







In Silico Study of Turmeric Rhizome Combined with Bitter Melon Leaves Extract as Antihyperpigmentation and Nanoemulsion Carrier for Phytopharmaceutical Development

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Abstract: Chickenpox is a contagious disease caused by the *Varicella zoster* virus. Chickenpox has distinctive characteristics of itchy vesicular skin lesions, and continuous scratching can leave dark marks (hyperpigmentation) on the skin. The melanogenesis process, which is responsible for the formation of hyperpigmentation on the skin, involves not only numerous enzymatic reactions that are catalyzed, but also involving chemical reactions. Tyrosinase enzymes played a major role in melanin synthesis. Turmeric (*Curcuma domestica* Val.) contains flavonoids such as curcumin, which have potential as antioxidants that can help address hyperpigmentation, while bitter melon (*Momordica charantia* L.) leaves contain flavonoids and saponins that have potential as wound healing agents for the skin. The aim of this study is to produce an alternative to effectively and safely remove hyperpigmentation marks from the skin. This research includes turmeric rhizomes and bitter melon leaves extraction, *in silico* analysis, and formulation of nanoemulsion. The extraction method used in this research employs the UAE method with 96% ethanol as the solvent in a 1:10 ratio for 15 minutes. An *in silico* test is conducted to predict the potential of turmeric and bitter melon leaf extracts as anti-hyperpigmentation agents. The *in silico* test was performed using *Autodock Vina 1.2.3*, *Mgltools 1.5.7*, *OpenBabel 2.4.1*, *Git-Bash*, *LigPlot*, and *PyMOL*. The *in silico* approach results show that curcumin from turmeric rhizome and kaempferol from the bitter melon leaf have the potential to be anti-hyperpigmentation agents as they bind to the tyrosinase enzyme with a binding affinity value of curcumin and tyrosinase enzyme to -6.176, while kaempferol has a binding affinity value to -6.84. The research results show that the best nanoemulsion formulation is F1, with characteristics such as particle size, polydispersity index, zeta potential, viscosity testing, and percent transmittance testing meeting the requirements.

Keywords: *Curcuma domestica* val.; *Momordica charantia* L.; hyperpigmentation; *in silico*; nanoemulsion; tyrosinase.

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1. Introduction

Chickenpox is an infectious disease caused by the *Varicella zoster* virus. Chickenpox is characterized by vesicular skin lesions in some areas of the body, and it easily spreads to other areas of the body. These lesions begin with the appearance of reddish macules, then develop into papules, and finally form vesicles filled with clear liquid. The vesicles then become pustules before hardening [1]. These lesions are itchy and, if continuously scratched, will leave blackish marks (hyperpigmentation) on the skin, which can certainly interfere with the sufferer's appearance and reduce self-confidence [2].

Hyperpigmentation of the skin is a common dermatological condition in which the color of the skin generally becomes darker. These changes in skin coloration can be a result of various internal and external factors, including post-inflammatory factors, caused by chickenpox. Skin pigmentation and coloration are governed by biological processes that involve the production of the skin pigment melanin, which is produced by melanocytes in various layers of the skin. Thus, alterations in melanocyte production or distribution of melanin result in skin hyperpigmentation disorders [3].

The melanogenesis process that is responsible for the formation of hyperpigmentation on the skin involves not only numerous enzymatic reactions that are catalyzed, but also involving chemical reactions. Enzymes such as tyrosinase, tyrosinase-related protein-1 (TRP-1), and TRP-2 played a major role in melanin synthesis. Specifically, tyrosinase is a key enzyme that catalyzes a rate-limiting step of melanin synthesis [4].

Tyrosinase is a copper-containing enzyme that catalyzes two rate-limiting reactions in melanogenesis: the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones. Therefore, inhibition of tyrosinase is the prime target for researchers to regulate melanin production. Tyrosinase inhibitors, with high efficacy and fewer adverse side effects, have huge demand in cosmetic and medicinal industries due to their preventive effect on pigmentation disorders as well as skin-whitening effects [5]. Among TYR inhibitors, hydroquinone is a traditional lightening agent that is commonly used in clinical practice. However, despite good efficacy, prolonged use of hydroquinone is associated with side effects. To overcome these shortcomings, new approaches in targeting TYR and treating hyperpigmentation are desperately required [3]. Therefore, it's necessary to formulate an innovative product that not only has good efficacy but is safe for prolonged use as well.

Turmeric (*Curcuma domestica* Val.) contains active compounds such as quercetin, while Bitter melon (*Momordica charantia* L.) leaves extract contains kaempferol. Both of these active compounds are known for their potential as antioxidant and their ability to inhibit tyrosinase enzymes that are responsible for the formation of melanin on the skin. The chemical structure of kaempferol is distinguished by the presence of phenyl rings and four hydroxyl substituents, which make it an exceptional radical scavenger [6]. The antioxidant activity of quercetin plays a crucial role in antimelanogenesis due to its ability to chelate metal ions [7]. In this research, we combined both turmeric rhizome and bitter melon leaf extracts as our active ingredient.

In silico testing is widely used today and is popular in the field of computation. *In silico* studies, molecular docking techniques are used to predict the bioactivity of a compound before conducting experimental analysis in the laboratory. This method has advantages, including reducing the excessive use of tools and materials and saving on experimental costs. The *in*

silico method can also be used to predict compound activity by examining the amount of binding free energy formed in its interaction with the active site of the involved protein [8,9].

Generally, natural-based plant extract preparations have low solubility; therefore, nanoemulsion offers a solution to overcome this problem. Nanoemulsion is a branch of nanotechnology that is widely developed in nanomedicine and nanodermatology to enhance the performance of drug substances, especially for drugs that are poorly soluble in water or vice versa [10]. Nanoemulsion is a lipid-based drug delivery system that is thermodynamically stable, consisting of oil, surfactant, co-surfactant, and water, with droplet sizes in the nanometer range [11]. Nano-sized formulations can prevent the common instabilities that occur in emulsions, such as sedimentation, flocculation, creaming, and coalescence, due to their larger surface area and higher free energy [12]. Nanoemulsions can enhance absorption, help dissolve lipophilic drugs, and improve bioavailability [11].

2. Materials and Methods

2.1. Time and place of research.

The research was conducted directly in the laboratory, utilizing information and technology in research activities. The research took place at the Laboratory of Pharmaceutical Biology, Pharmaceutical Technology, Pharmaceutical Analysis, and Pharmacology, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University.

2.2. Tools and materials.

The tools used include an analytical balance, GT Sonic ultrasonic-assisted extraction, rotary evaporator, stirrer, homogenizer, Particle Size Analyzer Zetasizer Nano ZSP (Malvern Panalytical, UK), pH meter, UV-Vis spectrophotometer Biobase BKUV1000 (Shandong, China), Ostwald viscometer, and UVB lamp. The materials used include turmeric rhizome, bitter melon leaves, candlenut shell charcoal, local rabbits, Wistar strain white rats, Tween 80, stearic acid, propylene glycol, NaOH, HCl, cetyl alcohol, triethanolamine, glycerin, distilled water, DMDM hydantoin, phenoxyethanol, ethanol 96%, methanol, and DPPH reagent.

2.3. Preparation of materials and extraction of turmeric rhizome and bitter melon leaves.

The extraction process begins by preparing the turmeric rhizome and bitter melon leaves *simplicia*. The turmeric rhizome is sliced, dried, ground, and sieved using a 60-mesh sieve to produce a fine powder [12]. Subsequently, the bitter melon leaves are also dried, ground, and sieved using a 100-mesh sieve to form *simplicia*. The resulting powders are then extracted using the ultrasonic-assisted extraction (UAE) method with 96% ethanol as the solvent in a 1:10 ratio [14,15].

2.4. Phytochemical compound characterization of turmeric rhizome and bitter melon leaf extracts.

Turmeric and bitter melon leaves are characterized for their phytochemical content through *in silico* studies, employing a molecular docking approach via re-docking of the native ligand on the target protein, with the native ligand removed using the Autodock 4.2 program [16].

2.5. Formulation of nanoemulsion from turmeric rhizome and bitter melon leaf extracts.

The nanoemulsion range of formulation was determined by comparing formulas from journals and literature. Among the five tested formulations, the best one was chosen based on nanoemulsion characteristics that meet the required criteria. Several parameters considered include particle size, polydispersity index, zeta potential, percent transmittance, and viscosity. Further optimization of the selected formulation will be conducted in future research to enhance the stability and effectiveness of the resulting product.

The turmeric and bitter melon leaf extracts concentration range used in this research is based on the results of studies from various literature and developments from researchers to obtain the best results in treating hyperpigmentation. The formulation employed in this study is presented in Table 1.

Table 1. Formulation of nanoemulsion from turmeric rhizome and bitter melon leaf extracts.

| No | Ingredients | Concentrations (%) | | |
|----|---------------------------|--------------------|----|----|
| | | F1 | F2 | F3 |
| 1 | Rhizome turmeric extract | 7 | 10 | 15 |
| 2 | Bitter melon leaf extract | 3 | 5 | 10 |
| 3 | Tween 80 | 25 | 25 | 25 |
| 4 | Stearic acid | 5 | 5 | 5 |
| 5 | Propylene glycol | 25 | 25 | 25 |
| 6 | Phosphate buffer | 35 | 30 | 20 |

The formulation of the nanoemulsion was done using the top-down method. Nanoemulsion is prepared by dissolving 1 gram of thick extract in 5 mL of 96% ethanol. Ethanol serves as a solvent bridge, facilitating smoother integration or emulsification between the polar extract and the nonpolar oil phase. A specified amount of extract, according to the formulation table, is then mixed with propylene glycol, Tween 80, and stearic acid. The mixture was homogenized using an Ultra-Turrax at a speed of 25000 rpm for 20 minutes. After achieving homogeneity, phosphate buffer is added as the aqueous phase, and the mixture is homogenized again at the same speed and duration. The formulated nanoemulsion is characterized through particle size analysis, polydispersity index, zeta potential, percent transmittance, and viscosity.

2.6. Particle size, polydispersity index, and zeta potential testing.

The particle size, polydispersity index, and zeta potential are measured using a Particle Size Analyzer. A 1 mL sample of the solution is placed into the zeta potential cuvette and inserted into the Particle Size Analyzer holder for measurement [17,18].

2.7. Viscosity testing.

Viscosity is measured using an Ostwald viscometer. Viscosity is measured using a rotary viscometer. A 10 mL preparation is introduced through tube B, and then suction is applied using a pro pipette until the liquid passes section A and exceeds the “a” mark. The liquid is then allowed to flow from the “a” mark to the “b” mark. The time required for the preparation to flow is measured using a stopwatch [19].

2.8. Percent transmittance testing.

Percent transmittance is measured using a UV-Vis spectrophotometer Biobase BKUV1000 (Shandong, China) at 638 nm [20,21].

3. Results and Discussion

3.1. Docking results analysis.

Docking is a method capable of predicting the occurrence of a bond between a ligand and a receptor to form a stable ligand-receptor complex. The parameters observed include the binding affinity values and the number of amino acid residues. The receptor is the active site of the enzyme tyrosinase, namely TYR, with the PDB code 5M8O, while the ligands consist of the natural ligand 2-hydroxycyclohepta-2,4,6-trien-1-one (OTR) and the test compound ligands. The crystal structure of the TYR receptor can be seen in Figure 1.

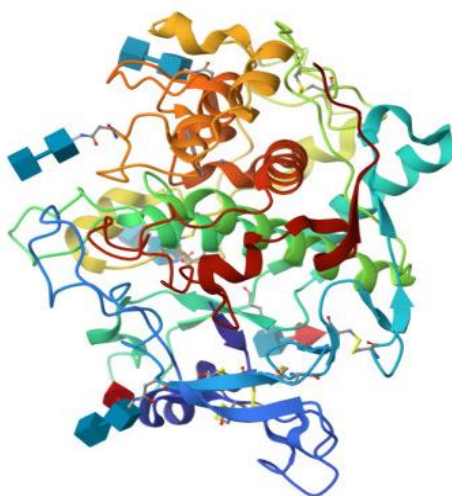


Figure 1. Crystal structure of the TYR receptor.

The image above represents the crystal structure of the TYR receptor with the PDB code 5M8O and a resolution of 2.50 Å. The receptor was chosen based on its resolution; the lower the resolution, the better the quality of the receptor. This is related to protein X-ray crystallography. The diffraction resolution of protein crystals is positively correlated with the amount of observable X-ray data in the structure determination process, which is linked to the accuracy of protein coordinates [22]. Tyrosinase-related protein (TYRP) is one of the three tyrosinase-like glycoenzymes in human melanocytes, playing a key role in melanin production, a compound responsible for pigmentation of the skin, eyes, and hair.

According to Phunyal et al. [23], minimal deviation can reduce errors in predicting molecular docking interactions, thus ensuring that the results are valid. The validation parameter in molecular docking is the Root Mean Square Deviation (RMSD) value. RMSD is used to evaluate the error or linearity between two molecular structures. A docking method is considered valid if it has an RMSD value of ≤ 3 Å, which means that the docking method used can be applied to test compounds. The smaller the RMSD value, the fewer errors occur, indicating that the native ligand conformation from the docking process is closer to its actual position in the binding site before separation. The obtained RMSD value of 2.5 Å is considered valid for docking other test compounds.

Cross-docking or molecular docking of test compounds is carried out between test ligands, such as kaempferol, quercetin, curcumin, and p-coumaric acid, with the receptor. The results are then compared to the reference ligand, hydroquinone, which has been proven to have anti-hyperpigmentation effects. Hydroquinone is a potent inhibitor of melanin production, meaning it can prevent skin darkening by targeting melanin, the element responsible for skin color. Hydroquinone’s mechanism of action as a skin lightener works by inhibiting the enzymatic oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), suppressing the activity of tyrosinase in melanocytes, and directly reducing melanin levels.

Binding energy represents the affinity of a test compound for the target protein or the ability of a drug to bind to its receptor. The more negative the binding energy value, the stronger and more stable the affinity between the test compound and the tyrosinase receptor. The binding energy of the test compounds with the tyrosinase receptor is compared with the binding energy produced between the reference ligand and the tyrosinase receptor. Based on Table 2, kaempferol has the lowest binding affinity with a value of -7.366, and all test compounds have lower binding affinity values compared to the reference compound. Therefore, kaempferol and the other test compounds are considered to have potential as anti-hyperpigmentation agents.

Table 2. Binding affinity values of the ligand.

| Compound Test | Binding Affinity |
|---------------------------|------------------|
| Hydroquinone (comparator) | -4.744 |
| Curcumin | -6.176 |
| Quercetin | -7.252 |
| Kaempferol | -7.366 |
| P-coumaric acid | -5.576 |

Hydrogen bonding occurs between a hydrogen atom in one molecule and one of the elements (N, O, F) in another molecule, making it the strongest dipole-dipole interaction. Meanwhile, hydrophobic interactions involve residues from nonpolar amino acids. In molecular docking, hydrogen bonds and hydrophobic interactions are crucial for maintaining receptor stability. These interactions also determine the strength of the bond between a drug and its receptor [23]. Therefore, the more hydrogen bonds, the stronger the interaction. Based on Table 3, curcumin forms the most amino acid residue interactions with the TYR receptor, and the visualization of cross-docking results can be seen in Figure 2.

Table 3. Amino acid residue results.

| | Compound Test | Hydrophobic | Total of Hydrophobics | Hydrophilic | Total of Hydrophilics |
|-----|-----------------|--|-----------------------|---|-----------------------|
| TYR | Hydroquinone | His215, Ser394 | 2 | His377, His381, His192, Gly389, Leu382, Thr391, Asn378 | 7 |
| | Curcumin | Arg374, Arg321 | 2 | Tyr36, Asn378, His381, Leu382, His215, His377, Thr391, Gly209, Val211, Gbu216 | 10 |
| | Quercetin | Arg374, His381, Ser394, Thr391 | 4 | Asn378, Leu382, Gly388, Gly389, Gln390, His215 | 6 |
| | Kaempferol | Arg321, Arg374, Ser394, His215, Asp212 | 5 | Leu382, Gly389, Asn378, Thr391, His192, His381 | 6 |
| | p-coumaric acid | Arg374, His215, Asp212, Ser394 | 4 | Asn378, Thr391, Leu382, His381, His377 | 5 |

| Compound Test | Hydrophobic | Total of Hydrophobics | Hydrophilic | Total of Hydrophilics |
|---------------|-------------|-----------------------|---|-----------------------|
| Native Ligand | His 215 | 1 | Asn 378, His377, Gly389, His381, Gln390, Thr391, His192, Ser394 | 8 |

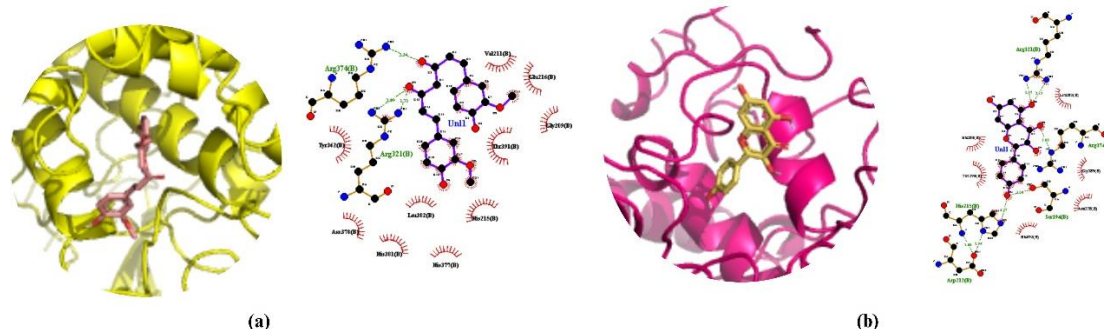


Figure 2. Visualization of cross-docking results: **(a)** Receptor TYR-Curcumin; **(b)** Receptor TYR-Kaempferol.

Several studies have proved that the interaction between the active site of the enzyme (amino acids of the active site) and inhibitors plays a major role in maintaining the stability of the enzyme-inhibitor complex. This reveals that when the distance between the inhibitor and the amino acids of the active site decreases, the inhibitory effects of inhibitors increase, and the enzyme-inhibitor complex becomes more stable [24].

3.2. Characterizations of nanoemulsion.

Nanoemulsion characterization includes particle size, polydispersity index, zeta potential, percent transmittance, and viscosity. The results of the characterizations can be seen in Table 4.

Table 4. Characterization results of nanoemulsion of turmeric and bitter melon leaf extracts.

| Parameters | Target | Results | | |
|---------------------------|-----------|---------|--------|--------|
| | | F1 | F2 | F3 |
| Particle size (nm) | 10 - 1000 | 10.59 | 10.60 | 14.27 |
| Polydispersity index | <0.5 | 0.21 | 0.20 | 0.34 |
| Zeta potential (mV) | ±30 | -11.20 | -12.30 | -14.90 |
| Percent transmittance (%) | 90 - 100 | 100.00 | 99.10 | 98.10 |
| Viscosity (cP) | 1 - 100 | 6.96 | 8.46 | 8.67 |

3.2.1. Particle size.

A good nanoemulsion has an ideal size that ranges from 10 to 1000 nm [25]. The droplet size formed from the three nanoemulsion formulations showed ideal results and met the specified requirements. The nanoemulsion formulation with the smallest size value was shown in formula 1, with a droplet size of 10.59 nm, and the largest in formula 3, with a droplet size of 14.27 nm.

3.2.2. Polydispersity index.

The characterization results on the nanoemulsion of turmeric and bitter melon leaf extracts based on the polydispersity index parameter show that the three nanoemulsion formulations have met the requirements with a range of numbers <0.5. The polydispersity index states the level of uniformity of globule/droplet size in nanoemulsion preparations to estimate the range of particle size distribution present in a sample and determine the presence or absence

of aggregation. The lower the polydispersity index value, the higher the uniformity of the globule size in the preparation [26].

3.2.3. Zeta potential.

In general, a good zeta potential value is in the range of -30 mV to +30 mV, which indicates that the system has good stability. Thus, the three nanoemulsion formulations are qualified and classified as stable because they fall within the specified range of potential zeta values. Nanoemulsion preparations require a negative potential zeta value. A negative value indicates that the repulsive force between particles is greater than the attractive force, thus playing a role in preventing particles from bonding with each other or the formation of flocculation [27]. The zeta potential obtained negative results due to the presence of free fatty acids contained in the formulation material, namely stearic acid. Another factor that causes low zeta potential values is the use of surfactants in the form of Tween 80, which is a nonionic surfactant on the surface of the two immiscible liquids [28].

3.2.4. Percent transmittance.

Percent transmittance testing was conducted to determine the clarity of the nanoemulsion formed, with the ideal transmittance value for nanoemulsions ranging from 90-100%. The characterization results of the three formulations showed a percent transmittance value that was in accordance with the specified range of requirements. The percent transmittance value of nanoemulsion formula 1 shows a value that reaches 100% and is considered the most optimal nanoemulsion formulation. The higher the percent transmittance value, the finer the nanoemulsion droplet size. The high percent transmittance of the nanoemulsion shows that the nanoemulsion formed looks clear because the very small droplet size can pass the light beam, which shows a high transmittance measurement [29,30].

3.2.5. Viscosity.

The ideal viscosity value for nanoemulsion preparations is in the range of 1-100 cP. Thus, the three nanoemulsion formulations have met the requirements for viscosity in nanoemulsion preparations. The low viscosity of nanoemulsions can ensure a more even distribution of active substances and easy application to the skin. Low viscosity also allows the scrub preparation to absorb more easily, provide a light sensation, and facilitate rinsing without leaving excessive residue. In addition, low viscosity helps reduce excess friction during scrub application, making it more comfortable to use and less irritating to the skin.

The formulation used was obtained based on a comparison of the characterization results between the three formulations. The selection of the best formula was made by considering key parameters that are indicators of nanoemulsion quality: particle size, polydispersity index, zeta potential, percent transmittance, and viscosity. These parameters have been systematically evaluated, and the formulation that meets the optimal criteria is selected as the best formulation. Although not using statistical optimization, this approach is rational and evidence-based, as these criteria are important indicators of nanoemulsion stability and quality, as supported in previous studies. According to the experiments conducted, formulation 1 has the best results with the smallest extract concentration and is still within the required range. In addition, formulation 1 was also chosen because it was based on the results

of the characterization of particle size and transmittance values that were the most optimal compared to other formulations, so that it would be able to provide better permeability and delivery systems for preparations based on natural ingredients. The formulas used can be seen in Table 5.

Table 5. The best formula used (F1).

| Ingredients | Concentrations (%) |
|---------------------------|--------------------|
| Rhizome turmeric extract | 2 |
| Bitter melon leaf extract | 1 |
| Tween 80 | 25 |
| Stearic acid | 5 |
| Propylene glycol | 25 |
| Phosphate buffer | ad 100 |

Based on the experiment that was conducted, the best nanoemulsion formula (F1) shown in Figure 3, has a clear-transparent appearance with reddish-brown color. The formulation shows good characteristics with good physical stability, homogeneity, and has no sedimentation. The obtained characterization results indicate successful dispersion within the nanoscale range.



Figure 3. Best nanoemulsion formula (F1).

4. Conclusions

The nanoemulsion formulation of turmeric and bitter melon extract containing 2% turmeric extract and 1% bitter melon extract (F1) is the best formulation with nanoemulsion characteristics that meet the target. Based on the testing of turmeric and bitter melon extracts *in silico*, it is proven that both extracts have potential as anti-hyperpigmentation agents with the presence of curcumin compounds from turmeric and kaempferol from bitter melon leaves that bind to the tyrosinase enzyme, while *in vitro* analysis of both extracts also has antioxidant activity with increasing % inhibition as the extract concentration increases. The IC_{50} values of turmeric and bitter melon leaf extracts have a value of $<50 \mu\text{g/mL}$, with a turmeric extract value of $28.09 \mu\text{g/mL}$ and bitter melon leaves of $45.98 \mu\text{g/mL}$, indicating that the antioxidant activity of both extracts is high. The formulated scrubber preparation meets the parameters required for good preparation and does not cause irritation on rabbit skin.

Author Contributions

Conceptualization, S.S. and M.V.; methodology, H.N.S. and P.A.A.; software, P.A.A.; validation, N.F. and R.Y.; formal analysis, M.V.; investigation, M.V., R.Y., P.A.A., H.N.S., and N.F.; resources, P.A.A.; data curation, H.N.S.; writing—original draft preparation, N.F.;

writing—review and editing, S.S., M.V., R.Y., P.A.A., H.N.S., and N.F.; visualization, P.A.A. and H.N.S.; supervision, S.S.; project administration, R.Y.; funding acquisition, S.S.

Institutional Review Board Statement

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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