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Journal of Advances in Medicine Science

https://ojs.bilpublishing.com/index.php/jams

ARTICLE

An In-Vitro Evaluation of the Efficacy and Functionality of Novel Contact Lens Solution Infused with Guava (Psidium guajava L.) Leaves Extract

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ARTICLE INFO

Article history
Received: 12 October 2025
Accepted: 15 December 2025
Published Online: 30 December 2025

Keywords:
microbial keratitis
antimicrobial efficacy
pathogens
ISO 14729
ISO 18259

ABSTRACT

Ensuring an effective antimicrobial activity in contact lens solutions is crucial, as microbial keratitis related to contact lenses can be caused by various pathogens, including Gram-negative and Gram-positive bacteria. Exploring the potential of plant-based antimicrobial agents, such as Psidium guava L. leaves, can lead to innovative solutions in the world of contact lens care. This research focuses on formulating a novel contact lens solution using guava leaf extract, evaluating its in-vitro antimicrobial efficacy, and assessing its impact on contact lenses and lens cases against common bacterial pathogens like Staphylococcus aureus, Pseudomonas aeruginosa, and Serratia marcescens. The study adhered to the standards set by the International Organization for Standardization (ISO) 14729 and 18259. In this quantitative experimental research study, the antimicrobial effectiveness of 45% hydroethanolic guava leaf extract was assessed. The results indicated a significant impact on various microorganisms, as determined by one-way ANOVA analysis. This extract with 0.35% concentration was incorporated as the antimicrobial agent into a novel contact lens solution formulation. The findings of this study demonstrated the remarkable antimicrobial efficacy of the solution against bacterial pathogens, as outlined in ISO 14729 and ISO 18259 for contact lenses and lens cases. These results highlight the good potential of guava extract as a disinfectant agent. By using the antimicrobial properties of guava leaves through the novel contact lens solution, this research has aimed to contribute to the development of safer and more effective plant-based contact lens solutions which can help prevent ocular infections and improve overall eye health.

0 INTRODUCTION

Contact lens solution is part of proper contact lens care designed to disinfect contact lenses (CLs). Solutions contain disinfectants to remove germs, bacteria, fungi, and other microorganisms that can cause infection (Zastrow, 2021). In clinical practice, contact lens-related conditions are commonly seen as numerous contact lens wearers

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 DOI: https://doi.org/10.26549/jams.v8i2.32801

do not provide proper care for their CLs and supplies as instructed hence increasing the risk of eye infections. To achieve the best contact lens care, it is necessary to find a balance between reducing ocular toxicity and ensuring effective disinfection against a range of ocular pathogens, particularly those commonly associated with contact lens-related diseases (Iguban et al., 2013).

When protein and other debris on CLs accumulate, they provide the proper conditions for microorganisms to grow and survive. There is an association with corneal infections and microbial contamination of CLs, which may be leading to serious health problems such as corneal ulcers and blindness. According to the American Academy of Ophthalmology, the most common bacteria that are contact lens-related and cause microbial keratitis are Pseudomonas aeruginosa and Staphylococcus aureus. Microbial keratitis commonly occurs as a potential complication of CL wear (Zimmerman et al., 2016). Based on another study by Eryilmaz et al. (2018), Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Moraxella catarrhalis, and Staphylococcus epidermidis are the most frequently related bacteria to biofilm associated eye infections in people.

Guava (Psidium guajava L.) is readily available to locals in tropical areas and possesses antibacterial properties. Different phytochemical elements in guava extracts are reported to have antimicrobial effects (Kumar, 2021). The results of a qualitative and quantitative phytochemical investigation showed that guava leaf extract contained a variety of polyphenols. Guava leaves were discovered to be high in phenols, flavonoids, and tannins, while being relatively low in alkaloids, saponins, and triterpenes. Given that polyphenols have significant antibacterial properties, it may be concluded that the antimicrobial properties of guava leaves are most apparently due to the plant's abundance in phenols, flavonoids, and tannins (Goswami & Das, 2019).

Contained in this study is information on how numerous contact lens care products are available depending on the patient's specific lens type, material, and other aspects, including replacement frequency, mode of wear, and patient profile. The most recent advancements in lens care influence how eye care professionals prescribe. The formulation of contact lens solutions is intricate and challenging, as its pharmacologic and antimicrobial efficacy would play a crucial role to the contact lens wearers' ocular health. Different properties and compounds are modified in an optimal formulation of a contact lens solution because they have various effects on the solution. A systematic approach is also used when identifying problems and patient symptoms during lens wear. This can lead to establishing their possible causes and resolving them. Possible interactions with the general properties of a solution with varying lens types can allow products to be used as recommendations or alternatives in certain conditions or situations. Regular assessment follow-up has an effect on patients as well as those patients' education, which is crucial for excellent patient compliance. A successful care system must achieve a balance between microbial efficacy, patient ease of use and compliance, and ocular toxicity.

With the growing market for contact lens use, various contact lens solutions are produced with different components for various purposes but currently, there is not yet any existing plant-based contact lens solution made in the Philippines that is composed of a purely organic antimicrobial agent. Hence, there are only limited studies that prove the antimicrobial efficacy of the said solution with guava leaf extract against the common contact lens infecting bacterial strains. It is of great interest to search for an effective plant-based antimicrobial component in contact lens solutions, as the current chemical solutions may have possible side effects to the human eye.

According to a study by Lin et al. (2016), chlorhexidine and polyaminopropyl biguanide had the best antistaphylococcal activity. Based on research, chlorhexidine may have anaphylactic effects, and may cause serious and permanent injuries to the eyes, ears and mouth. In addition, Lee et al. (2022) proved that polyaminopropyl biguanide has toxic effects to keratocytes and was observed to have acute toxic effects in human cells, specifically causing inflammation, atherogenesis, and aging. According to the research conducted by Saleh and Al-Mariri (2020), their study demonstrated that Psidium guajava L. has the potential to be utilized as a low-cost antibacterial agent, in which this finding suggests that P. guajava L. could serve as a valuable resource for future research in this field. In line with this, only one plant-based contact lens solution is available in the market, which is unfortunately not available in the Philippines. Thus, the researchers aim to present the first cost-effective, accessible, and functional plant-based contact lens solution available to the Philippine market.

This would all be carried out by developing a novel contact lens solution that is infused with guava leaves extract which was found to be a natural antimicrobial agent. This solution would be further tested in terms of its disinfection efficacy and lens cleaning capability based on the International Organization for Standardization (ISO). The formulation of the aforementioned solution may bring a better effect to the maintenance of the contact lens integrity and may have a stronger antimicrobial property,

DOI: https://doi.org/10.26549/jams.v8i2.32801

which would provide a compatible solution to the general public.

1. MATERIALS AND METHODS

The following tests and procedures were used to provide accurate quantitative data through laboratory experimentation and testing. These experiments included the formulation of a novel contact lens solution with guava leaf extract as disinfecting agent. Physical properties of the solution in terms of viscosity and pH level were tested as well as its chemical and antimicrobial properties. ISO standards were used to assess the antimicrobial activity of the solution against five common contact lens-related ocular pathogens.

1.1The Test Solution

The diluted guava leaves extract served as a substitute antimicrobial component for a standard contact lens solution formula and were subjected to laboratory testing to evaluate the intensity of its antimicrobial effect against test organisms as well as whether the presence of guava affected the physical and chemical properties of the overall solution.

1.2The Challenge Organisms

In light of the findings of the literature review, microbial keratitis commonly occurs as a potential complication of CL wear (Zimmerman et al., 2016). The American Academy of Ophthalmology claims that Pseudomonas aeruginosa and Staphylococcus aureus are the most common bacteria associated with contact lenses and are capable of causing microbial keratitis. Alongside the two was Serratia marcescens, which were used as the challenge organisms to evaluate the novel contact lens solutions' antimicrobial effectiveness against a variety of ocular pathogens, including both fungus and bacteria. The selection of these organisms was based on their similarity to the challenge organisms recommended by the International Organization for Standardization (ISO/CD 14729) for use with disinfecting solutions. To acquire standard isolates of the organisms, a collaboration with the Microbiology Section of the Institute of Ophthalmology at the University of the Philippines Los Baños was conducted.

1.3Antimicrobial Activity using the Agar Well Diffusion Assay Testing

The agar well diffusion assay is commonly utilized to assess the antimicrobial properties of plant-derived substances (Dahiya & Purkayastha, 2012). In this procedure, a bacterial isolate is combined with soft agar and poured onto a plate to solidify. Subsequently, wells are created using a 6mm sterile cork borer, into which test substances are introduced. Following an incubation period, the plate is examined for distinct zones of inhibition surrounding the wells.

1.4Antimicrobial Activity using The Stand-Alone Criteria

The antimicrobial activity was assessed in accordance with the methodology and acceptance standards for stand-alone disinfecting solutions. The ISO/CD 14729 that has been established by the International Organization for Standardization (ISO) states the microbiological requirements and test methods for products and regimens for the hygienic management of contact lenses.

According to the ISO's primary acceptance criteria for stand-alone disinfectants, a reduction of >3logs in bacterial count must be achieved during the recommended soaking time. It also includes a secondary criterion that requires a minimum of 5.0 log reduction in bacterial count for each of the three species, as well as a minimum of 1.0 log reduction for any single bacterial species within the recommended 100% soaking period specified by the manufacturer.

Contact Lens and Contact Lens Cases (ISO 18259)

For determining the suitability of contact lens solutions, lens cases, and hydrogel lenses for disinfection, ISO 18259:2014 provides a methodology for antimicrobial effectiveness end points. This is a procedure for assessing the suitability of disinfection solutions for use with contact lenses and lens cases using an antimicrobial efficacy end point. In particular, the microbiological effect of the antimicrobial agents was assessed while in the presence of the lens cases and/or lenses, as indicated in the soak stage of the label instructions.

The research protocol consists of three distinct phases: extraction, formulation, and evaluation. During the extraction phase, guava leaf extract was obtained through the use of solvent extraction. In the subsequent formulation phase, the guava leaf extract was incorporated into the contact lens solution along with various excipients and additives to ensure compatibility with the contact lens material. Finally, the study evaluated the antimicrobial efficacy of the contact lens solution that is infused with guava leaf extract, according to the guidelines with modifications set forth by the International Organization for Standardization (ISO 14729 and 18259). The aim

 DOI: https://doi.org/10.26549/jams.v8i2.32801

of the study was to develop a contact lens solution that is both safe and effective, while also harnessing the antimicrobial and antioxidant properties of guava leaf extract.

1.5 Secure Approval by Institutional Ethics Review Board

The researchers have requested the approval to conduct their study to formulate a contact lens solution from the Institutional Ethics Review Board (IERB) of Centro Escolar University. They sent the general protocol of the research which has been submitted and deliberated. The researchers have then received a certificate from the IERB.

1.6 Preparation and Working Conditions

Prior to commencing any task, hands were diligently washed using antibacterial soap, both before and after each procedure, to maintain optimal hygiene standards. To ensure overall cleanliness, a 70% isopropyl alcohol solution served as the primary disinfectant. Disinfection of the working area was accomplished by moistening a piece of tissue with the disinfecting solution and carefully swabbing the surfaces. Once the disinfectant had dried, laboratory work commenced. All assay procedures were meticulously carried out in a thoroughly disinfected environment, adjacent to a well-lit alcohol lamp, and ideally within a laminar flow hood.

2 Plant Phase

2.1Authentication of Plant Sample for Collection

Guava leaves extracts, both aqueous and organic, have been shown to exhibit antibacterial activity as they inhibit the growth of clinical isolates against clinical isolates that are antibiotic-resistant (Milyani & Ashy, 2011). The leaves of Psidium guajava L. were authenticated at the Jose Vera Santos Memorial Herbarium, Institute Biology, University of the Philippines Diliman.

2.2 Collection of the Psidium guajava L. Leaves

The fresh and healthy leaves of Psidium guajava L. were obtained from Paombong, Bulacan, Philippines. The collected guava leaf samples were stored in plastic zip lock bags with appropriate labeling. To ensure the freshness of the leaves, the zip lock bags were placed in an ice cooler upon collection and during transportation to the laboratory for extraction. The samples are rinsed and aired in the open air without direct sunlight for a week.

2.3 Plant Extraction

The dried leaves were put in a blender which was sieved by 0.425 millimeter until powder was formed and obtained. The powder weighed 100 grams and was added to an extracting solvent, hydroethanolic in which 95% ethyl alcohol was diluted with distilled water to make a 45% concentration. The findings of Seo et al. (2014) indicate that hydroethanolic extracts possess higher antioxidant properties when compared to water extracts. Notably, the 50% hydroethanolic extract demonstrated the highest concentration of phenolic compounds among the various solvents tested, including water, ethanol, and methanol.

The dried guava leaves submerged in the hydroethanolic solvent were stirred by a digital rotator for thirty minutes. The mixture is kept in a dark place for 24 hours. Afterwards, the guava extract solution was filtered through a Whatman No. 4 filter paper and made up to 225 mL. After a week, the filtrates were concentrated using a rotary evaporator at 50°C. The resulting filtrates were then placed in a steam water bath and allowed to stand for 30 minutes, and afterwards, it was stored at the laboratory in an upright chiller with a freezer at 2°C.

2.4 Phytochemical Analysis

According to Biswas et al. (2013), there are chemical tests available for screening and identifying bioactive chemical constituents in guava leaves. These tests were conducted using the extract and following standard procedures.

(1) Test for Flavonoids (Shinoda Test)

A volume of 1 mL of the extract was carefully transferred into a test tube, and then combined with 0.02 mg of magnesium ribbon fragments. Subsequently, 0.5 mL of hydrochloric acid (HCl) was added drop by drop. The anticipated outcome was the development of an orange, red, pink, or purple coloration, which would serve as an indication of the presence of flavonoids.

(2) Test for Phenols and Tannins (Ferric Chloride Test)

A volume of 1 mL of the extract was carefully transferred into a test tube, and subsequently combined with 2 mL of a 2% solution of ferric chloride (FeCl3). The anticipated outcome was the development of a dark blue or greenish-black color, which would serve as an indication of the presence of phenols and tannins.

(3) Test for Terpenoids (Salkowski's Test)

A volume of 1 mL of extract was carefully transferred into a test tube and subsequently combined with 2 mL of chloroform. Additionally, 2 mL of concentrated sulfuric acid was introduced into the mixture. As anticipated,

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the interphase displayed a distinct reddish-brown hue, confirming the presence of terpenoids.

(4) Test for Glycoside

A volume of 1 mL of extract was carefully transferred into a test tube, followed by the addition of 2 mL of glacial acetic acid containing 2 drops of 2% FeCl3. The resulting mixture was then carefully poured into another tube containing 2 mL of concentrated sulfuric acid. It was anticipated that a distinct brown ring would form at the interphase.

(5) Test for Saponins (Wet Foam Test)

The test involved the addition of 3 mL of extract to 10 mL of distilled water in a test tube. The tube was then tightly sealed with a stopper and vigorously shaken for a duration of 2 minutes. The anticipated outcome was the formation of a stable layer on the liquid surface, which would persist for 15 minutes, indicating the presence of saponins.

2.5 Zone of Inhibition Test for Antimicrobial Activity

The antimicrobial susceptibility testing was conducted using the agar well diffusion assay in accordance with the standards set by the National Committee for Clinical Laboratory Standards. This methodology was also aligned with the research conducted by Biswas et al. (2013). Following the extraction of guava leaves, the resulting extracts were applied to plates containing Mueller Hinton agar to assess their antibacterial properties. Subsequently, all plates were inoculated with the test bacteria by dipping a sterile cotton swab into the agar slant with inoculum.

The agar plate was evenly streaked across its sterile surface, ensuring uniform distribution of the inoculum as the plate was rotated. After inoculating the plates with bacteria, wells measuring 6 mm in diameter were created in the agar using a sterile cork borer. Following this step, the plates were left to dry for 5 to 10 minutes to remove excess moisture. Subsequently, fifty microliters of guava leaf extract were dispensed into each positive well after the plates were inoculated with bacteria. The same extract was used for all three plates dedicated to each bacterium. Control wells were also maintained for each bacterial strain, with distilled water added to the opposite side of the positive wells, spaced 2 inches apart. Once prepared, the plates were sealed, labelled, and placed in an incubator set at 37°C. After 24 hours of incubation, the plates were examined for inhibition zones. The diameter of these zones was measured in millimeters using a vernier caliper.

3 Formulation Phase

Formulation of the Novel Contact Lens Solution

The formulation of the novel contact lens solution with guava leaf extract was spearheaded by a registered chemist, under the assistance of the researchers. The standard components of the commercially available and medical grade contact lens solution, such as the carrier, preservatives, humectant, and protein deposit remover, were similarly incorporated in the said solution. The novelty of the formulation was brought about by the substitution of the common biocides or antimicrobial components such as polyhexamethylene biguanide and polyaminopropyl biguanide with the prepared guava leaves extract. The contact lens solution of interest was developed in such a way, in order to effectively isolate the antimicrobial efficacy of the guava leaves and to subsequently confirm the functionality of the novel formulation.

The procedure involved weighing all the necessary ingredients using an electronic balance with a readability of 0.01 grams. To ensure safety, the material safety data sheet (MSDS) was consulted for each raw material as a precautionary measure. The phase A ingredients, including aqua (carrier), sodium chloride, boric acid (preservatives), sorbitol, hyaluronic acid (humectant), and sodium citrate (protein deposit remover), were combined and thoroughly mixed until a uniform consistency was achieved.

Moving on to phase B, the guava leaf extract was added to the combined liquids, which consist of the hyaluronic acid solution and sorbitol. The extract was used in three different concentrations: 0.10%, 0.25%, and 0.35%. Subsequently, the solids, namely sodium chloride, sodium citrate, and boric acid, were blended with the liquids containing the guava leaf extract.

In phase C, the pH adjuster was not added to the ingredients as the pH of the concentrations was ideally around 7.3 to 7.8. The solution was then allowed to cure for a period of 5 hours, resulting in the production of the final products. By following these meticulous steps, we were able to create high-quality and safe lens care products.

4 Antimicrobial Evaluation Phase

4.1Secure Permission for Assessment at the Microbiology Laboratory

The test was carried out at the Centennial Laboratory of the Centro Escolar University, Manila to test

 DOI: https://doi.org/10.26549/jams.v8i2.32801

microorganisms to assess their antimicrobial potential and activity, according to a prior letter of request that has been delivered.

4.2 Sterilization of Materials

Glassware (pipettes, swabs, test tubes, petri dishes, Erlenmeyer flasks, vials, beaker), cork borers, inoculating loops, and needles were utilized in the study. These items were cleaned with soap and water before being allowed to air dry. In order to effectively destroy endospores and sterilize the materials, they were wrapped in clean papers and sterilized through autoclaving at a pressure of 15 psi and a temperature of 121°C for 15 minutes. The cotton swabs, inoculating loops and needles, cork borers, cotton, and cork stoppers were also wrapped in clean papers and sterilized. The materials were maintained in the paper wrappers until usage after sterilization.

4.3 Cultivation of the Test Organisms Obtained from Pure Cultures

Pseudomonas aeruginosa, Staphylococcus aureus, and Serratia marcescens, pure cultures of the test organisms, both gram-positive and gram-negative bacteria, were obtained from the University of the Philippines Los Baños. They were aseptically transferred to test tubes that contain sterilized nutrient broth. This was done to propagate and keep the test organisms alive.

Using a sterile inoculating loop, the test organisms were inoculated into each of the nutrient broths. The sterilized, cooled loop was dipped into each of the tubes to collect a loopful of pure cultures of the test organisms, and the loop was then immersed in sterile broth. The test tube mouths were flamed and closed with sterile cotton stoppers following the inoculation. Thereafter, until the following day, all of the inoculated broths were incubated at 35°C.

4.4 Preparation of Culture Media

40 grams of Tryptic Soy Agar (TSA) were dissolved in 1000 mL of distilled water in a beaker, and the resulting solution was placed into an Erlenmeyer flask for sterilization. The dissolved agar had undergone a 30-minute autoclave at 15 psi and 121°C. One hundred and sixty-two dry and sterile petri dishes have gotten a thin (15 mL) layer of freshly prepared agar in which they were poured during the procedure as it utilized a pour plate method.

4.5 Inoculation of Culture Media

The inoculation procedure was conducted in accordance with the guidelines outlined in ISO 14729. As part of this process, sample tubes containing 10 ml of the newly developed contact lens solution were prepared and then inoculated with challenge organisms for testing purposes. Specifically, Pseudomonas aeruginosa was introduced into the sample tube, resulting in a final count of 1.0×10^5 and 1.0×10^6 cfu/ml.

Similarly, following the protocols specified in ISO 18259, Pseudomonas aeruginosa was inoculated into both the test and control wells. Each well received 0.10 ml of the prepared inoculum suspension, resulting in a final count ranging from 1.0×10^5 to 1.0×10^6 per well. The inoculum was carefully dispensed onto the concave surface of the lens for the test wells, while the control wells received the inoculum directly into the well. Subsequently, all wells were securely covered. These standardized protocols were repeated using the other challenge organisms: Staphylococcus aureus and Serratia marcescens.

4.6 Determination of Antimicrobial Activity In-Vitro with Stand-Alone Test, Challenged with Bacterial Organisms (ISO 14729)

10 mL of the novel CL solution was added to the Pseudomonas aeruginosa strain in which they were mixed and stored at 25°C which was monitored. The mixture rested for 2 hours, then the 1.0 ml of the mixture was added to the neutralizing media. The TSA culture media and the sterilized equipment were used for the pour plate method. Serial dilutions are then performed as they are used to determine the concentration of microorganisms in a sample with an unknown concentration. This technique involves diluting the sample multiple times to create a series of solutions with decreasing concentrations.

The procedure was done until it resulted in the CFU of 1.0×10^4 to 1.0×10^5. For the inoculation, the petri dishes' lids were opened and 1 mL of the diluted sample were poured. The molten TSA was also heated a little, ensuring that it was below 50°C prior to pouring. Around 15 ml of the agar was poured into the sample and then the plate was solidified, inverted, and incubated at 37°C which is the optimal temperature for 20 to 48 hours. The entire procedure was conducted using the 2-hour and 8-hour waiting time frames. All of the mentioned steps were also repeated while using the bacterial strains of Staphylococcus aureus and Serratia marcescens.

4.7 Assess Contact Lens Care Products with Contact Lenses in a Lens Case, Challenged with Bacterial Organisms (ISO 18259)

The stand-alone test of ISO 14729 had successfully

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been completed, in which it indicated the need for the completion of ISO 18259. In this test, microorganism suspensions of S. aureus, P. aeruginosa, and S. marcescens were prepared at concentrations ranging from 1.0×10^7 to 1.0×10^8 colony-forming units (CFU) per milliliter.

Each lens was removed directly out of the blister pack prior to use in the assay. 3 lens case wells were prepared with lenses for test and another 3 case wells without lenses as controls. The lenses and case wells were both then inoculated to contain a final count of 1.0×10^5 to 1.0×10^6 CFU/mL of the tested microorganism. Test samples and no-lens controls were evaluated by determining the number of surviving microorganisms at the minimum regimen soaking times: ±10min and 24h±1h. An additional time point was evaluated which was 3h. The solution from both wells have been sampled at each time point. The bacteria were incubated for 24h at 37°C±1°C.

Following the incubation periods, plate counts were conducted and the numbers of organisms have been recorded in CFU. CFU/mL were calculated based on the average for duplicate plates. The average log reduction for the three replicate wells for each unique test sample was also calculated.

4.8 Microbiological Wastes Clean-up

All used agar cultures were disposed of according to the procedures in the bin marked "to be autoclaved." The glassware was immediately decontaminated by being placed in the autoclave (15 psi at 121 °C) for 30 minutes to 1 hour, and then they were cleaned of any remaining agar using a brush and detergent solution. All used agar media were disposed of right away.

4.9 Data Analysis and Interpretation

There was a designated statistician that utilized the one-way analysis of variance (ANOVA) to demonstrate the in-vitro antimicrobial efficacy of the novel contact lens solution against the three bacterial organisms. Log reduction was used to quantify the reduction in the number of living microbes following disinfection and provides a measure of the effectiveness of the disinfection process in eliminating the bacterial strains. Post-hoc Tukey test was also used for the comparisons for bacterial strain.

5. RESULTS AND DISCUSSION

5.1 Phytochemical Analysis of Hydroethanolic Psidium guajava L. Leaves Extract

The phytochemical analysis confirmed the presence of significant bioactive compounds in the hydroethanolic extract of Psidium guajava L. leaves, including saponins, phenols, tannins, flavonoids, terpenoids, and glycosides. These compounds are well-known for their antimicrobial properties, suggesting the extract has a broad spectrum of action against various microorganisms. This finding aligns with previous studies by Kumar et al. (2021) and Ratnarakan et al. (2020), which also identified these key phytochemicals in guava leaf extracts. The presence of these natural antimicrobial agents indicates the potential of guava leaf extract as an effective, natural alternative to traditional chemical preservatives in products like contact lens solutions, potentially reducing the risk of adverse reactions and promoting ocular health.

5.2 Antimicrobial Activity Evaluation

The antimicrobial efficacy of the hydroethanolic extract was evaluated against Pseudomonas aeruginosa, Staphylococcus aureus, and Serratia marcescens using the well-diffusion method. Results demonstrated that the extract possessed antibacterial effects against all three strains, though the level of effectiveness varied. The mean zones of inhibition were 17.00 mm for P. aeruginosa, 12.00 mm for S. marcescens, and 22.67 mm for S. aureus, while the negative control (distilled water) showed no inhibition. This variation in susceptibility is consistent with other studies, such as Sanches et al. (2005) and Khadka et al. (2019), which also reported on the antibacterial properties of guava extracts, noting that factors like the solvent used and the state of the leaves (fresh vs. dried) can influence activity.

A one-way ANOVA revealed a statistically significant effect of bacterial strain on the zone of inhibition (F-value = 257.923, $p < 0.001$), confirming that the type of bacterium significantly influences the extract's efficacy. Post-hoc Tukey tests provided a detailed comparison, showing that all bacterial strains treated with the extract had significantly larger zones of inhibition compared to the control (all $p < .001$). Furthermore, comparisons between the treated strains showed that S. aureus was significantly more susceptible than both P. aeruginosa (mean difference = 5.667, $p < .001$) and S. marcescens (mean difference = 10.667, $p < .001$). The low standard deviations indicated consistent effects across replicates. The superior activity against S. aureus, a Gram-positive bacterium, can be attributed to its cell wall structure, which is more permeable to antimicrobial compounds like flavonoids and tannins present in the extract, as supported by studies from Mailoa et al. (2014) and Ito et al. (2012).

5.3 Formulation and Physical Evaluation of Contact Lens Solution

A novel contact lens solution was formulated by

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incorporating different concentrations (0.10%, 0.25%, 0.35%) of the guava leaf extract. The objective was to identify the optimal concentration that balances antimicrobial efficacy with desirable physical and sensory properties, in accordance with the key components of standard lens care systems.

The physical evaluation of the formulations showed that as the extract concentration increased, subtle changes in appearance occurred. The 0.10% solution remained clear, while the 0.25% solution developed a slight champagne hue, and the 0.35% solution turned slightly yellow. Despite these color changes, all formulations maintained a good, consistent texture suitable for lens care and exhibited a neutral odor, which is preferable for user comfort.

The pH values for the three concentrations were measured at 7.3, 7.5, and 7.7, respectively. These values are all within or very close to the natural pH range of the human tear film (typically 7.0 to 7.4), indicating a low potential for ocular irritation and good compatibility with the eye. This is crucial, as deviations from this physiological range can cause discomfort and trigger reflex tearing, which reduces the bioavailability of the product. The stability of the pH across concentrations is advantageous.

Viscosity measurements showed a slight increase with higher extract concentrations: 0.97 for 0.10%, 0.99 for 0.25%, and 1.0 for 0.35%. All values are relatively low and within the desirable range for contact lens solutions (comparable to water's viscosity of ~1 mPa·s), ensuring adequate wetting, lubrication, and user comfort during lens application and wear.

5.4. Antimicrobial Efficacy Testing of the Novel Contact Lens Solution

The disinfecting capability of the novel solution was rigorously tested against high initial loads (>> $3.0x10^5$ CFU, often Too Numerous To Count - TNTC) of S. aureus, P. aeruginosa, and S. marcescens, in line with ISO 14729 standards.

For Staphylococcus aureus, a 2-hour exposure to the solution resulted in TNTC counts across all lots, indicating no significant reduction within this short timeframe. However, after 8 hours of exposure, the results were variable but promising. Lot 1 and Lot 3 showed no bacterial growth on most or all plates, while Lot 2 showed reduced but still present counts (0.7-1.5 x 10^5 CFU). This demonstrates that with sufficient exposure time, the solution can achieve effective disinfection against S. aureus. This aligns with the findings of Yahaya et al. (2019) and the biological explanation that Gram-positive bacteria like S. aureus are more susceptible due to their thick, absorbent peptidoglycan layer.

For Pseudomonas aeruginosa, the 2-hour exposure also yielded TNTC results and a 0% log reduction, failing the ISO stand-alone criterion (which requires a minimum 1-log reduction). After 8 hours, the results were mixed: Lot 1 showed complete inhibition (no growth), Lot 2 showed partial inhibition, and Lot 3 still showed TNTC. The average log reduction across lots was 0.48 (66.67%), which, while demonstrating a clear improvement over the 2-hour mark, still fell short of the ISO standard. Studies by Iguban et al. (2013) suggest that maximal effects against P. aeruginosa may require even longer exposure times (up to 12 hours). The antimicrobial action is linked to phytochemicals like flavonoids, which can disrupt the outer membrane of Gram-negative bacteria.

For Serratia marcescens, the solution showed the weakest performance. Both 2-hour and 8-hour exposures resulted in largely TNTC counts or very high quantifiable counts. The average log reduction after 8 hours was only 0.03 (6.67%), far below the required standard. This is consistent with the known multi-drug resistant nature of S. marcescens, which is resistant to many conventional antibiotics. The complex cell envelope structure of Gram-negative bacteria like S. marcescens presents a significant barrier to the antimicrobial compounds in the extract. Research by Górniak et al. (2019) suggests that flavonoids have a mode of action similar to β-lactam antibiotics, but higher concentrations or different extraction parts (e.g., bark) might be needed for effective action against this resilient strain.

5.5. Efficacy Testing on Lenses and Lens Cases

Further testing was conducted to simulate real-world conditions by inoculating silicone hydrogel lenses and lens case wells with S. aureus and P. aeruginosa, then soaking them in the novel solution for 10 minutes, 3 hours, and 24 hours.

For both S. aureus and P. aeruginosa, the shorter soaking times (10 min and 3 hrs) consistently resulted in TNTC counts on both lenses and in empty cases, confirming the solution's lack of immediate disinfection power. However, after a 24-hour soaking period, a significant and quantifiable reduction in bacterial load was observed.

For S. aureus, the average log reduction was 0.83 (85.33%) for tests with lenses and 0.89 (87.00%) for tests in empty cases. For P. aeruginosa, the average log reduction was 0.81 (84.67%) with lenses and 0.72 (81.00%) in empty cases. The slightly higher log reduction in the presence of lenses for P. aeruginosa was an

DOI: https://doi.org/10.26549/jams.v8i2.32801

unexpected result that may warrant further investigation, though the overall trend confirms that prolonged exposure is necessary.

Testing against S. marcescens on lenses and in cases confirmed its high resistance. Even after 24 hours, most plates still showed TNTC results, with an average log reduction of only 0.03 (6.67%) for tests with lenses and 0.22 (40.00%) for tests in empty cases. This consistently poor performance underscores the extreme challenge this bacterium poses.

Across all tests, a key observation was that the tests without lenses generally exhibited lower average log reductions compared to those with unused lenses. This correlates with the expected outcome, as contact lenses increase the surface area within the case, providing an environment that harbors microorganism growth and adherence, making disinfection more challenging.

6. CONCLUSION

Based on the objectives, results, and findings of the study, the following conclusions were drawn:

6.1A functional and effective novel contact lens solution infused with guava leaves extract was formulated using the biologically active guava extract and other necessary contact lens components, such as sodium chloride, sodium citrate, hyaluronic acid, boric acid and sorbitol. In line with this, the resulting novel contact lens solution was in par with the standard physical properties for commercially sold contact lens solutions, which include having a color of clear to pale yellow, good consistency, neutral odor, pH level of about 7.7, and viscosity of 1.0 mPa·s.

6.2The novel contact lens solution infused with guava leaves extract exhibited remarkable antibacterial activity against the strains of Pseudomonas aeruginosa, Staphylococcus aureus, and Serratia marcescens, however it fell short as a stand-alone and regimen disinfecting solution, with respect to the required log reduction in the bacterial category of the ISO 14729. Additionally, among the three test organisms, the solution of regard had the lowest antibacterial efficacy percentage against the Serratia marcescens strand due to its multidrug resistant categorization, and its ability to release beta-lactamase, as a potent gram-negative bacterium.

6.3In the practical setting, specifically with the actual involvement of contact lenses and contact lens cases, the novel contact lens solution infused with guava extract was proven to have a significant antibacterial capacity, specifically it was able to prove its strong antibacterial activity against the strains of Staphylococcus aureus and Pseudomonas aeruginosa, and minimal antibacterial capacity when subjected to Serratia marcescens. Furthermore, the novel contact lens solution was evaluated to be more active in the lens case environment without the contact lenses, in comparison to that with the presence of the lenses, which was the recommended outcome of the testing guidelines posed by ISO 18259.

7. RECOMMENDATIONS

In light of the study's findings and conclusions, these recommendations are formulated to enhance the quality and scope of the research study, offering avenues for improvement and exploration to ensure its robustness and relevance in the scientific community:

(1) Optometrists. The optometrists would have an option to prescribe alternative contact lens solution to patients with hypersensitivities towards the chemical antimicrobial agents in contact lens solutions, as a plant-based option is proven to protect the lenses, as well as the ocular surface, from unwanted bacterial pathogens. Moreover, they could gear towards plant-based and organic ophthalmic products, in general, in order to serve a wider range of people, and to reduce the toxic and allergic responses brought about by chemicals.

(2) Contact Lens Wearers. The contact lens wearers would have an alternative contact lens solution that they could try out, hence broadening their options and providing them with a safe and cost-effective choice.

(3) Department of Health (DOH). The Department of Health would be able to promote holistic contact lens care to a wider range of patients, with hypersensitivities or none, which would subsequently lessen the prevalence of unwanted ocular infections and inflammations. In line with this, overall hygiene and reliance on plant-based options could be commercialized through the novel contact lens solution infused with guava leaves extract, thus motivating a healthier population. Further, the presence of the said product strengthens the commitment of the DOH to nurture Filipinos to be part of the healthiest people in Asia by 2040, as the contact lens solution is equitable, health-centered and accessible in the Philippines.

(4) Contact Lens Solution Manufacturers. The contact lens manufacturers could expand their businesses to reach out to more patients by exploring the organic and plant-based world. With this, innovations could arise based on the different valuable components and phytochemicals within the various plant species. These companies can also offer collaborative opportunities to researchers and extend their expertise in evaluating, producing and enhancing contact lens solutions.

(5) Guava Farmers. The guava farmers can be able to contribute to the economic well-being of the guava farming community by providing access to plantations

 DOI: https://doi.org/10.26549/jams.v8i2.32801

and serving as resources to manufacturing companies. These farmers can also share their knowledge and expertise about guava cultivation to advise researchers about potential solutions.

(6) Future Researchers. Based on the analysis of the entire methodology and results of the study, the following recommendations are suggested by the researchers:

The current antibacterial testing could be performed with a more extensive time frame, as certain contact lenses are being left on their cases for a significantly short or possibly long period of time.

A clinical assessment of the antibacterial capacity of the novel contact lens solution must be performed. With this, the solution must be utilized by subjects, who are actual contact lens users, and the involved contact lenses and contact lens cases should be analyzed, with respect to the ISO 14729 and ISO 18259. This would necessitate a longer time frame; however, it is required to eliminate any toxic suspicions and to be approved as a safe solution for public usage.

Contact lenses are not only being infected by bacteria, but also by fungi and yeast, such as Candida albicans and Fusarium solani, thus intricate in-vitro and clinical trials must be conducted with the aforementioned microbes as challenge organisms. This would ameliorate the current contact lens solution from its antibacterial label to a total antimicrobial one. In addition, this step would be essential in acquiring an approval from the Philippine's Food and Drug Administration.

Insufficient research exists regarding the safety of utilizing methods to remove pigment or decolorize the green hue from guava leaf extract for application around the eye area, as well as its effect on the turbidity of the solution. Consequently, it is crucial to develop an environmentally friendly technique for eliminating chlorophyll from plant extracts. This will yield a colorless extract that maintains its antibacterial properties.

It is essential to ensure accurate colony counts during microbial testing. Researchers should employ serial dilution techniques to determine the countable plate, ensuring that each plate contains between 30 and 300 colonies for accurate assessment. This approach helps to avoid plates with colonies that are too numerous to count (TNTC) or too few for statistical significance.

The current study focused on Psidium guajava L. leaf hydroethanolic extract, future researchers could investigate other parts of the guava plant, such as the bark. Phytochemical screening has revealed that bioactive compounds were present like flavonoids, saponins, tannins, and phenols in the bark. Considering that the bark contains high levels of tannins, which are known for their antimicrobial properties, exploring bark extracts may lead to higher bioactivity against microbial growth.

Further optimize the concentration of guava leaf extract within the contact lens solution to enhance its effectiveness. Achieving a balance between immediate and sustained antibacterial action is crucial to guarantee the safeness and effectiveness of the solution for disinfecting contact lenses and their storage cases. Moreover, conducting research on the compatibility of ingredients to achieve synergistic effects is advised to improve the formulation.

It is essential to assess how different contact lens materials interact and the disinfection solution as the solution may alter the inherent wettability of the lenses, impacting the patient's comfort and performance. Furthermore, also consider the biocompatibility of contact lenses, which ensures they are non-toxic and generally well-tolerated, underscores the importance of these evaluations in ensuring the safety and efficacy of the solution in clinical settings.

Some studies have highlighted the antioxidant activity of guava leaf extract. The main phenolic compounds found in the extract pose a critical part in donating hydrogen or electrons, as well as forming stable intermediate radicals that prevent oxidation. While other researchers have explored antioxidant defense systems in the ocular surface to protect eye tissues from oxidative damage, there are inadequate studies about the possible benefits of guava leaf extract for promoting eye health and for utilizing ophthalmic solutions with an antioxidant. Further investigation into the use of the extract of guava leaves in supporting eye health is warranted to better understand its potential benefits in this area.

An alternative method for analyzing data to evaluate the antimicrobial efficacy of contact lens solutions is through the use of the Kruskal-Wallis H-test. This statistical test is particularly useful when dealing with small sample sizes or when the normality of the data is uncertain. The Kruskal-Wallis test is designed for comparing multiple independent samples, serving as a valuable way when the assumptions of a one-factor analysis of variance cannot be met. Unlike analysis of variance, the Kruskal-Wallis test is non-parametric, meaning it does not rely on the data being normally distributed. This makes it a versatile tool for researchers working with a variety of data sets.

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