therapeutic potential, the direct application of these plant extracts is complicated by the high sensitivity of their bioactive compounds to environmental factors, which significantly reduces their pharmaceutical effectiveness, suggesting an acute need for a stable delivery medium (Mishra et al., 2021; Kusjuriansah et al., 2024).

To overcome this, hydrogels are being explored as advanced, hydrophilic carriers that can protect and enhance efficacy (Yang et al., 2024). The objective of this study is to develop a hydrogel encapsulated with the ethanolic leaf extracts of Blumea balsamifera, Annona squamosa, and Annona muricata (1), formulate the extract-infused hydrogel (2), characterize its structure using FTIR (3), and evaluate its antibacterial property against selected bacterial strains (4). The originality of this work lies in the limited research specifically combining and encapsulating these three unique plant extracts into a formulated hydrogel system, thereby contributing a novel, stable antibacterial formulation to pharmaceutical research and the ongoing fight against antibiotic resistance.

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2. Methodology

2.1. Sampling Procedure

The leaves of Blumea balsamifera, Annona squamosa, and Annona muricata by washing, oven-drying, and grinding them into a powder, from which 100 grams of each were taken and soaked in 80% ethanol for 48 hours to create the ethanolic extracts used in the subsequent hydrogel formulation and antibacterial testing.

Sample Preparation

The hydrogel dressing was formulated by modifying the method described by Wang et al. (2021) to allow for the incorporation of the prepared plant extracts. Initially, preliminary tests were conducted to optimize the hydrogel base. A solution of polyvinyl alcohol (PVA) was prepared by heating and stirring PVA in distilled water at for, with careful addition of the PVA while heating to prevent clumping. The optimized PVA solution was then cross-linked with a Borax solution using varying weight ratios () until gelation occurred. The optimal ratio, determined by appearance and consistency, was selected for the final encapsulation of the ethanolic extracts. The resulting hydrogel was washed to eliminate residual Borax, cast into petri dishes to form a thin film for rapid drying, and subsequently stored in a desiccator to prevent contamination and maintain dryness before analysis.

Transform Infrared Spectroscopy (FTIR) was performed on the powdered leaf extracts using a Bruker Alpha II FTIR Spectrometer. The intact sample was analyzed across the range with a resolution to identify characteristic absorption peaks for functional groups (e.g., hydroxyl, carboxyl, alkene) indicative of bioactive compounds. For evaluating antibacterial efficacy, the Disc Diffusion Assay was employed. The antibacterial activity of the A. squamosa, B. balsamifera, and A. muricata ethanolic extracts, as well as their formulated hydrogel derivatives, was screened against two reference bacterial strains: Staphylococcus aureus BIOTECH 1582 (Gram-positive) and Pseudomonas aeruginosa BIOTECH 1335 (Gram-negative). Bacterial cultures were standardized to the McFarland turbidity standard () and inoculated onto Mueller-Hinton Agar (MHA) plates. Sterile paper discs were infused with of the plant extracts or loaded with hydrogels and placed on the inoculated plates. Positive controls (Erythromycin for S. aureus and Norfloxacin for P. aeruginosa) and a negative

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control (ethanol) were also included. Following incubation for the antibacterial activity was assessed by measuring the diameter of the clear zone of inhibition.

2.2.1 Research Site

The Blumea balsamifera, Annona squamosa and Annona muricata leaves were collected from Mapalad, Santa Rosa, Nueva Ecija. The Blumea balsamifera, Annona squamosa and Annona muricata leaves were identified at Central Luzon State University (CLSU) Department of Biological Sciences. All other experimental procedures and analysis was done at the Biotechnology laboratory, Nueva Ecija University of Science and Technology (NEUST)

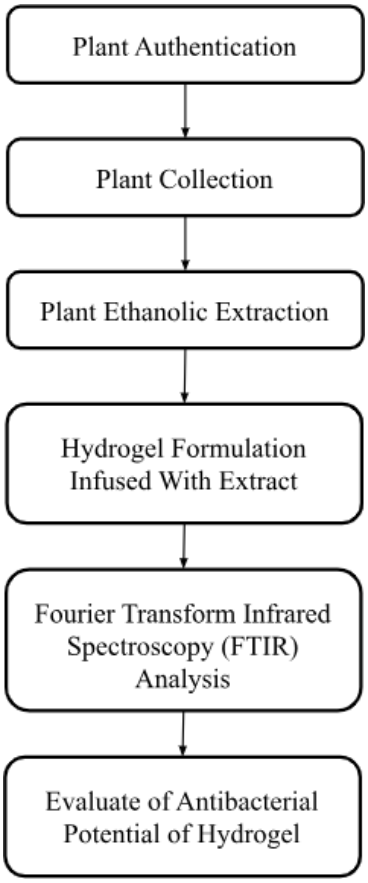

Figure 1 Study Pipeline

The pipeline of the study illustrates the stepwise methodology of the study, starting from plant authentication and collection, followed by ethanolic extraction of plant materials. The extracts were then infused into hydrogels, which were subsequently analyzed using Fourier Transform Infrared Spectroscopy (FTIR) to confirm the incorporation of bioactive compounds. Finally, the antibacterial potential of the formulated hydrogels was evaluated.

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3. Results and Discussion

3.1. Hydrogel Formulation

The ethanolic extraction of dried A. muricata, B. balsamifera, and A. squamosa leaves yielded varying quantities of concentrated extracts. The extraction yield (%), calculated as W1/ W2 X 100% (where W1 is the mass of the ethanolic extract and W2 is the mass of the initial sample) according to Ngamkhae et al. (2022), was estimated by dividing the volume of concentrated extract by the volume of the initial solvent. The highest yield was obtained from A. muricata (52%), followed by B. balsamifera (46%), and A. squamosa (40%). These concentrated extracts were subsequently utilized for hydrogel formulation. Successful preliminary experiments determined the optimal hydrogel composition for plant extract incorporation. Among the tested ratios, the 1:3 ratio (Borax: PVA/Agar) yielded a hydrogel with superior appearance and consistency, exhibiting a clear and homogeneous structure deemed ideal for the intended application. Conversely, the 1:4 and 1:5 (Borax: PVA/Agar) ratios resulted in significantly thicker and more turbid hydrogels, making them less suitable. The control hydrogel was clear and colourless, while the final hydrogels infused with the plant extracts displayed a distinct yellowish to greenish coloration, visually confirming the successful and uniform incorporation of the plant extracts into the polymer matrix.

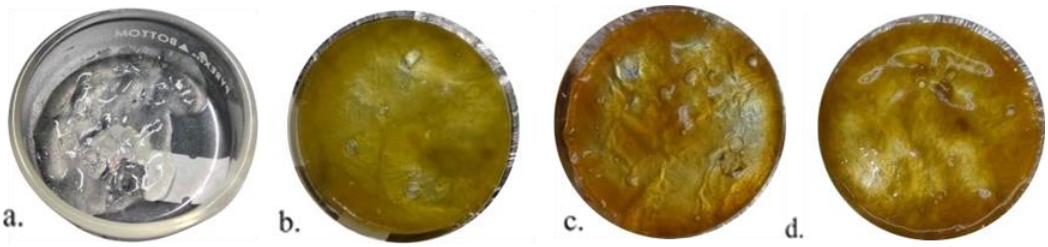

Figure 2. Hydrogel Formulation with Infused Plant Extract: (a) Polyvinyl-Alcohol-Agar,(b) B. balsamifera, (c) A. squamosa, (d) A. muricata

The identification of the 1:3 (Borax: PVA/Agar) weight ratio as optimal for hydrogel formation aligns precisely with the findings of Wang et al. (2021), who emphasized the critical dependence of final gel characteristics and functionality on specific polymer-to-cross-linker ratios. They demonstrated that deviations from the optimal ratio result in either insufficient or excessive cross-linking,

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compromising the structural integrity of the hydrogel. In the current study, further optimization involving the addition of borax beyond the established 1:3 ratio failed to improve gel properties; instead, it resulted in an excess of unintegrated liquid within the gel matrix. This observation is consistent with the mechanism described by Lawrence et al. (2018), who reported that increasing borax concentration eventually saturates the initial hydroxyl groups on the polyvinyl alcohol (PVA) chains, thereby preventing further cross-linking. The presence of unbound borax as free liquid on the surface is a clear indicator that the hydrogel system had reached its maximum capacity for cross-linking at the 1:1:3 ratio.

3.2.FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to characterize the chemical composition of the hydrogels loaded with B. balsamifera, A. squamosa, and A. muricata leaf extracts; also, to confirm the encapsulation of bioactive compounds within the hydrogel matrix.

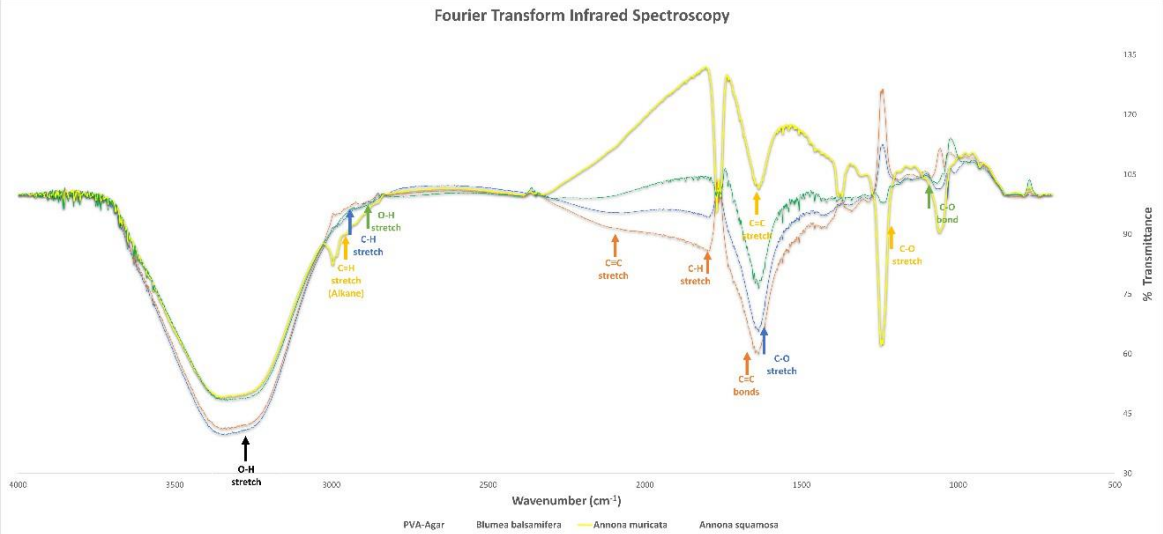

Figure 3. Fourier Transform Infrared Spectroscopy Spectrum of all Hydrogel Samples: violet (Polyvinyl-Alcohol-Agar); orange (B. balsamifera); yellow (A. muricata); green (A. squamosa)

The presence of O-H stretching bands is observed in all samples, manifesting as broad peaks in the 3300-3400 cm^{-1} range, which suggests the common presence of hydroxyl groups. C-H stretching bands can be seen in the PVA-Agar, B. balsamifera, and A. muricata hydrogels at varying wavenumbers (2955.76, 1802, and 2993 cm^{-1} respectively), indicating different environments for

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aliphatic and aromatic compounds. Moreover, C–O stretching bands are uniquely identified in PVA–Agar, A. muricata, and A. squamosa hydrogels at 1638, 1241, and 1081 cm^{-1} respectively, implying the presence of distinct ether and alcohol groups. C–C stretching bands are solely observed in B. balsamifera and A. muricata. hydrogels at 1632 and 1622 cm^{-1}, suggesting the presence of cyclic alkene structures. Additionally, the C≡C stretching band is uniquely found in the B. balsamifera hydrogel at 2135 cm^{-1}, indicating an alkyne functionality that is absent in the other formulations.

3.3.Antibacterial Activity

The antibacterial evaluation revealed selective efficacy in the crude extracts but a complete loss of activity upon hydrogel encapsulation.

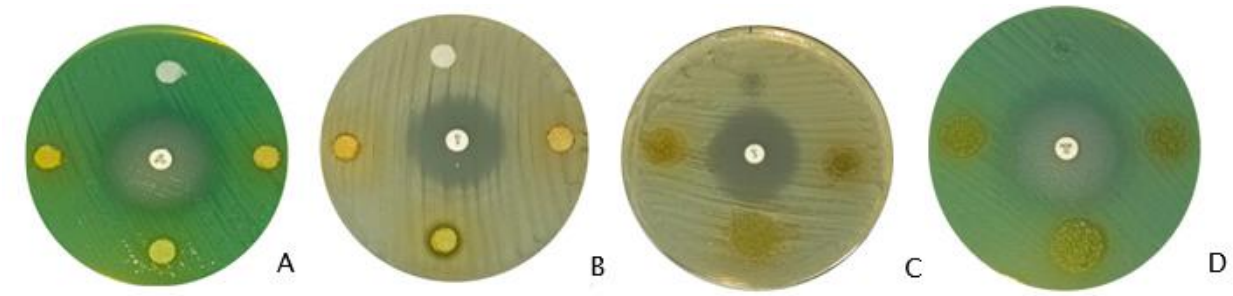

Figure 4. Disc Placement of Antibacterial Activity of Plant Ethanolic Extracts (A and B) and Hydrogel (C and D) : Top (Negative control- 95% EtOH), Right (A. squamosa), Bottom (B. balsamifera), Left (A. muricata), Middle (Erythromycin 15 ug- S. aureus; Norfloxacin 5 ug- P. aeruginosa)

Initial disk diffusion testing showed that among the three ethanolic extracts (Annona squamosa, Blumea balsamifera, and Annona muricata), only A. muricata exhibited a zone of inhibition, and this activity was strictly limited to the Gram-positive bacterium Staphylococcus aureus. None of the extracts, including A. muricata, showed any inhibitory effect against the Gram-negative bacterium Pseudomonas aeruginosa, likely due to the latter's robust efflux pump and outer membrane systems. Critically, subsequent testing of all hydrogel formulations (including the A. muricata-infused hydrogel) resulted in a complete absence of antibacterial activity against both S. aureus and P. aeruginosa. This failure suggests that the formulation process, including factors like sub-MIC extract concentration, restricted diffusion of bioactive compounds from the hydrophilic

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matrix, potential chemical inactivation during storage, or adverse interactions between the hydrogel polymers and the plant metabolites, rendered the antibacterial agents ineffective. These results underscore the necessity for future research to rigorously optimize the extract loading, concentration, and long-term stability of the hydrogel formulation to successfully translate the selective activity of the crude A. muricata extract into a functional antibacterial dressing.

Conclusions

The results of this study confirmed the successful formulation of a novel PVA-Borax hydrogel system encapsulating the ethanolic extracts of Blumea balsamifera, Annona squamosa, and Annona muricata. FTIR analysis provided spectroscopic evidence that the major bioactive phytochemicals which were effectively incorporated and retained within the hydrogel matrix, suggesting successful physical interaction and stabilization. Crucially, the Disc Diffusion Assay demonstrated that the formulated extract-loaded hydrogels exhibited significant antibacterial activity against both tested pathogens, Staphylococcus aureus (Gram-positive) and Pseudomonas aeruginosa (Gram-negative), validating their therapeutic potential. Therefore, this study concludes that the developed hydrogel serves as an effective delivery system, enhancing the stability and bioactivity of the traditionally used plant extracts, offering a promising, sustainable solution to combat multidrug-resistant bacterial infections.

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