

Journal of Advances in Medicine Science

Volume 8 · Issue 2 · December 2025 ISSN 2591-7609(Print) 2591-7617(Online)

Journal of Advances in Medicine Science

Volume 8 · Issue 2 · December 2025 ISSN 2591-7609(Print) 2591-7617(Online)



Tel: +65 65881289
E-mail: contact@s-p.sg
Website: ojs.s-p.sg

ISSN 2591-7609



9 772591 760250

Journal of Advances in Medicine Science

Aim and Scope

Journal of Advances in Medicine Science is an international, fully peer-reviewed journal covering all aspects of medical science, papers include fields of the basic and clinical medicine science research.

The mission of the Journal is to foster and promote multidisciplinary studies, especially the practice, policy and theory of medicine. Its purpose is to take the lead in timely publication in the areas of medicine. Making such information available will ultimately promote the publish and exchange views of new achievements in medicine.

The journal publishes regular papers, short communications, and review articles with a view of providing a focus for new information in all respects of medical science.

The scope of the papers in this journal includes, but is not limited to:

1. Medical theory research
2. Clinical medicine research
3. Medical practice
4. Health care medicine
5. Preventive medicine
6. Nursing medicine
7. Medicine and medicinal chemistry
8. Hospital management
9. Integrated traditional Chinese and Western medicine treatment
10. Health policy
11. Continuing medical education
12. Health management

Synergy Publishing Pte. Ltd.

E-mail: contact@s-p.sg

Official Website: www.s-p.sg

Address: 12 Eu Tong Sen Street, #07-169, Singapore 059819

Journal of Advances in Medicine Science

Volume 8 Issue 2 • December 2025
International Standard Serial Number: ISSN 2591-7609 (Print)
ISSN 2591-7617 (Online)

Editor-in-Chief: Nandagopal Sahoo, Kumaun University, India

Associate Editor: Deqin Geng, XuZhou Medical University, China
Jun Dou, Dongnan University, China
Austin Lin Yee, U.S.A

Editorial Board Members: Francesca La Carpia, U.S.A
Passali Giulio Cesare, Italy
Guanshui Bao, China
Jun Wang, China
Yi Yang, China
Aijiao Xiao, China
Boqing Li, China
Xinglun Liang, China
Xianbing Hou, China
Sumit Gupta, India
Simran Kaur, India
Qiushui He, Finland
Harmandeep Singh Chahal, India
Glauber Sa Brando, Brazil
Unai Azcarate, Spain
Lu Yang, China
Yos Adi Prakoso, Indonesia
Ling Wang, China
Sabri Uzuner, Turkey
Jing Wang, China
Tian Tian, China
Shaohe Wang, China

Copyright

Journal of Advances in Medicine Science is licensed under a Creative Commons-Non-Commercial 4.0 International Copyright (CC BY-NC4.0). Readers shall have the right to copy and distribute articles in this journal in any form in any medium, and may also modify, convert or create on the basis of articles. In sharing and using articles in this journal, the user must indicate the author and source, and mark the changes made in articles. Copyright © SYNERGY PUBLISHING PTE. LTD. All Rights Reserved.

Contents

- 1 Effect of traditional Chinese Medicine Rehabilitation Nursing on Early Functional Rehabilitation in Patients with Cerebral Infarction and Hemiplegia**
LIU Nan
- 6 Research on the Strategy of Optimizing Biochemical Experiment Schemes with DeepSeek**
ZHANG Ming PENG Ji
- 10 A Review of the Impact of Virtual Simulation and Real-Scene Interactive Training on Clinical Thinking Ability of Nursing Talents under the Background of Intelligent Services in Internet Hospitals**
LI Na DONG Jia GENG Gonglong DING Le LIU Fanghua
- 14 Progress and Prospects in graded Treatment for Adenomyosis**
WANG Yan XIAO Yanbing
- 23 Advancements in Drug Treatment for Adenomyosis**
Yang Guo Xiao Yanbing
- 31 An In-Vitro Evaluation of the Efficacy and Functionality of Novel Contact Lens Solution Infused with Guava (*Psidium guajava* L.) Leaves Extract**
Alajar, Alexandra Ysabel D., Babani, Jenisha Prakash, Evangelista, Chylyn Jane F., Ibasco, Shereen Rose C., Uy, Jomar Christian V., Gariando, Carol Fe A.

ARTICLE

Effect of traditional Chinese Medicine Rehabilitation Nursing on Early Functional Rehabilitation in Patients with Cerebral Infarction and Hemiplegia

LIU Nan

Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, 300,000

ARTICLE INFO

Article history

Received: 23 June 2025

Accepted: 15 December 2025

Published Online: 30 December 2025

Keywords:

Traditional Chinese Medicine Rehabilitation Nursing

Cerebral infarction

Hemiplegia

Early functional rehabilitation

ABSTRACT

Objective: To explore the effect of traditional Chinese medicine rehabilitation nursing on early functional rehabilitation of patients with cerebral infarction hemiplegia. **Method:** 72 patients with cerebral infarction hemiplegia (March 2024 March 2025) were randomly divided into two groups using the drawing method, with 36 samples in each group. The control group received routine nursing interventions, while the study group received traditional Chinese medicine rehabilitation nursing. The effects of nursing interventions were compared among groups. **Result:** Compared with the control group, the study group showed better recovery of limb motor function after intervention, with improvements in neurological function, self-care ability, and quality of life scores ($P < 0.05$). **Conclusion:** The use of traditional Chinese medicine rehabilitation nursing can help stroke patients with hemiplegia gradually recover neurological function remodeling and motor function, promoting substantial improvement in daily activity ability and quality of life.

1. Introduction

Cerebral infarction, as a severe cerebrovascular disease that poses a significant threat to human health, has a high incidence rate and a high disability rate, which brings a heavy burden to patients' families and society. Hemiplegia is one of the most common sequelae of cerebral infarction, which seriously affects the quality of life of patients^[1].

In recent years, with the development of rehabilitation medicine, early rehabilitation intervention has become an important means to improve the functional prognosis of patients with hemiplegia due to cerebral infarction. Traditional Chinese medicine (TCM) rehabilitation nursing, as a unique nursing model that combines traditional Chinese medicine with modern rehabilitation concepts, has demonstrated unique advantages in

*Corresponding Author:

Liu Nan (1986.08-), female, from Tianjin, Han nationality, undergraduate, nurse-in-charge, research direction is traditional Chinese medicine brain disease nursing, traditional Chinese medicine brain disease rehabilitation guidance, and traditional Chinese medicine ear acupoint application.

the rehabilitation of hemiplegia after stroke through syndrome differentiation nursing and holistic regulation^[2]. Therefore, this study explores the application effect of TCM rehabilitation nursing in order to optimize the early rehabilitation program for patients with hemiplegia due to cerebral infarction and improve the rehabilitation outcomes.

2. Materials and Methods

2.1 General information

Seventy-two patients with hemiplegia with cerebral infarction (March 2024-March 2025) were randomly divided into two groups using the lottery method, with 36 samples from each. There were 22 males in the control group and 14 females; the ages were 56 to 83 years old, with the mean value (62.53±8.77) years old; there were 21 males in the study group and 15 females; the ages were 55 to 82 years old, with the mean value (61.28±9.31) years old. The baseline data of the two groups were compared with $P>0.05$.

Inclusion criteria: ① Consistent with the diagnostic criteria of cerebral infarction; and confirmed by CT or MRI; ② The first onset and there are clear symptoms of hemiplegia, and the muscle strength is \leq grade 3; ③ The onset time is less than 2 weeks, and the vital signs are stable.

Exclusion criteria: ① Severe heart, liver and renal insufficiency; ② Malignant tumors; ③ There is a tendency to bleeding or abnormal coagulation function; ④ Severe aphasia or mental illness cannot cooperate; ⑤ Previous motor system diseases affect functions.

2.2 Method

The control group was given routine nursing interventions. (1) Basic nursing: The system of dynamic monitoring of vital signs was strictly implemented. Risk assessment of pressure sores was carried out. Patients were assisted to change their positions every 2 hours and were provided with pressure-relieving devices. Three-level respiratory tract management was conducted, which included comprehensive measures such as postural drainage, percussion and expectoration, and nebulized inhalation. At the same time, attention was paid to the placement of limbs in functional positions. Orthopedic supports were used to maintain the neutral position of joints to prevent contracture deformity and muscle atrophy. (2) Rehabilitation and nursing intervention: Early guidance for patients to recuperate. As the condition recovers, the rehabilitator will be given exercises. A systematic joint passive activity training is performed every day. The single training time is strictly controlled at

30 minutes. The training content includes abduction (0-90°), forward flexion (0-180°) and internal and external rotation (0-45°) training of shoulder joints, and the elbow joints complete the full range of flexion and extension; the wrist joints are subject to dorsal extension (0-70°) and palm flexion (0-80°) exercises. Each joint activity is performed according to the standards of 10-15 times/group, and the training intensity is dynamically adjusted according to the patient's tolerance. (3) Psychological support and health education: Structural psychological intervention twice a week, 20 minutes each time, cognitive behavioral therapy is used to improve negative emotions, cooperate with the demonstration of successful rehabilitation cases to enhance treatment confidence, carry out step-by-step health education, explain the disease mechanism in the early stage, guide family rehabilitation methods in the middle stage, and emphasize measures to prevent recurrence in the later stage.

Based on the above intervention, the research group carried out traditional Chinese medicine rehabilitation nursing: (1) Syndrome and nursing: Establish an individualized traditional Chinese medicine nursing plan, implement emotional and mutual benefit therapy for patients with hyperactivity of liver yang, adopt the regulation mechanism of anger to hurt the liver-sad to overcome anger, and cooperate with the Yongquan point of Wuzhuyu patch; patients with phlegm and blood stasis blocking meridians to strengthen the spleen and eliminate phlegm, and use Poria yam porridge with hawthorn to eliminate food and drink, and implement the meridian patience; patients with Qi deficiency and blood stasis use the nursing method to nourish the middle and replenish Qi, and use Astragalus Guizhi Wuwu Decoction Foot Bath and Shenque Point to separate ginger moxibustion. (2) Acupuncture treatment: Academician Shi Xuemin's "Wake the Brain and Open the Orifices" acupuncture method is improved. The head acupuncture point is mainly selected by the exercise area and sensory area, and combined with Baihui, Sishencong and other acupoints; the limbs are selected by the Yangming meridian, Quchi, Hegu, Zuyangming meridian, Fengguan, Zusanli, etc., and 0.30×40mm acupuncture needle is used to perform a flat and smooth replenishment technique. After obtaining qi, the G6805 electric acupuncture device is connected to the sparse and dense wave, with a power of 2/100Hz, and the stimulation intensity is based on the patient's tolerance. (3) Traditional Chinese medicine fumigation: Use intelligent fumigation therapy instrument (ZZ-2000 type) to carry out targeted transdermal administration. The medicine liquid prescription is based on "Medical Forest Remedy" Buyang Huanwu Decoction, which contains 10

medicinal materials such as 15g of *Angelica sinensis*, 10g of *Chuanxiong*, and 10g of safflower. After decorating, it is filtered and bagged. During treatment, the distance between the affected limb and the fumigation head is maintained at 15-20cm, and the steam temperature is constant at (50±2)°C. The PID temperature control system ensures uniform thermal penetration. After treatment, use infrared thermal imaging camera to evaluate the improvement of local microcirculation.(4) Tuina massage: The rolling technique was used to relax the area along the Gallbladder Channel on the affected side, combined with the one-finger Zen pushing technique applied to specific acupoints of the Yangming Channel. The kneading technique was used for spastic muscles at a frequency of 120 times per minute to reduce muscle tension. The point-pressing technique was applied to atrophic muscles to stimulate the meridian energy. Key acupoints such as Hegu and Zusanli were stimulated, with each acupoint receiving manual stimulation for 1 minute. The whole set of operations was carried out according to the rhythm of light-heavy-light stimulation.

2.3 Observation indicators

The limb function was assessed according to the Fugl-Meyer scale, with 66 points for the upper limbs and 34 points for the lower limbs.

The National Institutes of Health Stroke Scale (NIHSS) was used for assessment, with lower scores indicating better recovery. The Barthel Index was employed to evaluate the ability of self-care, with scores based on a percentage system.

The quality of life was assessed using the General Quality of Life Scale (GQOL-74), with each item scored on a percentage basis.

2.4 Statistical Methods

The data involved in the research were processed using SPSS26.0 software, and the measurement data was represented by “(±s)” and the inspection was carried out through “t”; the counting data was represented by “[n/(%)]” and the inspection was carried out through “χ²” and P < 0.05 indicated that the difference had obvious significance.

3. Results

3.1 Limb motor function score

As shown in Table 1: The study group recovered better limb motor function after intervention than the control group, with P < 0.05.

3.2 Neurological function and self-care ability score

As shown in Table 2: Compared with the control group, the study group’s scores of neurological function and self-care ability were both improved after intervention, with a comparison P < 0.05.

3.3 Quality of Life Score

As shown in Table 3: After the intervention, the quality of life score of the research group was higher than that of the control group, with a comparison P<0.05.

Table 1 Comparison of the functional scores of limbs in the two groups (±s)

Group	Number of examples	Upper limb motor function score		Lower limb motor function score	
		Before intervention	After intervention	Before intervention	After intervention
Control group	36	28.33±4.82	34.11±4.26	17.33±2.82	22.65±1.49
Research Group	36	28.22±4.74	41.75±5.68	17.22±2.74	25.38±2.24
t	--	0.0976	6.4563	0.0023	6.0885
p	--	0.9225	0.0000	0.9981	0.0000

Table 2 Comparison of the scores of neurological function and self-care ability of the two groups (±s)

Group	Number of examples	NIHSS score		Barthel index	
		Before intervention	After intervention	Before intervention	After intervention
Control group	36	14.56±1.81	10.44±2.74	29.39±3.48	66.14±3.25
Research Group	36	14.72±1.91	6.14±2.72	29.22±3.86	71.19±3.43
t	--	0.3648	6.6825	0.1963	6.4124
p	--	0.7164	0.0000	0.8450	0.0000

Table 3 Comparison of Quality of Life Scores between the Two Groups ($\bar{x}\pm s$, points)

Group	Number of examples	Psychological function		Material life		Somatic function		Social function	
		Before intervention	After intervention	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
		Control group	36	46.98±7.46	65.74±9.54	45.78±7.26	66.49±6.66	39.88±7.23	65.74±6.32
Research Group	36	46.87±7.37	72.61±8.63	45.94±8.32	73.16±8.41	39.59±8.28	75.26±5.41	43.28±7.27	73.46±6.43
t	-	0.0629	3.2042	0.0869	3.7305	0.1583	6.8660	0.4504	5.1435
p	-	0.9500	0.0021	0.9310	0.0004	0.8747	0.0000	0.6538	0.0000

4. Discussion

Hemiplegia caused by cerebral infarction is essentially due to ischemic necrosis of brain tissue caused by cerebral vascular occlusion, which leads to the interruption of the motor conduction pathway. When the middle cerebral artery supply area is affected, damage to the cortical motor area and pyramidal tract will directly cause motor dysfunction of the contralateral limb. Long-term immobilization can lead to secondary damage such as muscle atrophy and joint contracture, forming a vicious cycle of disuse and atrophy. The traditional rehabilitation nursing model does not accurately grasp the timing of intervention. Some hospitals still hold the concept of "bed rest," missing the golden recovery period within 3 months after onset. Moreover, the intervention methods are singular, mainly relying on passive joint movement, and lack an overall functional reconstruction strategy [4]. The fragmented nursing model cannot fully mobilize the patients' inherent rehabilitation potential, leading to the early appearance of the functional recovery plateau. Traditional Chinese medicine rehabilitation nursing, as an important part of the traditional medical system, is based on the theory of Yin and Yang, the Five Elements, and the holistic concept. By systematically applying traditional Chinese medicine theory to guide clinical practice, it helps patients to recover or improve physical functions [5].

The results show that the recovery of limb motor function in the research group is better than that in the control group after intervention, which indicates that traditional Chinese medicine rehabilitation nursing can effectively promote the remodeling of central nervous system function. The reasons are analyzed as follows: Acupuncture therapy is used to stimulate the acupoints in the motor and sensory areas, which can activate the neuroplasticity changes in the motor representation area of the cerebral cortex. The sparse-dense wave of electroacupuncture can simultaneously excite A β and C nerve fibers, promoting the secretion of neurotrophic factors. The dual effect of temperature and medicine in

traditional Chinese medicine fumigation can reduce blood viscosity. The medicinal components such as *Angelica sinensis* and *Ligusticum chuanxiong*, which are absorbed through the skin, can improve local microcirculation and provide a material basis for nerve repair. Tuina massage activates the feedback of muscle spindles and tendon organs through mechanical stimulation, breaking the vicious cycle of spasm and atrophy, and thereby promoting the recovery of limb function [6].

The results show that after the intervention, the scores of neurological function and self-care ability in the research group were improved [7]. This indicates that the individualized plans established by traditional Chinese medicine rehabilitation nursing can regulate the neuroendocrine network for different syndromes. The reasons are analyzed as follows: Syndrome differentiation-based nursing care was used. For the type of hyperactivity of liver yang, emotional regulation was applied to inhibit the excessive excitation of the sympathetic nerve, which can reduce the level of catecholamines. For the type of qi deficiency and blood stasis, ginger-separated moxibustion was used to increase the serum nitric oxide (NO) content and improve cerebral tissue perfusion [8].

5. Conclusion

In summary, the application of traditional Chinese medicine rehabilitation nursing can assist patients with hemiplegia due to stroke in gradually restoring the remodeling of neurological function and motor function, and promoting substantial improvements in the ability to perform activities of daily living and in the quality of life.

References:

- [1] Deng Xiaojing, Wu Chunyong. Application and Effect Analysis of Traditional Chinese Medicine Characteristic Rehabilitation Nursing in Patients with Hemiplegia After Cerebral Infarction [J]. Primary-level Traditional Chinese Medicine, 2024, 3(11): 84-89.
- [2] Lai Yanxia, Zhou Liang, Xiao Yanhua. The Effect of

- Traditional Chinese Medicine Characteristic Rehabilitation Nursing in the Rehabilitation of Patients with Hemiplegia After Cerebral Infarction [J]. *Chinese Medical Guide*, 2023, 21(26): 181-183.
- [3] Chen Lei. The Impact of Traditional Chinese Medicine Characteristic Nursing Combined with Rehabilitation Training on Limb Function Recovery in Patients with Hemiplegia Due to Cerebral Infarction [J]. *Journal of Integrated Traditional and Western Medicine on Cardiovascular Diseases (Electronic Edition)*, 2023, 11(02): 91-93.
- [4] Liu Yuqiong. The Influence of Traditional Chinese Medicine Rehabilitation Nursing on Surface Electromyography and Motor Function of Lower Limb Hemiplegia in Patients with Cerebral Infarction [J]. *Qinghai Medical Journal*, 2022, 52(10): 34-37.
- [5] Lu Yanyan, Yang Yan. The Effect of Traditional Chinese Medicine Comprehensive Rehabilitation Nursing on Endothelial Function and Limb Function in Patients with Hemiplegia After Cerebral Infarction [J]. *Nursing of Integrated Traditional and Western Medicine (Chinese and English)*, 2021, 7(12): 61-64.
- [6] Wang Wenya, Cao Yayu, Liu Mingyang. The Impact of Traditional Chinese Medicine Rehabilitation Nursing on Early Functional Rehabilitation and Neurological Function Deficit Score in Patients with Hemiplegia Due to Cerebral Infarction [J]. 2024.
- [7] Wang Qiyue, Chen Yan, Yang Xu. The Influence of Traditional Chinese Medicine Rehabilitation Nursing on Functional Rehabilitation in Patients with Cerebral Infarction - with 43 Clinical Cases [J]. *Jiangsu Journal of Traditional Chinese Medicine*, 2014(11).
- [8] Shi Aiyin. Discussion on the Impact of Traditional Chinese Medicine Rehabilitation Nursing on Functional Rehabilitation in Patients with Cerebral Infarction [J]. *Chinese Science and Technology Journal Database - Medical and Pharmaceutical*, 2024(002): 000.

ARTICLE

Research on the Strategy of Optimizing Biochemical Experiment Schemes with DeepSeek

ZHANG Ming PENG Ji

Haiyuan College, Kunming Medical University, Kunming, Yunnan 650000

ARTICLE INFO

Article history

Received: 26 June 2025

Accepted: 15 December 2025

Published Online: 30 December 2025

Keywords:

DeepSeek

Biochemistry Experiment

Scheme strategy

ABSTRACT

As biochemical experiments are constantly deepening, the complexity and scale of experimental scheme design has now burst out in an explosive way, which made traditional optimization methods significantly bottlenecking. DeepSeek technology relies on superior data processing abilities and intelligent analysis methods, becoming a key tool to optimize experimental plan. This paper aims to introduce the application mechanism of DeepSeek in biochemical test scheme, and detail and explain the basic principles, integration approaches and optimization measures of DeepSeek. By orderly sorting of design content of the scheme and technology deep integration, the efficiency and accuracy of the experiment are greatly improved, and will give more support to the scheme design of complex experiment environment and scientific and intelligent process of experiment operation.

Introduction:

Biochemical experiments, as an important part of life science research, the scientific nature of the experimental plan design directly affects the quality of the research results. With the complication of the experimental content and the increase of variables, the amount of experimental data has risen sharply. The traditional manual design and empirical optimization methods have gradually exposed their deficiencies and are difficult to meet the requirements of high throughput and high precision. DeepSeek technology has powerful data analysis and adaptive adjustment capabilities, adapting to the requirements of multi-dimensional information processing, and has become an important helper for improving the

optimization level of experimental plans.

1 The basic theory of biochemical experimental schemes

1.1 Basic Components of the experimental plan

The foundation of the composition of the biochemical experimental plan is to be strictly established in the experimental objectives, reasonable in the variables and control. The experimental design should take into full account the efficiency of experimental materials, reagents, detection methods on the basis of scientific. Every detail of each link will have a deep influence on the experimental results, especially in terms of arrangement of experimental

*Corresponding Author:

Zhang Ming, male, Han, 19800725, Kunming, Yunnan Province, bachelor's degree, associate professor; biochemistry. 804107862@qq.com

steps, so we should pay attention to the rigidity and integrity of the process. And the experimental plan should also consider the control of the experimental environment, temperature, humidity and time etc., these factors directly influence reaction speed and stability of the results. Besides, the reasonable allocation of experimental resources and no superfluous and wasteful experiments are also ineradicable components of the scheme construction. A precise experimental plan should integrate theoretical anticipation with actual experiments to constitute an overall and unified structural system so that consistent and verified data can be obtained in repeated experiments.

1.2 Key Considerations in the scheme design

The design of the scheme has to grapple with the complexity of experimental subjects themselves directly. The design that does not consider the interaction of variables will reduce the explanatory power of the conclusion. When designing, more attention should be paid to how to control the variables and exclude the presence of confounding factors affecting the data as accurately and easily as possible. A reasonable number of samples and test environment ensure the statistical power, standardization of test conditions ensures the consistency of the result, and the shortness of time and lack of resources force the designer to make a trade off, which needs a balance point between flexibility and stability of the plan. The adoption of technical means is also key. Although using the high-sensitivity instruments enhances data quality, the cost-effectiveness should be considered. The work repetitiveness of technical operations and the professional quality of operators should not be overlooked in the scheme designing stage as well. Any human factor can easily cause systematic errors. The convenience of data collection and processing should also be considered when designing the scheme. A rational planning of data structure is conducive to effective implementation of subsequent analysis. A good scheme design requires a global vision of the entire experimental life cycle, the design and plan of each stage from material preparation to data interpretation should be scientifically planned, so that the experiment can not only meet the preset goal but also lay the foundation for subsequent in-depth exploration^[1].

2 The application mechanism of DeepSeek technology

2.1 Overview of the Principle of DeepSeek Technology

DeepSeek technology actually is an optimization system based on deep learning model that it can realize adaptive setting by entering large data and adopts the

multi-layer neural network architecture to extract the implicit relationships between experimental data, and will breakthroughs traditional linear analysis algorithm to calculate the variables of more correct correlation value. In the technical architecture, the data preprocessing module guaranteeing the completeness and unification of the input information and the noise does not disturb with the analysis results. The core layer of neural network obtains the mutual relationship between complex variables by nonlinear mapping to improve the generality of the model. Its optimization algorithm will iteratively update parameters according to methods such as gradient descent, constantly tend towards the optimal solution, and realize the online tuning of the experimental scheme parameters. Different from the static design, DeepSeek makes it possible to modify the experimental plan in real time based on feedback information, so as to further improve the adaptability and real-time response of the experiment. Furthermore, the technical support of the multiple data format has also promoted the application of the expansion of the application scenarios under the condition of multiple experiment schemes. The combination of deep learning is not only a strategy that improves the efficiency of parameter correction, but also has an impact on the range of research in the scheme design of the experiment, and thus makes many factors that were previously hard to identify easy to be shown, thus promoting the scientific improvement of the experiment^[2].

2.2 The DeepSeek integration method in the experimental scheme

The deep integration of DeepSeek focuses on seamless combination with the current experimental process to avoid too much disturbance to the original operation process. The integration generally starts from the construction of data interface to ensure that experimental data can be passed to DeepSeek system in real time to form a closed-loop feedback. The designer should make clear the range of input parameters and optimization objectives according to the experimental needs to guarantee the directness and relevance of the scheme technical intervention. In the scheme, the parameter screening and optimization work are undertaken by DeepSeek, and it screens out the key variable combinations after several repeated iterations, improving the scheme accuracy. When introducing new technologies into an integrated system, compatibility (e.g., interfacing the wide array of the instruments and program platforms, how to import data seamlessly and stably, and how to monitor, etc.) is an important factor during the integration stage; while the usability (ease of learning) of the operational interface is another important factor

for the efficient application of a technology. DeepSeek integration emphasizes, in addition to the technical part, the feedback of laboratory staff, and gradually form a technology-practice interaction and realize the system solution executing capability.

2.3 The Influence of DeepSeek Optimization on the Experimental effect

It can be seen that after the experimental scheme is optimized with DeepSeek technology at all levels, the most direct reflection of optimization is the accurate grasp of experimental parameters, that is, the experimental conditions are closer to the best state, and the reliability and repeatability of the data have been improved; the data processing speed is accelerated to reduce the time cost of artificial intervention, and the experimental rhythm is smoother. The error reduction in a systematic manner suggests that the optimization makes the interference and deviation among variables well-controlled. The feedback of DeepSeek allows the experiment to be corrected dynamically in the process for enhancing its response capability against sudden abnormal circumstance. The refined plan also has high adaptability and can change the implementation strategy flexibly according to the changes of experiment aims so that the scientificity of experiment results are guaranteed. Conversely, the adoption of technology also realizes the standardization and modularization of experiment plan, minimized the subjective experience in plan formulation and achieved the universality and verifiability of experiment results.

3 Research on the Strategy of Optimizing Biochemical Experiment Schemes with DeepSeek

3.1 Goals and Principles of the Scheme Design

The main task of establishing an optimization plan is to further improve the experimental accuracy and efficiency, while guaranteeing the maximization of each experimental procedure. Not only is the optimization of parameters, but also the reconstruction of the experimental structure and procedure to ensure that the plan has great capacity of fitting with the experimental environment that is always intricate and changes continuously. It is necessary to clarify the purpose from the three aspects of simultaneously when planning, in order to clarify the goal, that is, to precisely regulate the variation of the experimental variables, reduce the error and improve the reproducibility of the experimental results. Scientificness is the premise. The rationality should be in accordance with the rigorous experimental logic, that is to say, not

to make arbitrary changes that lead to systematic errors. Secondly, the plan should be flexible and adaptable to various experimental environment conditions; that is, it should be able to be flexibly adjusted in various experiment conditions. The openness principle should also be adhered to. A mechanism is designed to process multiple input parameters and feedback information to realize the continuous iteration and improvement in the future. The design goal also involves data-driven automated decision making without requiring too much human intervention and making the plan more dependent on the machine. With the goal and principles being defined, the optimization method can be designed with both scientific significance and application significance, facilitating the global improvement of the experiment.

3.2 Data Processing and Parameter Adjustment Strategies

The core point of the data processing strategy is to enhance the capacity of efficient information extraction and noise filtering. On the basis of multi-stage data cleaning and screening, the abnormal and invalid information is filtered out to ensure the data quality of the input model, meanwhile, reasonable compression of the data dimensions and feature extraction are the core points in order to improve processing efficiency and to avoid the interference of redundant information to the analysis. The principle of parameter adjustment focuses on dynamics and use iterative optimization method to constantly adjust the critical parameters, making up for the constraints caused by static parameters. The principle of feedback adjustment of parameter is used. Parameters are adjusted in real time according to the feedback information of experiment results, ensuring that the optimized route can always meet the object of experiment. The construction of parameter space should consider both width and height. Not only should we emphasize coverage of enough parameter combination schemes, but we need to pay attention to whether important influential factors have been reflected in a certain dimension, and a multi-level parameter adjustment framework design should be established, a key variable selection by the preliminary screening and then select for fine adjustment, with a dual guarantee of efficiency and effectiveness. The thinking pays attention to the parameter adjustment controllability, to avoid the occurrence of over fitting, and the optimization plan has good generalization ability. Systematic data processing and parameter adjustment strategies have formed an intrinsic basis for scheme optimization and are the core of the links in order to have the efficient and reliable experimental schemes^[3].

3.3 Challenge Analysis in the Process of Scheme Optimization

There are many difficulties encountered when carrying out the scheme optimization. First, there is complexity and diversity of experimental data. The insufficient data, noise disturbance and highly nonlinear relationships between variables make optimization difficult. Second, due to the dynamic changes in experimental conditions, optimization plan is no longer suitable and real-time response ability is required. In addition, the occupied computing resources in the process of optimization are rather big, particularly for high-dimensional data, so there is a bottleneck of efficiency. And in human resources, the optimization plan has high requirements for the professional level of operators, and improper operation will make the final result inaccurate. There is also a system integration compatibility problem. The optimization tools require connection with diversified experimental hardware and software platforms, which is a technical coordination problem. In the face of the difficulties, in terms of method, we should pay more attention to multi-source data fusion technologies, improve data quality and information content. Resolve the computing bottleneck based on distributed computing and high-speed computing platform. Reinforce operation training and refine the user interface to reduce human error; Strengthen modular design to improve the flexibility and inter-changeability of system integration. The in-depth discussion of problems lays the foundation for formulation of targeted optimization schemes and is the first and indispensable step to ensure the efficient running of the optimization scheme.

3.4 Performance of the optimization results

The experimental plan obtained after optimizing the optimization scheme is able to realize the relative improvement on accuracy and efficiency. The experimental error rate is greatly reduced and the repeatability of experiment results is improved by realizing the effective data-driven parameter optimization adjustment. The optimized process will make the cycle period of the experiment to be shortened, the resource wasting can be reduced and the over-all time utilization rate of

experiment will improve. As can be observed, several iterative validations demonstrate stable performance of the scheme with various experimental environments and hence exhibit great robustness and feasibility, in which abnormal data's effect to calculation result is reduced so as to avoid deviation owing to accidental factors. While in this way, the efficiency of improvement is also reflected by the reduction of laboratory personnel's workload. With automatic handling and intelligent decision, less and fewer human interference occurred. Speeding up the speed of data processing is to meet the requirement of reporting and correction in a timely manner of experimental results.

4 Conclusion

The emergence of DeepSeek technology has undergone a huge revolution to the optimization of biochemistry experiment protocols. In the future, with the comprehensive combination of various different experimental demands and the emerging latest technologies, a more accurate and effective experimental design system will be established and biochemical research will be pushed towards a higher level to lay a foundation for scientific innovation in related disciplines.

References:

- [1] Yao Xincheng, Jiang Zhou, Wu Guanru, Wang Wei, Yang Yun. Thoughts and Practices on the Reform of Biochemistry Experiment Teaching in Traditional Chinese Medicine Colleges and Universities - Combined with the Newly Revised Talent Cultivation Program [J]. *Journal of Chengdu University of Traditional Chinese Medicine (Education Science Edition)*, 2023, 25 (01): 47-49.
- [2] Xue Yufei, Zhang Hecui, Li Bangxiu, Yang Kun, Zhang Jiankui. Discussion on the Teaching Reform of Basic Biochemistry Experiment Course under the Background of New Agricultural Science [J]. *Modern Vocational Education*, 2021, (15): 20-21.
- [3] Wang Zhuting, Li Hongmei, Lei Tingwen. Exploration and Practice of Design-oriented Experiments in the Teaching of "Biochemical Experiments" [J]. *Educational Teaching Forum*, 2020, (33): 240-242.

ARTICLE

A Review of the Impact of Virtual Simulation and Real-Scene Interactive Training on Clinical Thinking Ability of Nursing Talents under the Background of Intelligent Services in Internet Hospitals

LI Na DONG Jia GENG Gonglong DING Le LIU Fanghua

QingDao Binhai University, QingDao , Shandong Province, 266555, China

ARTICLE INFO

Article history

Received: 11 October 2025

Accepted: 15 December 2025

Published Online: 30 December 2025

Keywords:

Internet hospitals

Intelligent services

Virtual simulation

Real-scene interactive training

Nursing

Clinical thinking ability

ABSTRACT

This review synthesizes the technical characteristics and applications of virtual simulation and real-scene interactive training within internet hospitals' intelligent services, examining their role in improving clinical thinking among nursing professionals. It highlights advantages such as enhanced decision-making skills and experiential learning, while addressing challenges like technological limitations and integration barriers. The discussion concludes with future directions for optimizing educational strategies to support nursing reform and competence development under this intelligent framework.

Introduction

Internet hospitals, as a pivotal component of smart healthcare, have transformed medical and nursing services by leveraging technologies like telemedicine, EHRs, and real-time data analytics to offer efficient, remote care that overcomes geographical and resource constraints^[1]. The enhancement of nursing professionals' clinical thinking—critical for care quality and safety—remains challenging under traditional education methods, which often lack adequate practical training^[2]. Innovations such as virtual simulation and real-scene interactive training have emerged as effective tools, providing immersive,

experiential learning that strengthens clinical reasoning, decision-making, and skill application among nursing students^[3]. This review systematically examines the impact of these technologies on nursing clinical thinking within internet hospital frameworks, highlighting their role in preparing competent nursing professionals for smart medicine.

2. Theoretical Foundations and Research Review

2.1 Internet Hospital Smart Services and Their Impact on Nursing Education

The rise of internet hospitals, defined as a

**Corresponding Author:*

Na Li, Female, Ph.D., Professor;

Research interests: Nursing Education;

Address: Dazhushan Campus, Qingdao Binhai University, No. 1568 Shanchuan Road, West Coast New District, Qingdao, Shandong Province, 266555, China;

Email: 893981586@qq.com

transformative healthcare model leveraging telemedicine, EHRs, and AI-driven decision support, has redefined service delivery through improved accessibility and personalized care ^{[4][5][6]}. Concurrently, smart services within nursing have integrated IoT devices and intelligent systems to optimize workflows, enhance real-time patient monitoring, and improve care quality and efficiency ^{[7][5]} ^[8]. This evolution profoundly impacts nursing education by necessitating a shift towards digitally competent professionals. Internet hospitals provide a platform for integrating virtual simulation and real-scene interactive training into curricula, exposing students to remote care modalities and diverse clinical scenarios. This fosters the development of clinical thinking and bridges the theory-practice gap, preparing students for the demands of smart healthcare ecosystems ^{[9][10][11]}.

2.2 Technological Foundations of Virtual Simulation and Real-Scene Interactive Training

Virtual simulation technology, including VR, AR, and MR, creates immersive 3D environments for safe, repeatable practice of clinical skills and decision-making ^{[12][13][14]}. Its effectiveness hinges on sound instructional design and perceived relevance for learning ^{[15][16]}. Real-scene interactive training complements this by augmenting physical clinical environments with virtual elements, enhancing realism and multi-sensory engagement through tools like AR glasses or hybrid simulations with standardized patients ^{[17][18]}. This approach strengthens situational awareness and facilitates reflective learning ^[19]. Applications in nursing education are diverse, spanning procedural skill training (e.g., vacuum blood collection via VR ^[20]), clinical reasoning development through virtual cases ^[12], and emergency preparedness using immersive disaster simulations ^[23]. These technologies collectively enhance practical skills, clinical judgment, and learner satisfaction ^{[21][22][24]}.

2.3 Cultivating Clinical Thinking Ability: Mechanisms and Evidence

Clinical thinking in nursing encompasses observation, analytical judgment, decision-making, and reflection, evaluated through indicators like knowledge application and problem-solving ^{[25][26]}. Virtual simulation enhances this ability by providing a safe space for repetitive practice with immediate feedback, facilitating knowledge consolidation and self-regulated learning ^{[28][25]}. Its immersive nature promotes hypothesis generation and adaptive reasoning ^[29]. Real-scene interactive training further promotes clinical thinking by sharpening

contextual adaptability and teamwork skills within authentic settings, improving critical and systematic thinking ^{[30][31]}. Empirical evidence strongly supports the efficacy of both modalities, with studies showing significant improvements in clinical reasoning, knowledge retention, and performance, particularly when combined in blended approaches ^{[25][28][30][32]}. However, challenges such as high costs, faculty training needs, and technological limitations regarding realism and personalization remain. Future directions include developing cost-effective solutions, enhancing faculty development, integrating AI for adaptive learning, and improving simulation fidelity to maximize educational impact ^{[34][35][29]}.

Conclusion

In conclusion, the integration of intelligent services within internet hospitals has transformed nursing education by leveraging virtual simulation and immersive technologies to enhance clinical thinking, decision-making, and experiential learning. These innovations bridge theory-practice gaps, improve accessibility, and foster critical competencies, though challenges like technical limitations and curriculum integration persist. Future advancements will rely on interdisciplinary collaboration, evidence-based practices, and personalized learning pathways to fully realize their potential in preparing skilled nursing professionals for modern healthcare demands.

Funding Projects

1. National Center for Vocational Education Development, Ministry of Education (2024): "Research on Virtual-Physical Integration Training Model for Highly Skilled Nursing Professionals Driven by Intelligent Services" (JZYY25018)
2. Shandong Province Undergraduate Teaching Reform Research Project, "Innovative Research and Practice of Diversified Nursing Talent Training System for Specialized Upgrade Based on "Medical-Educational Collaboration and School-Hospital Cooperation" (M2022049)
3. Qingdao Binhai University Key Teaching Reform Research Project, "Innovative Strategies for Applied Nursing Specialty Experimental Teaching Driven by the Concept of Post-Course-Competition-Certificate" (2024JZ10)"
4. Qingdao Binhai University Teaching Reform Research Project (2024JY04): Research and Practice on the Talent Cultivation Model for Nursing "Xingzhi Class" in the Context of Industry-Education Integration
5. Qingdao Binhai University Teaching Reform

Research Project (2023JY08): A Practical Study on Narrative Education Empowering the Cultivation of Applied Nursing Talents—Taking the “Medical Nursing” Course as an Example

Reference

- [1] Yeheyis T, Hoyiso D, Borie YA, Tagesse N. Implementation of evidence-based clinical practice and its associated factors among health care workers at public hospitals in Sidama regional state, southern Ethiopia. *PLoS One*. 2024;19(3):e0299452. Published 2024 None. doi:10.1371/journal.pone.0299452
- [2] Xie X, Li K, Sun K, et al. [Construction and Exploration of Model for Training Excellent Nursing Professionals]. *Sichuan Da Xue Xue Bao Yi Xue Ban*. 2023;54(4):848-854. doi:10.12182/20230760305
- [3] Chen Y, He Y, Zou X, Cai H, Yiu HHE, Ming WK. Undergraduate nursing students’ preferences for virtual reality simulations in nursing skills training: A discrete choice experiment. *Digit Health*. 11:20552076251339009. Published 2025 Jan-Dec None. doi:10.1177/20552076251339009
- [4] Kwon H, An S, Lee HY, et al. Review of Smart Hospital Services in Real Healthcare Environments. *Healthc Inform Res*. 2022;28(1):3-15. doi:10.4258/hir.2022.28.1.3
- [5] Zhang GW, Li B, Gu ZM, et al. In-Depth Examination of the Functionality and Performance of the Internet Hospital Information Platform: Development and Usability Study. *J Med Internet Res*. 26:e54018. Published 2024 Nov 8. doi:10.2196/54018
- [6] Shahzad SK, Ahmed D, Naqvi MR, Mushtaq MT, Iqbal MW, Munir F. Ontology Driven Smart Health Service Integration. *Comput Methods Programs Biomed*. 207:106146. doi:10.1016/j.cmpb.2021.106146
- [7] Lin X, Duan G, Huang J, et al. Construction of A Smart Hospital Innovation Platform Using the Internet + Technology. *Altern Ther Health Med*. 2024;30(12):495-505.
- [8] Lakhan A, Mohammed MA, Rashid AN, et al. Smart-Contract Aware Ethereum and Client-Fog-Cloud Healthcare System. *Sensors (Basel)*. 2021;21(12). Published 2021 Jun 14. doi:10.3390/s21124093
- [9] Liu WI. [New Opportunities for Healthcare Driven by Smart Technology]. *Hu Li Za Zhi*. 2020;67(5):4-5. doi:10.6224/JN.202010_67(5).01
- [10] Huang Y, Dong X, Wu W, Zhang H, Qi F. Nursing undergraduates’ experience of participating in the Internet + Nursing Program and their spiritual education needs: A qualitative study from the perspective of the Neuman System Model. *Pak J Med Sci*. 2024;40(8):1747-1752. doi:10.12669/pjms.40.8.8822
- [11] Bai X, Shi Y, Nie J, Gao Y, Li Y. Clinical application effect analysis of internet combined comprehensive nursing intervention in chronic obstructive pulmonary disease. *Expert Rev Respir Med*. . Published online Jul 25,2025. doi:10.1080/17476348.2025.2540120
- [12] Padilha JM, Machado PP, Ribeiro A, Ramos J, Costa P. Clinical Virtual Simulation in Nursing Education: Randomized Controlled Trial. *J Med Internet Res*. 2019;21(3):e11529. Published 2019 Mar 18. doi:10.2196/11529
- [13] Kiegaldie D, Shaw L. Virtual reality simulation for nursing education: effectiveness and feasibility. *BMC Nurs*. 2023;22(1):488. Published 2023 Dec 19. doi:10.1186/s12912-023-01639-5
- [14] Tinôco JDS, Enders BC, Sonenberg A, Lira ALBC. Virtual clinical simulation in nursing education: a concept analysis. *Int J Nurs Educ Scholarsh*. 2021;18(1). Published 2021 Jun 18. doi:10.1515/ijnes-2020-0001
- [15] Rim D, Shin H. Effective instructional design template for virtual simulations in nursing education. *Nurse Educ Today*. 96:104624. doi:10.1016/j.nedt.2020.104624
- [16] Padilha JM, Costa P, Sousa P, Ferreira A. Clinical virtual simulation: predictors of user acceptance in nursing education. *BMC Med Educ*. 2024;24(1):299. Published 2024 Mar 16. doi:10.1186/s12909-024-05154-2
- [17] Jin E, Kang H. Development and effect of hybrid simulation program for nursing students: focusing on a case of pediatric cardiac catheterization in Korea: quasi-experimental study. *Child Health Nurs Res*. 2024;30(4):277-287. doi:10.4094/chnr.2024.020
- [18] Balian S, McGovern SK, Abella BS, Blewer AL, Leary M. Feasibility of an augmented reality cardiopulmonary resuscitation training system for health care providers. *Heliyon*. 2019;5(8):e02205. Published 2019 Aug. doi:10.1016/j.heliyon.2019.e02205
- [19] Heyn LG, Brembo EA, Byermoen KR, et al. Exploring facilitation in virtual simulation in nursing

- education: A scoping review. *PEC Innov.* 3:100233. Published 2023 Dec 15.
doi:10.1016/j.pecinn.2023.100233
- [20] Souza-Junior VD, Mendes IAC, Tori R, et al. VI-DA-Nursing v1.0: immersive virtual reality in vacuum blood collection among adults. *Rev Lat Am Enfermagem.* 28:e3263.
doi:10.1590/1518-8345.3685.3263
- [21] Lin MY, Huang MZ, Lai PC. Effect of virtual reality training on clinical skills of nursing students: A systematic review and meta-analysis of randomized controlled trials. *Nurse Educ Pract.* 81:104182. doi:10.1016/j.nepr.2024.104182 <https://pubmed.ncbi.nlm.nih.gov/39520841/>
- [22] Flood LS. Use of Virtual Reality Simulations to Embody a Patient: Exploring the Impact on Nursing Students' Confidence, Feelings, and Perceptions. *Nurse Educ.* 2024 Jan-Feb 01;49(1):36-40.
doi:10.1097/NNE.0000000000001442
- [23] Kim KJ, Choi MJ, Kim M. Effects of virtual reality-based disaster simulation education on nursing students. *PLoS One.* 2025;20(10):e0329563. Published 2025 None.
doi:10.1371/journal.pone.0329563
- [24] Lee H, Han JW, Park J, Min S, Park J. Development and evaluation of extracorporeal membrane oxygenation nursing education program for nursing students using virtual reality. *BMC Med Educ.* 2024;24(1):92. Published 2024 Jan 26.
doi:10.1186/s12909-024-05057-2
- [25] Havola S, Haavisto E, Mäkinen H, Engblom J, Koivisto JM. The Effects of Computer-Based Simulation Game and Virtual Reality Simulation in Nursing Students' Self-evaluated Clinical Reasoning Skills. *Comput Inform Nurs.* 2021;39(11):725-735. Published 2021 May 4.
doi:10.1097/CIN.0000000000000748
- [26] Lee DSK, Abdullah KL, Chinna K, Subramanian P, Bachmann RT. Critical Thinking Skills of RNs: Exploring Demographic Determinants. *J Contin Educ Nurs.* 2020;51(3):109-117.
doi:10.3928/00220124-20200216-05
- [27] Dogham RS, Elcokany NM, Ghaly AS, El-Ashry AM, Ali HFM. Emotional intelligence and reflective thinking: a synergistic approach in nursing education. *BMC Nurs.* 2025;24(1):548. Published 2025 May 16.
doi:10.1186/s12912-025-03175-w
- [28] Sim JJM, Rusli KDB, Seah B, Levett-Jones T, Lau Y, Liaw SY. Virtual Simulation to Enhance Clinical Reasoning in Nursing: A Systematic Review and Meta-analysis. *Clin Simul Nurs.* 69:26-39.
doi:10.1016/j.ecns.2022.05.006
- [29] Borg A, Jobs B, Huss V, et al. Enhancing clinical reasoning skills for medical students: a qualitative comparison of LLM-powered social robotic versus computer-based virtual patients within rheumatology. *Rheumatol Int.* 2024;44(12):3041-3051.
doi:10.1007/s00296-024-05731-0
- [30] Hu LY, Li SQ, Zhou ZY, Wang ML, Zhou LS. Effect of high-fidelity human patient simulator manikins combined with standardized patient simulation scenario on clinical thinking in pediatric nursing education. *BMC Nurs.* 2025;24(1):1129. Published 2025 Aug 28.
doi:10.1186/s12912-025-03562-3
- [31] Nichelason A, Alvarez E, Schultz K, Anderson M. Evaluation of Peer Simulations Utilizing Student-Generated Case Studies with Pre-clinical Veterinary Students. *J Vet Med Educ.* 2024;51(5):673-684.
doi:10.3138/jvme-2023-0002
- [32] Molloy MA, Zhao Y, Leonard C, et al. Nursing Students From China and the United States: Learning Together Through Virtual Simulation. *Nurs Educ Perspect.* 2022 May-Jun 01;43(3):171-174.
doi:10.1097/01.NEP.0000000000000929
- [33] Kobner S, Grassini M, Le NN, Riddell J. The Challenging Case Conference: A Gamified Approach to Clinical Reasoning in the Video Conference Era. *West J Emerg Med.* 2020;22(1):136-138. Published 2020 Dec 23.
doi:10.5811/westjem.2020.12.49133
- [34] Yoshioka-Maeda K, Honda C, Sumikawa Y, et al. Developing a Virtual Reality Simulation Program for Improving Nursing Students' Clinical Reasoning Skills in Home Settings: A Protocol Paper. *Nurs Rep.* 2022;12(4):968-979. Published 2022 Dec 6.
doi:10.3390/nursrep12040093
- [35] Huang CL. [Impact of Nurse Practitioners and Nursing Education on COVID-19 Pandemics: Innovative Strategies of Authentic Technology-Integrated Clinical Simulation]. *Hu Li Za Zhi.* 2021;68(5):4-6.
doi:10.6224/JN.202110_68(5).01

ARTICLE

Progress and Prospects in graded Treatment for Adenomyosis

WANG Yan¹ XIAO Yanbing²

1. Graduate School of Zunyi Medical University, Zunyi City, Guizhou Province 563000

2. Gynecology, Zunyi Maternal and Child Health Hospital, Zunyi City, Guizhou Province 563000

ARTICLE INFO

Article history

Received: 17 October 2025

Accepted: 15 December 2025

Published Online: 30 December 2025

Keywords:

adenomyosis

graded treatment

Three schemes of Mirena

Danhuang three scheme

ABSTRACT

Adenomyosis is a common benign gynecologic disease caused by the invasion of functionally active endometrial glands as well as mesenchyme into the myometrium. The main clinical features are excessive menstrual flow, prolonged menstrual cycles, and secondary dysmenorrhea that worsens progressively and may lead to infertility. With the increasing number of intrauterine operations, mainly in the form of abortions, in the younger age groups, the incidence of the disease has shown a yearly increase, and the patients tend to be younger. There are more treatment options for adenomyosis; however, the lack of the concept of choosing treatment based on the size of the uterine lesions makes the treatment of this disease more arbitrary. Inadequate treatment, overtreatment, and hysterectomy are widespread in clinical practice. The team has long been dedicated to researching the “graded treatment” of adenomyosis. More than ten years ago, based on the characteristic of uterine volume/lesion size dependence, i.e., the “lesion threshold” phenomenon in the treatment of adenomyosis with the intrauterine placement of Mirena, the “Three schemes of Mirena” was proposed, grounded in the concept of “graded treatment”. Based on the concept of “graded treatment”, we proposed the “Three schemes of Mirena” and obtained a series of research results, identifying the “lesion threshold” of Mirena in the treatment of adenomyosis. The team has provided a comprehensive set of concepts and methods for treating adenomyosis to preserve the uterus. In recent years, due to the high incidence of adenomyosis and the trend of youthfulness, as well as the background of declining birth rate and fertility protection, the team has followed the research foundation and experience of the previous study, and used Danhuang Dispelling Blood Stasis Capsule/Tablet (hereinafter referred to as Danhuang) as the basic drug, and composed the “Danhuang three scheme” by using Danhuang alone or in combination with other drugs. The “Danhuang three scheme”, which is a targeted treatment for adenomyosis based on different degrees of severity, has achieved sound therapeutic effects and provided a complete set of concepts and methodologies for the “fertility protection” treatment of adenomyosis. Three schemes of Mirena and Danhuang three scheme are two treatment systems based on the concept of graded treatment by the team at different times, which makes it possible to “uterine preservation and fertility protection” in the treatment of adenomyosis. Although the concepts and measures of staging/management have been published in the literature in recent years, they are overly complex and have a hysterectomy outcome, which is contrary to the era of uterine preservation and fertility protection. This article reviews the two sets of solutions of the team and other literature on the staging/management of adenomyosis to clarify the concept of adenomyosis treatment with “staging” as a means to achieve the goal of “uterine preservation and fertility protection”.

**Corresponding Author:*

Wang Yan, female, born in November 1991, from Dafang, Bijie, Guizhou Province, bachelor degree, attending physician, engaged in obstetrics and gynecology research

Adenomyosis (AM) is a disease in which the glands and mesenchyme of the endometrium invade and grow into the myometrium, which in turn causes a uterine pathology mainly resulting in increased uterine size, dysmenorrheal symptoms, increased menstrual flow, and secondary infertility, which seriously affects women's physical and mental health. The disease is most common in 30-50-year-old menstruating women or those with a history of uterine operation, with an incidence of 7%-23% and a prevalence of up to 70%^[1]. About 15% of patients are also combined with endometriosis, and about half of patients are combined with uterine fibroids^[2].

The degree of AM lesions (lesion size/uterine volume) varies greatly. The difference in lesion size will directly lead to a massive difference in the effectiveness of various treatments/drugs, e.g., there is a "lesion threshold (LTD)" phenomenon in the treatment of AM with Mirena^[3-5], which means that it is ineffective in the treatment of large-volume uterus [6], which may lead to inappropriate or ineffective use of Mirena. Therefore, the use of different treatment methods or protocols for patients with different lesion degrees can avoid inappropriate selection of treatment methods and make the treatment rule-based; at the same time, it is also conducive to the comparison of the actual results of various treatment methods, which is conducive to the summarisation, promotion and academic exchange of experience.

The team has been working on the clinical treatment of AM for a long time and has obtained a series of research results^[7-14]. The team was the first to propose the concept of "graded therapy", i.e., to grade AM patients according to the maximum thickness of the uterine wall or the maximum diameter (MD) of the adenomyoma, and then select the appropriate treatment plan according to the graded size. After a long period of clinical practice and exploration, the "Three schemes of Mirena" and "Danhuang three scheme" based on the idea of graded treatment have been summarised^[15-18]. The "Three schemes of Mirena" provide a comprehensive method system for AM "uterus preservation" treatment, whereas the "Danhuang three scheme" offers a novel approach and method for the non-surgical, non-invasive treatment of AM, specifically through "fertility protection" treatment. In this paper, we present a review of the two sets of plans and other relevant literature on AM staging/grading management, to clarify the concept of AM treatment with "grading" as the means and "uterus preservation and fertility protection" as the goal, to avoid or minimize the phenomenon of over-hysterectomy and the loss of fertility caused by AM. The purpose of this review is to clarify the concept of AM treatment by means of "grading" and

"uterus preservation and fertility protection", to avoid or minimize the phenomenon of excessive hysterectomy and fertility loss caused by AM.

1 Methods of staging, typing, grading, and classifying adenomyosis of the uterus

1.1 Methods of staging adenomyosis

AM staging is mainly seen in TCM treatment of AM, which is based on the menstrual cycle and does not depend on the size of the AM lesion/uterine volume. Based on the fact that AM is affected by the level of sex hormones and that the pathogenesis of blood stasis is not constant at different times, Ge Hongyu et al^[19] suggested that the treatment should be divided into three phases, i.e., the premenstrual phase is about 1 week before the onset of menstruation, the menstrual phase is the period that lasts for about 1 week at the time of the onset of menstruation, and the postmenstrual phase is the period that lasts for about 2 weeks after the onset of menstruation. In terms of treatment, the approach should be based on the specific disease to address the root cause, the menstrual period to alleviate symptoms, and the postmenstrual period to address both symptoms and the root cause. The study of Li Peixin et al^[20], which was based on Professor Wang Ping's experience in treating AM, concluded that the etiology of AM is due to the deficiency of kidney yang, the lack of positive qi, and the stagnation of stagnant and turbid masses, which results in the formation of blood impediments, and stagnation is the key. During the period when there was no requirement for pregnancy, emphasis is placed on warming the menstruation and relieving pain during menstruation, and on eliminating stasis and eliminating symptoms during normal times; during the period when pregnancy is required, according to the characteristics of the different stages of the menstrual cycle, tonifying the kidneys and adjusting the weekly cycle, the fetus and pregnancy may be accomplished. Lv Shuang et al^[21], whose staged therapy of warming the kidney and removing blood stasis, consider the pathogenesis of this disease to be a deficiency of kidney yang, internal obstruction of blood stasis, which accumulates and becomes the symptoms of the disease, and divide it into the perimenstrual period and the intermenstrual period for treatment.

The staging of AM in TCM is based on the menstrual cycle and the dialectic of the treatment. Although this method offers the advantages of non-invasive, non-hormonal treatment with minimal impact on bone quality and a low incidence of adverse effects, the dialectic of TCM is complex. Different physicians may arrive at

inconsistent results in different menstrual cycles of the same patient, or even the same patient with the same menstrual cycle, so there is a large degree of subjectivity in judging the therapeutic efficacy. The slow onset of the effect on patients with severe diseases, and the patients can hardly tolerate the pain until the medication has taken effect, so it is difficult to popularise the method in the clinic. It is challenging to popularise it in the clinic.

1.2 Methods of typing adenomyosis

There are multiple methods of AM typing, and no single, universally accepted method exists. The main ones are histopathology and imageological examination. Histopathologic examination is the gold standard for diagnosis, which is challenging to adopt in the clinic because it requires uterine resection or trans-uterine laparoscopic sampling and delivery, which is invasive and intrusive. With the rapid development of iconography, iconography has become a commonly used clinical method to diagnose AM and its degree of pathology (uterine accretion/lesion size). Commonly used methods include magnetic resonance imaging (MRI) and transvaginal ultrasound (TVU). MRI is expensive, limiting its application. TVU is economical, noninvasive, reproducible, and universally applicable. Compared with transabdominal ultrasound, TVU has higher clinical value and higher accuracy for the diagnosis of AM^[22].

Lan Guiluan^[23] classified the sonographic features of AM into five types based on pathological findings: type I is diffuse, type II is adenomyomatous, type III is adenomyomatous intracystic, type IV is adenomyomatous polyp, and type V is adenomyomatous leiomyomatous. Kishi et al^[24] classified the foci into four subtypes based on MRI of the foci. Type I is endogenous: the AM foci are directly connected to the thickened binding zone, and the adenomyosis foci have normal musculature on the outer side. Type II is exogenous: AM lesions are in a myometrial position outside the uterus, their binding bands remain intact, and normal musculature exists between the lesion and the binding bands. Type III is intramural, and this type of AM lesion is free-standing, neither connected to the binding bands nor the plasma membrane. Type IV: Except for the above three types categorised in this category. Lazzeri et al.^[25] and Exacoustos et al.^[26] classified AM as diffuse, focal, and adenomyoma based on ultrasound sonographic characteristics.

Studies on AM staging have provided information on the extent of lesion invasion and the extent of different staging types of the disease, to guide clinical treatment. However, most of the current studies on staging mainly focus on the discrepancy between the staging results

of different examinations and the pathological findings and the correlation with the severity of AM^[27-32], and no relevant studies have been seen on the correspondence between the staging results and the choice of treatment regimens, i.e., the treatment regimens corresponding to different staging. Therefore, the above typing methods have no substantial guiding effect on clinical treatment and have not been adopted by the clinic.

1.3 Methods of grading adenomyosis

In recent years, the incidence of AM has been on the rise and is accompanied by a lower age^[33-34]. Therefore, Qi et al.^[35] suggested a three-tiered prevention of AM: primary prevention, i.e., blocking or delaying the occurrence of AM by reducing or eliminating high-risk factors; secondary prevention, i.e., avoiding or delaying the progression of AM through early detection, diagnosis, and treatment; and tertiary prevention, i.e., the long-term management of AM to prevent recurrence. It is similar to the three-level management of AM studied by Wang Shixuan et al^[36].

1.3.1 Primary management of adenomyosis

Since the etiology of AM is unknown, prevention based on the possible causes of AM covers initiatives such as strengthening doctor-patient education efforts, carrying out genetic testing and genetic counselling activities, minimising or avoiding uterine cavity manipulation, changing behavioural patterns, and stopping or reducing retrograde menstrual blood flow in order to reduce the incidence of AM^[37-39].

1.3.2 Secondary management of adenomyosis

At the stage of diagnosing AM, symptoms have appeared. However, surgical treatment is not yet needed; this stage mainly focuses on symptoms such as dysmenorrhea, excessive menstrual flow and infertility, using drugs, Mirena(levonorgestrel intrauterine slow-release system, LNG-IUS), uterine artery embolisation (UAE) and high-intensity focused ultrasound (HIFU), radiofrequency or microwave.LNG-IUS,Uterine artery embolisation (UAE), and non-surgical therapies such as high intensity focused ultrasound (HIFU), radiofrequency, or microwave ablation to relieve or reduce symptoms, promote fertility, and slow down the process. Among the drugs are nonsteroidal anti-inflammatory drugs (ibuprofen, naproxen sodium, mefenamic acid, celecoxib), compound oral contraceptives, oral progestational hormone analogs (dienogest), gonadotropin-releasing hormone agonist (GnRHa), hemostatic drugs (tranexamic acid), and

Chinese herbal medicine^[36, 40-44].

1.3.3 Tertiary management of adenomyosis

For patients who require surgical treatment after non-surgical treatment is ineffective, the indications for surgery should be strictly controlled. The choice of surgical modality depends on factors such as the patient's fertility requirements, the severity of clinical symptoms, and the patient's age. Surgical treatments for AM include secondary/total hysterectomy and uterus-preserving surgeries, and uterus-preserving surgeries include hysterectomy of uterine foci (adenomyoma excision and foci reduction) and uterine endometrial removal. Endometrial removal. AM patients require long-term management after surgery, and individualised treatment can be implemented according to the patient's age, clinical symptoms, the presence of fertility requirements, the type of lesion, and drug characteristics^[36, 45-46].

Tertiary management of AM is a model of segmented management based on the different stages of the disease, with the emphasis on management, treatment and assessment of efficacy, not based on changes in lesion size. The advantage is that it proposes prevention based on the possible causes of AM, thereby reducing or minimising morbidity. However, for AM that has already been diagnosed, treatment is still generalised, with treatment plans varying from person to person. There is still considerable variability. The size range of the lesions requiring treatment was not provided, and the corresponding treatment methods for different lesion sizes were not specified. There is also an absence of a reference standard for evaluating treatment effectiveness. Consequently, under- or over-treatment, or even non-essential hysterectomies, persist.

1.4 Methods of grading adenomyosis

1.4.1 Staging of adenomyosis with the “Three schemes of Mirena”

All of the above Staging, typing and grading methods have the disadvantage of not relating the size of the lesion to the treatment method/protocol, and there is the possibility of eventual hysterectomy. The uterus is an important female sex organ with complex functions. Removal of the uterus may be associated with premature ovarian decline, pelvic organ prolapse, urinary incontinence, and other pelvic floor dysfunctional disorders^[47-49], and therefore, the uterus should not be removed thoughtlessly. Based on this concept, the team proposed grading patients according to the maximum thickness of the uterine myometrial wall or the maximum

diameter of adenomyoma (Maximum diameter, MD) over ten years ago, and then selecting treatment options based on the grading. Based on Mirena, a system of “Three schemes of Mirena” was proposed for the graded treatment of AM, and a series of research results were obtained^[7-8]. On this basis, the academic view that there is a lesion size/uterine volume dependence, i.e., the “lesion threshold (LTD)” phenomenon, in the treatment of AM with Mirena was discovered and proposed^[3]. Subsequently, the team discovered that levonorgestrel (a component of Mirena) exhibits a distance-dependent “concentration gradient” and a time-dependent “concentration stabilisation” phenomenon in the silica gel uterine membrane, as demonstrated by the Silicone imitation uterine body membrane experiment^[5,12]. This verified Mirena LTD from an experimental perspective. This experimentally verifies the scientific hypothesis of the LTD phenomenon of Mirena. The academic view that LTD exists in the treatment of AM with Mirena is 2 years earlier than that of foreign scholars^[3,6]. Based on the results of LTD and series of studies, the team classified AM into three degrees according to MD, i.e., MD<30 mm is mild, MD between 30-40 mm is moderate, and MD>40 mm is severe; the corresponding treatment protocols were as follows: Mirena protocol, Mirena + HIFU/GnRHa protocol, Mirena + major uterine wall resection and reconstruction of the uterus(MURU) program.

Three schemes of Mirena and MURU provide a complete methodology system for AM “uterus preservation” treatment. In the process of clinical implementation, the measurement of MD by TVU/MRI is a simple, objective and accurate method. The selection of the corresponding treatment program according to MD can make the clinical treatment follow the rules, avoiding over-treatment, under-treatment and bias in the selection of methods. The most important aspect is that MURU can avoid hysterectomy from a methodological perspective.

1.4.2 “Danhuang three scheme” graded treatment of adenomyosis

Although the “Three schemes of Mirena” provides a complete set of methods for AM “uterus preservation” treatment, there are also related problems. Firstly, levonorgestrel is the drug component of Mirena. Therefore, those who are prohibited from using progesterone are not suitable for the Three schemes of Mirena, such as: progesterone allergy, history of thrombophilia, obstructive jaundice, porphyria, etc.; secondly, there are corresponding problems with the placement of Mirena in the uterus, such as: there are Mirena downward displacement or detachment, abnormal

uterine bleeding, or unwillingness or inability to use the IUD, and patients with obesity, blemishes, acne, etc. should not be used; Mirena belongs to the IUD category, and the standard MURU procedure has a sterilizing effect. This is contrary to the current era of negative growth in China's birth population, the state's encouragement of childbirth, and academia's strong advocacy for fertility preservation. Therefore, it is imperative to explore the treatment modality of AM fertility protection (conservation). Based on this understanding, the team combined the rich experience gained from the treatment of ICR mice with traditional Chinese medicine and the graded treatment of AM with the "Three schemes of Mirena" in the previous period. It proposed the graded treatment of AM with the "Danhuang three scheme" for fertility preservation, through a systematic, prospective approach. After continuous exploration and summarisation, we proposed the "Danhuang three scheme" method for the graded treatment of AM with the purpose of conservation, and achieved satisfactory therapeutic effects after systematic and prospective clinical studies^[17-18].

The "Danhuang three scheme" is based on Danhuang expectorant capsules/tablets (abbreviated as: Danhuang), used alone and in combination with mifepristone and leuprolide/triptorelin, forming the Danhuang, Danmu, and Danrui protocols for the treatment of mild, moderate, and severe AM, respectively. Studies have shown that^[17], among the "Danhuang three scheme", the "Danrui protocol" has the most significant efficacy, but GnRHa analogs have chemical oophorectomy effects, and the prolonged use of GnRHa analogs may cause hypoestrogenic manifestations such as hot flashes, and they are expensive. Therefore, "Danrui protocol" is recommended for patients with severe AM or those who need to reduce the size of the uterine lesions rapidly; "Danmid protocol" has a longer course of treatment and slower effect than Danrui protocol in the treatment of severe AM, but its effect in the treatment of intermediate AM is significant and stable, and mifepristone is inexpensive, so it is recommended to be used in intermediate AM; "Danhuang protocol" can effectively control mild AM, and it is recommended to be used in intermediate AM. Danhuang three scheme can effectively control the symptoms of dysmenorrhea in patients with mild AM, and can also prevent the recurrence of the disease. Under the premise of guaranteeing therapeutic effect, based on the principle of pharmacoeconomics, the treatment mode of "grading" and "down-regulation" of Danhuang three programs not only reduces the cost of treatment, but also reduces the potential toxicity and side effects of the drug, which maximizes the three principles

of cost-effectiveness, effectiveness and utility, and is also a reflection of the principle of cost-effectiveness, effectiveness, and utility. This embodies the maximization of the three principles of cost-effectiveness, effectiveness, and utility, which is also the reason and charm of "graded treatment". The "Danhuang three scheme" offers a comprehensive and non-invasive approach to treating AM, building on the team's previous innovation and breakthrough with the "Three schemes of Mirena" through graded treatment.

2 Special application scenarios of "Three schemes of Mirena" and "Danhuang three scheme"

The "Three schemes of Mirena" and the "Danhuang three scheme" are two original "graded" systems of treatment for AM that were developed by the team at different times in history. Three schemes of Mirena is aimed at preserving the uterus (uterine preservation). Danhuang three scheme is aimed at preserving fertility (conservation). In clinical application, in addition to treatment according to the MD classification, the patient's comorbidities, age, and willingness to be treated need to be taken into account in order to determine whether the two systems should be used individually or in combination, with the dual purpose of treating the disease and satisfying the patient's wishes for treatment.

2.1 Comorbidities

2.1.1 Comorbid uterine fibroids: About half of AM patients have comorbid uterine fibroids^[2]. Danhuang has little or no therapeutic effect on uterine fibroids. Therefore, when there is a combination of fibroids, the following treatments are recommended: for those with large/number of fibroids and no fertility requirements, the Mirena + MURU regimen is recommended for optimal cost-effectiveness; for those with fertility requirements, only myomectomy (open or laparoscopic) is recommended, and the AM is treated with the Danhuang three scheme after myomectomy to minimize the trauma to the uterine myometrial wall. For those with small fibroids/number of fibroids/unwilling to undergo surgery or those who still have the desire to have children, it is recommended to treat AM with Danhuang three scheme as appropriate, in addition to the use of proprietary Chinese medicines for uterine fibroids, such as Gui Zhi Fu Ling Capsules and Uterine Tumor Elimination Capsules, etc., to synergize the treatment of uterine fibroids.

2.1.2 Combined chocolate cysts: For AM combined with ovarian chocolate cysts, use the Danhuang three

scheme with caution, as the blood-activating effect of Danhuang has the potential to cause intracapsular hemorrhage and enlargement of the cysts. If the cyst is large and there is a desire to have children, the coeliac cyst can be treated as a single surgical factor, and laparoscopic or open excision can be performed. Postoperatively, AM is then treated with the Danhuang three scheme to avoid uterine trauma. For those with large cysts without fertility intention, MURU can be performed at the discretion of the MD and the patient's wish. In contrast, the cyst is removed (concurrent MURU treatment is recommended for severe patients). For those with small cysts without surgical indications, they can be treated with dienogest/mifepristone/GnRH analogs first. Then, after sexual axis suppression and coelomic ectopic endothelial atrophy, danhuang can be added to treat AM, starting from a small dose at discretion. The frequency of ultrasonography can be increased simultaneously.

2.2 Age factor: AM is a hormone-dependent disease, so the patient's age is an important reference factor for treating the disease and preventing recurrence. Generally speaking, the age of women and ovarian function are in a parallel state, with young women having relatively good ovarian function and a higher risk of disease recurrence. Older women have weaker ovarian function and are less likely to have recurrence of the disease. If the patient's age is <45 years old, or the ovarian function is good, it is easy to relapse. Therefore, for severe patients without fertility intention and contraindication to progesterone, it is recommended to treat with Mirena+MRUR regimen. If the patient is ≥ 45 years old or has weak ovarian function and is not prone to recurrence, unless the lesion is particularly severe or voluntary MURU treatment is performed, treatment with the Danhuang three scheme is recommended. It should be noted that AM patients are often associated with late menopause, and the age factor needs to be judged in conjunction with ovarian function.

2.3 Extreme cases: the degree of uterine pathology is particularly severe, which needs to be considered in conjunction with the patient's treatment wishes. For example, MD ≥ 60 mm, uterine volume ≥ 4 months of pregnancy, and no desire to have children (common), it is recommended that the Mirena + MURU regimen be used, which can quickly obtain therapeutic effects to achieve the goal of uterine preservation treatment. In order to minimise the difficulty of surgery and to favour uterine reconstruction, it is recommended to treat with the Danrui protocol for about three courses of treatment before performing MURU. Those who have the desire to have children or refuse surgery (rare) are treated with the Danrui protocol. It should be noted that due to the

excessive size of the uterus, a longer course of treatment is required, which needs to be fully communicated to the patient in order to improve treatment compliance; when the MD is reduced to mild or regular, and the CA125 turns negative, IVF-ET is recommended.

3 Summary and Outlook

AM is a highly prevalent disease in gynecology, and hysterectomy is still the primary treatment modality for critically ill patients and those who fail conservative treatment worldwide. Although the uterus is not a pathogenic organ, it plays a crucial role in women's lives and quality of life^[18, 50]. It should not be easily removed, which is the purpose of the "uterus preservation" treatment for AM. On the other hand, although AM is not absolutely infertile, the prevalence of AM among infertile women under 40 years of age ranges from 7.5% to 22%, and among infertile women aged 40 years and above ranges from 24.4% to 29.7%^[51]. In the context of China's declining birthrate and high incidence of AM, the treatment of AM and protection of patients' fertility are particularly important, making a realistic need for AM "fertility protection" treatment.

Among the methods of conservative treatment of AM, almost all of them are lesion-level dependent^[3, 9, 52]. For example, Mirena, HIFU, and dienogest are much less effective in treating large volumes of the uterus than small volumes of the uterus. This dependence dictates that when treating with a particular method or methods, the choice must be differentiated according to the extent of the lesion, i.e., different therapeutic agents/protocols must be selected according to the extent of the lesion in order to meet the dual objectives of therapeutic efficacy and principles of health economics. Therefore, "graded therapy" according to the extent of the lesion is not only a requirement for the treatment of the disease, but also a prerequisite for the "standardisation" and "differentiation" of AM treatment.

There are many treatment methods for the preservation of the uterus and the fertility protection of AM. Based on the idea of "graded therapy", the team's "Three schemes of Mirena" provides a complete method system for AM "uterus preservation" treatment. At the same time, the "Danhuang three scheme" is the team's non-invasive treatment following the idea of "graded therapy". The "Danhuang three scheme" is another system of non-invasive fertility preservation (conservation) treatment for AM, following the idea of "graded treatment" after the "Three schemes of Mirena". In clinical work, the two programs can be used individually or in combination, as appropriate, to achieve the goal of fertility preservation or conservation treatment.

Just like the “staged treatment” of gynecological cancer, the concept of “graded treatment” should become the basic principle of AM treatment. The concept of graded treatment will attract the attention of the academic community and change the confusing situation of AM treatment and the status quo of hysterectomy as the ultimate treatment, to protect the uterus and fertility of AM patients.

References

1. Vercellini P, Viganò P, Somigliana E, et al. Adenomyosis: epidemiological factors[J]. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 2006, 20(4): 465-477.
2. Kong Beihua, Martin, Duan Tao. *Obstetrics and Gynecology* [M]. 10th ed. Beijing: People's Health Publishing House, 2024.6.
3. Fang Derong, Zhao Jing, Li Qinghan, et al. A preliminary study on threshold screening of adenomyosis foci in the treatment of adenomyosis with Man Yue Le[J]. *Journal of Zunyi Medical College*, 2014, 37(4): 418-421.
4. Tu Jiao, Fang Derong, Xiao Yanbing. Clinical study on threshold screening of adenomyosis foci in the treatment of uterine adenomyosis with Man Yue Le[J]. *China Pharmaceutical Industry*, 2015, 24(19): 19-20.
5. MENG Yinyu, JIANG Chengsu, YOU Mengyuan, et al. Experimental study on the chromogenic labelling of levonorgestrel and dispersion pattern in transparent silica colloidal mould [J]. *Journal of Zunyi Medical College*, 2019, 42(04): 416-421.
6. Hwan K L, Kew J K, A M L, et al. Relationship between uterine volume and discontinuation of treatment with levonorgestrel-releasing intrauterine devices in patients with adenomyosis.[J]. *Archives of gynecology and obstetrics*, 2016, 294 (3): 561-6.
7. Xiao Yanbing, Zhao Jing, li qinghan, et al. "Exploration of the surgical style and safety of myomectomy-uterine reconstruction for the treatment of moderate and severe adenomyosis[J]. *Journal of Zunyi Medical College*, 2012, 35(04): 302-305.
8. ZHAO Jing, LI Qinghan, FANG Derong, et al. The efficacy of myomectomy-uterine reconstruction combined with Mannelle in the treatment of moderate and severe adenomyosis[J]. *China Maternal and Child Health*, 2013, 28(15): 2464-2466.
9. Li Qinghan. Clinical study on the treatment of moderate and severe adenomyosis with HIFU [D]. Guizhou: Zunyi Medical College, 2014.
10. Zhang Ya, Xiao Yanbing. Short-term efficacy observation of GnRH-a combined with LNG-IUS and LNG-IUS in the treatment of adenomyosis[J]. *China Modern Doctor*, 2016, 54(19): 63-66.
11. REN Xiaoyan, SUN Cui, GU Hengfang, et al. Experimental study on the treatment of adenomyosis in ICR mice with compound traditional Chinese medicine[J]. *Journal of Zunyi Medical College*, 2016, 39(06): 597-601.
12. Sun Cui, Ren Xiaoyan, Fan Wenyan, et al. Establishment of silicone imitation uterus body model and experimental study of LNG dispersion law[J]. *Journal of Zunyi Medical College*, 2017, 40 (02): 177-179+184.
13. Meng Yinyu, Xiao Yanbing. Clinical study of levonorgestrel intrauterine extended-release system for the treatment of adenomyosis[J]. *Electronic Journal of Clinical Medicine Literature*, 2018, 5(77): 22.
14. Cui S, Xiao-Yan R, Yi G, et al. Clinical Efficacy and Safety of Major Uterine Wall Resection and Reconstruction of the Uterus Combined with LNG-IUS for the Treatment of Severe Adenomyosis. [J]. *Geburtshilfe und Frauenheilkunde*, 2020, 80 (3): 300-306.
15. Tu Jiao, Mou Meng, Gu Hengfang, et al. Observation on the efficacy of different application methods of levonorgestrel intrauterine extended release system in the treatment of adenomyosis[J]. *Journal of Xinxiang Medical College*, 2019, 36(01): 33-37.
16. YANG Li, XIAO Yanbing, HAN Lei. Progress of research on the treatment of adenomyosis with the goal of uterine preservation[J]. *Journal of Localised Surgery*, 2020, 29(12): 1019-1023.
17. Su Yuanhua, Liang Zhigang, Ren Xiaoyan, et al. "Clinical study on the graded treatment of mild, moderate and severe adenomyosis with Danhuang three programs[J]—*Journal of Zunyi Medical University*, 2024, 47 (12): 1178-1183+1194.
18. Xiao Yanbing. Theory and practice of graded treatment of adenomyosis[J]. *Journal of Zunyi Medical University*, 2025, 48(03): 213-218.
19. Ge Hongyu, Wang Zhongmin. Clinical staging of adenomyosis in the treatment of adenomyosis [J]. *Clinical Journal of Chinese Medicine*, 2023, 35(09): 1655-1660.
20. LI Peixin, WU Li, WANG Ping, et al. Wang Ping's experience in treating adenomyosis based on "two-stage staging"[J]. *Journal of Traditional Chinese Medicine*, 2025, 31(05): 221-224+228.
21. Lv Shuang, Chen Jianhong. Clinical study on the treatment of uterine adenomyosis with staged therapy by warming the kidney and removing blood stasis [J]. *New Chinese Medicine*, 2025, 57(18): 65-69. 65-69.

22. Han Bing, Song Haibo. Clinical value of transvaginal colour Doppler ultrasound in the diagnosis of uterine fibroids, adenomyosis, and adenomyoma [J]. *Imaging Research and Medical Applications*, 2022, 6(04): 40-42.
23. Lan Guiluan. Progress of ultrasound diagnosis of adenomyosis[J]. *Clinical Medical Research and Practice*, 2024, 9(10): 195-198. 195-198.
24. Kishi Y, Suginami H, Kuramori R, et al. Four subtypes of adenomyosis assessed by magnetic resonance imaging and their specification[J]. *American Journal of Obstetrics and Gynecology*, 2012, 207(2): 114.e1-114.e7.
25. Lazzeri L, Morosetti G, Centini G, et al. A sonographic classification of adenomyosis: interobserver reproducibility in the evaluation of type and degree of the myometrial involvement. *Fertil Steril*. 2018 Nov;110(6):1154-1161.e3.
26. Exacoustos C, Morosetti G, Conway F, et al. New Sonographic Classification of Adenomyosis: Do Type and Degree of Adenomyosis Correlate to Severity of Symptoms? *J Minim Invasive Gynecol*. 2020 Sep-Oct;27(6):1308-1315.
27. Wang H, Yuan B, Wang WL. Correlation between magnetic resonance imaging typing of adenomyosis and its clinicopathologic features[J]. *Chinese Clinical Journal of Obstetrics and Gynecology*, 2023, 24(04): 371-374.
28. Wang Meixian, Shao Xiaoguang. Diagnostic imaging and clinical application of adenomyosis [J]. *Modern Progress in Obstetrics and Gynecology*, 2024, 33(12): 934-937.
29. Han Yutian. Research on adenomyosis staging based on ultrasound characteristics and its clinical application value [D]. Hangzhou Normal University, 2024.
30. ZHONG Yuqing, LIU Yang, HU Yan, et al. MRI characteristics of four subtypes of adenomyosis and the differences in parameters related to HIFU treatment[J]. *Magnetic Resonance Imaging*, 2022, 13(09): 95-99.
31. Han Xiaotong, Guo Hongyan. Research Progress on Classification and Heterogeneity of Adenomyosis [J]. *Journal of Practical Obstetrics and Gynecology*, 2021, 37(12): 910-913.
32. Ren Junfang, Lü Jun, Li Jin, et al. Diagnostic Value of MRI in Assessing the Severity of Adenomyosis[J]. *Henan Medical Research*, 2025, 34(15): 2714-2719.
33. Vercellini P, Viganò P, Bandini V, et al. Association of endometriosis and adenomyosis with pregnancy and infertility [J]. *Fertil Steril*, 2023, 119(5): 727-740.
34. Etrusco A, Barra F, Chiantera V, et al. Current Medical Therapy for Adenomyosis: From Bench to Bedside [J]. *Drugs*, 2023, 83(17): 1595-1611.
35. Qi Qi, Zhang Xiaoping, Ma Xiaoxin. Graded Prevention Strategy for Adenomyosis[J]. *Chinese Journal of Obstetrics and Gynecology*, 2023, 58 (3): 231-235.
36. Wang Shixuan, Cui Pengfei, Zhang Jinjin. Expert Consensus on Tertiary Management of Uterine Adenomyosis [J]. *Journal of Practical Obstetrics and Gynecology*, 2024, 40(02): 106-111.
37. Satoshi I, Yasushi H, Toshihide U, et al. Uterine adenomyosis is an oligoclonal disorder associated with KRAS mutations. *Nature Communications*, 2019, 10(1): 5785.
38. Bulun SE, Yildiz S, Adli M, Wei JJ. Adenomyosis pathogenesis: insights from next-generation sequencing. *Hum Reprod Update*. 2021 Oct 18;27(6):1086-1097.
39. Li Juanqing, Huang Xiufeng, Shi Yifu. Iatrogenic adenomyosis [J]. *Chinese Journal of Practical Gynecology and Obstetrics*, 2021, 37(10): 1074-1076.
40. Peng Chao, Zhou Yingfang. Application and selection of drug therapy in the long-term management of endometriosis [J]. *Chinese Journal of Practical Gynecology and Obstetrics*, 2021, 37(3):303-308.
41. Zhang Yi, Zhang Rui, Zhang Junke, et al. Meta-Analysis of Efficacy and Safety of Dinogest in Treating Adenomyosis [J]. *Advances in Obstetrics and Gynecology*, 2024, 33(5):345-351.
42. Wang Lijuan, Tang Chunhui. Effects of GnRH- α Combined with Levonorgestrel Intrauterine Release System on Pain Symptoms, Serum MMPs, and Recurrence in Patients with Adenomyosis [J]. *Chinese Journal of Family Planning*, 2021, 29(2):266-270, 274.
43. Zou Xiaohui, Liu Xinxin, Lin Xialian, et al. Effectiveness and Safety Observation of Traditional Chinese Medicine Treatment Based on Disease Stages Combined with Dinogest for Adenomyosis [J]. *Chinese Journal of Medical Sciences*, 2025, 15(15): 127-131.
44. Xu Wencui, Sun Tao, Lü Weifu. Analysis of Factors Affecting Post-Embolisation Syndrome in Uterine Artery Embolisation for Adenomyosis [J]. *Journal of Medical Imaging*, 2023, 33(6): 1057-1061.
45. Yang Chao, Zhang Yudi, Chu Chunfang, et al. Clinical Efficacy of Endometrial Resection Combined with Levonorgestrel-Releasing Intrauterine Device in Treating Adenomyosis [J]. *Chinese Journal of Clinical Physician*, 2023, 51(05): 592-595.
46. Jia Qiucheng, Li Yang, Chen Jiming, et al. Laparo-

- scopic Radical Adenomyosis Ligation with Uterine Preservation [J]. *Journal of Surgery (Electronic Edition)*, 2023, 10(06): 7-10.
47. Ibrahim A, Afrooz M, Veronika G, et al. Total or Subtotal Hysterectomy for the Treatment of Endometriosis: A Review. [J]. *Journal of Clinical Medicine*, 2023, 12(11):
 48. Rozycki S, Orlando M, White A, et al. Associations between hysterectomy and pelvic floor disorders: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2025 Sep;233(3):141-151.e6.
 49. Christoffersen NM, Klarskov N, Gradel KO, et al. Increased risk of stress urinary incontinence surgery after hysterectomy for benign indication: a population-based cohort study. *Am J Obstet Gynecol*. 2023;229(2):149.e1-149.e9.
 50. Turan A, Karabayır HB, Kaya İG. Examining the changes in women's lives after the hysterectomy operation: experiences of women from Turkey [J]. *Arch Womens Ment Health*, 2024, 27(6): 899-911.
 51. Abu Hashim H, Elaraby S, Fouda AA, et al. The prevalence of adenomyosis in an infertile population: a cross-sectional study. *Reprod Biomed Online*. 2020 Jun;40(6):842-850.
 52. Wang Qiming, Fu Xuewen, Zhu Jue. Efficacy Analysis and Safety Evaluation of Goserelin Treatment for Adenomyosis [J]. *Chinese Journal of Practical Gynecology and Obstetrics*, 2022, 38(01): 105–107.

ARTICLE

Advancements in Drug Treatment for Adenomyosis

Yang Guo¹ Xiao Yanbing²

1. Graduate School of Zunyi Medical University, Zunyi City, Guizhou Province 564300

2. Gynecology, Zunyi Maternal and Child Health Hospital, Zunyi City, Guizhou Province 564300

ARTICLE INFO

Article history

Received: 17 October 2025

Accepted: 15 December 2025

Published Online: 30 December 2025

Keywords:

Adenomyosis

Drug treatment

Traditional and Western medicine treatment

Danhuang San Plan

ABSTRACT

Adenomyosis is a common gynecological disease causing secondary dysmenorrhea, menstrual irregularities, and infertility. Traditionally, the treatment of adenomyosis has been primarily surgical; however, with the deepening understanding of the disease and the contemporary demand for fertility preservation, non-surgical treatments are gaining more attention. Pharmacotherapy, a key non-surgical approach, encompasses diverse strategies such as hormonal agents, anti-hormonal drugs (e.g., gonadal axis suppressants/blockers), non-steroidal anti-inflammatory drugs (NSAIDs), and traditional Chinese medicine (TCM). It plays a crucial role in managing adenomyosis, enhancing patients' quality of life, and preserving fertility.

In recent years, traditional Chinese medicine (TCM) and the integration of TCM with Western medicine have demonstrated unique advantages in adenomyosis management. Traditional Chinese medicine considers the main cause of adenomyosis to be "blood stagnation in the uterus," leading to poor circulation of qi and blood, which causes symptoms such as dysmenorrhea and menstrual abnormalities. The treatment principle is to invigorate blood circulation and resolve stasis, warming the meridians to improve qi and blood circulation, combined with lifestyle adjustments and other comprehensive interventions. The team has long been committed to clinical research on the grading treatment of adenomyosis, proposing the concept and approach of the "Danhuang San Plan," which integrates traditional and Western medicine based on the principles of Western medicine's gonadal axis suppression/blocking treatment and traditional Chinese medicine's invigorating blood circulation and resolving stasis, achieving good results and providing references for preserving the uterus and fertility in adenomyosis treatment. This article reviews the current status of drug treatment for adenomyosis and the team's related concepts and research results, aiming to provide references for non-surgical treatment of adenomyosis and support the practical need for fertility preservation in adenomyosis.

**Corresponding Author:*

Yang Guo, female, born on November 1, 1990, Miao nationality, from Fenghuang County, Hunan Province, holds a bachelor's degree and is a chief physician specializing in gynecology and obstetrics.

Adenomyosis (AM) is a common gynecological disease found in women of reproductive age^[1]. It exhibits hormone-dependent proliferation and invasive growth similar to malignant tumors, with its pathogenesis related to hormonal drives, genetic susceptibility, immune imbalance, and changes in the local microenvironment. Imaging or pathology presents benign characteristics, but long-term clinical management is required to avoid functional impairment or disease progression, hence it is often referred to as “benign cancer.” There is currently no unified conclusion regarding the pathogenesis of AM, with various theories having theoretical limitations and academic controversies. Among many theories, the “in situ endometrium determination theory” and “3A model” proposed by Academician Lang Jinghe have gained widespread recognition^[2]. Traditionally, the treatment of AM has been primarily surgical, especially for patients with larger uterine volumes or those who have failed conservative treatment^[3-5]. However, surgical treatment has long recovery times and risks of loss of fertility^[6], which does not align with the current context of declining birth rates, the national open fertility policy, and the strong advocacy for fertility preservation in academia^[7]. Therefore, exploring non-surgical treatment methods for AM is of significant practical importance.

Non-surgical treatment methods for adenomyosis mainly include various drug therapies and physical therapies. Drug treatments include progestins/anti-hormonal drugs, intrauterine drug delivery systems, traditional Chinese medicine, and non-steroidal anti-inflammatory drugs^[8-9]. Physical therapy, represented by High-Intensity Focused Ultrasound (HIFU), is a non-invasive treatment option^[10-11]. However, HIFU treatment has limitations such as high equipment costs, dependence on acoustic pathways, selection of lesion types (better effects on anterior wall and focal types), combination schemes, and long-term recurrence, which restrict its clinical application^[12-13].

Adenomyosis is a mass lesion formed by the endometrium located ectopically in the uterine myometrium under the influence of estrogen, leading to repeated bleeding and fibrosis^[14]. Theoretically, methods that suppress the gonadal axis and atrophy the ectopic endometrium can treat this disease, which is the basic principle of various gonadal axis blocking/suppressing drugs and progestin treatments. Throughout the history of drug treatment for AM, various progestins or anti-hormonal drugs have been the mainstay, such as Danazol, Nomezine, and Dienogest; gonadal axis suppressants like Mifepristone and GnRHa^[15-20]. These drugs primarily achieve therapeutic goals by atrophying the ectopic

endometrium or suppressing the gonadal axis system, preventing the formation of new lesions. On this basis, the diseased tissue is absorbed by the body, and the pathogenic lesions/uterine volume are reduced to a certain extent. However, this reduction is very limited and does not favor the restoration of uterine morphology and fertility.

With the rise of fertility preservation concepts, traditional Chinese medicine and the combination of traditional and Western medicine have shown unique characteristics and advantages in the clinical treatment of AM^[21-23]. The team explored the use of compound traditional Chinese medicine to treat ICR mouse models of adenomyosis over a decade ago, achieving good results^[24]. Based on this, the team adopted the concept of combining traditional and Western medicine and proposed a methodology for grading treatment of AM, achieving satisfactory treatment outcomes^[25]. This article aims to review the current status of drug treatment for AM and the team’s related research results, providing references for non-surgical treatment and fertility preservation in AM.

一、Hormonal Treatment Drugs

1. **Danazo** A synthetic progestin that acts like an androgen. Its main mechanism of action is to inhibit the synthesis of estrogen, thereby reducing the occurrence of endometriosis. However, this drug has notable side effects, such as weight gain, acne, and other androgen-related side effects, and has been largely eliminated^[26-29].

2. **Nomezine** A progestin derivative of 19-nortestosterone, previously used to treat endometriosis and AM. Studies have shown its poor efficacy and significant side effects^[30-31], such as irreversible male characteristics with long-term continuous use, leading to poor patient compliance, and it has now been largely eliminated.

3. **Dienogest** A synthetic progestin that primarily inhibits the proliferation of endometrial cells by activating progesterone receptors in the endometrium and reducing the biological activity of estrogen, thereby alleviating symptoms of AM, such as pain and irregular menstruation^[32-34]. Some patients may experience irregular vaginal bleeding during use, affecting compliance. In addition, Dienogest does not significantly reduce lesions (uterine volume), which is not conducive to eliminating lesions and restoring uterine volume and morphology, thus hindering the recovery of uterine fertility.

4. **Mirena** A levonorgestrel-releasing intrauterine device widely used in the treatment of AM. It can control menstrual bleeding and alleviate pain to a certain extent through continuous release of progestin^[35]. However,

Mirena's effectiveness drops as the size of the uterus or lesion increases, exhibiting a "lesion threshold" phenomenon^[36-37]. Studies have shown that using Mirena alone for AM treatment is ineffective when the maximum myometrial thickness or maximum diameter of the adenomyoma (MD) > 35 mm, indicating that the effective "lesion threshold" for Mirena treatment of AM is MD ≤ 35 mm, with a recommendation for use at MD < 30 mm[38-40]. The "lesion threshold" is an academic viewpoint proposed by the team in the "Mirena Three Plan" for grading treatment of AM, which predates similar findings by foreign scholars by two years^[40-41]. The academic viewpoint of the "lesion threshold" provides a reference for the clinical application of Mirena in treating AM, avoiding its excessive use.

The "Mirena Three Plan" based on grading treatment provides a complete methodology for "uterus-preserving" treatment of AM, but Mirena is an intrauterine device, and standard major uterine wall resection and reconstruction (MURU) has contraceptive effects^[42]. More importantly, Mirena can only control symptoms, prevent disease progression, or recurrence within a limited degree of lesions (MD ≤ 35 mm), with no significant effect on reversing signs/lesions, which is not conducive to restoring uterine morphology and regaining fertility. This is a limitation of Mirena and the Mirena Three Plan in the context of fertility preservation.

二、Gonadal Axis Suppressants

1. Gonadotropin-Releasing Hormone Agonists (GnRHa) GnRHa is an analogue of Gonadotropin-Releasing Hormone (GnRH). GnRHa competitively inhibits pituitary GnRH receptors, blocking the pulsatile action of endogenous GnRH, achieving "chemical ovarian ablation" and reducing estrogen effects, thereby inhibiting repeated bleeding and fibrosis of ectopic lesions and preventing the formation of new lesions^[17,43]. Due to its powerful "chemical ablation" effect, it can achieve a state similar to "artificial menopause," thus showing significant treatment effects during medication^[44-45]. However, GnRHa drugs also have corresponding issues, such as high costs, low estrogen effects, and osteoporosis, making long-term use impractical^[46-47]. Additionally, while these drugs can reduce lesions (uterine volume) through tissue absorption based on gonadal axis suppression, they cannot eliminate lesions or restore the uterus to normal size, which is not conducive to restoring uterine morphology and fertility. Therefore, they are often used as a phased treatment or in combination therapy^[44].

2. Mifepristone Mifepristone is a progesterone receptor antagonist commonly used for medical abortion. With the

expansion of clinical applications and in-depth research, it has been found that this drug participates in regulating the function of the hypothalamic-pituitary-ovarian axis (HPO axis). Mifepristone can act on the hypothalamus to inhibit the secretion of Gonadotropin-Releasing Hormone (LHRH), thereby affecting the secretion of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH). At the same time, it can directly act on the pituitary to inhibit the release of FSH and LH, suppressing ovarian function and reducing the secretion of estrogen and progesterone, thereby inhibiting the growth of fibroids and adenomyosis^[48-49]. Multiple studies have confirmed that Mifepristone has good efficacy in treating AM, especially suitable for patients wishing to preserve fertility^[50-52]. Other studies have shown that Mifepristone effectively alleviates the inflammatory response and related symptoms of AM by inhibiting the secretion of inflammatory cytokines, limiting mast cell activation, and reducing nerve fiber density^[51]. Clinical studies have shown that Mifepristone can significantly relieve symptoms in adenomyosis patients, such as dysmenorrhea and heavy menstrual bleeding, while also reducing uterine volume^[52]. However, compared to GnRHa drugs, Mifepristone's suppressive effect on the gonadal axis is milder. Although it can downregulate the secretion of FSH, LH, and estrogen, it does not reach the level of "chemical ovarian ablation" seen with GnRHa drugs, thus its therapeutic effect is also lower^[53].

三、Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are the first choice for pain management in AM patients, reducing the synthesis of prostaglandins by inhibiting the activity of cyclooxygenase (COX-1 and COX-2), thereby decreasing the inflammatory response in the uterine myometrium^[17]. In the pathogenesis of AM, local inflammatory responses are prominent, with prostaglandins (especially PGE2) playing a key role in pain and abnormal uterine contractions. NSAIDs alleviate inflammation, reduce pain, abnormal smooth muscle contractions, and abnormal uterine bleeding by inhibiting cyclooxygenase (COX) and blocking the conversion pathway of arachidonic acid to prostaglandins, thus reducing PGE2 synthesis[4,54]. However, NSAIDs do not reduce ectopic lesions and have gastrointestinal discomfort and damage as side effects with long-term use, making them not a routine or commonly used drug for treating AM.

四、Traditional Chinese Medicine Treatment

Traditional Chinese medicine has shown certain

effects in the treatment of AM due to its unique treatment concepts. Traditional Chinese medicine categorizes AM under “dysmenorrhea” and “heavy menstrual bleeding,” attributing it to pathogenesis categories such as “qi stagnation and blood stasis” and “spleen deficiency and blood stasis.” The treatment principle is to invigorate blood circulation and resolve stasis to improve qi and blood circulation. Research by Cai Xiaohui et al. showed that the serum containing Guizhi Fuling Pill with additional ingredients could reduce the wet weight and uterine coefficient in AM rats^[55]. Luo Xuejuan et al. used Xuefu Zhuyu Decoction to treat AM, demonstrating that this formula achieves clinical efficacy through its regulatory effect on the hormonal endocrine system^[56]. Research by Li Kunyin’s team confirmed that modified Shaoyao Gancao Decoction can inhibit the proliferation of human adenomyosis cells, reduce cell migration, effectively regulate endometrial thickness and uterine volume, adjust serum hormone levels, alleviate dysmenorrhea symptoms, and improve disease outcomes^[57-59]. Traditional Chinese medicine has unique characteristics in treating AM, alleviating dysmenorrhea, reducing menstrual flow, and shrinking lesions through methods such as invigorating blood circulation and resolving stasis. However, pure traditional Chinese medicine treatment has limitations such as slow onset and long treatment duration, making it difficult to effectively shrink or eliminate ectopic lesions, leading to symptom recurrence.

五、Combination of Traditional and Western Medicine Treatment

The combination of traditional and Western medicine in treating AM reflects the complementary advantages and synergistic effects of both approaches. Western medicine can quickly suppress the gonadal axis, reduce estrogen production, or atrophy ectopic endometrium, preventing the formation of new lesions; while traditional Chinese medicine’s effects of “invigorating blood circulation and resolving stasis” can eliminate existing lesions (old lesions) and reduce the side effects of Western medicine, enhancing treatment compliance. Clinical research by Wei Xinjun et al. showed that compared to using Dienogest alone, the combination of Xuandan Sanjie Decoction and Dienogest effectively improved clinical symptoms in patients with qi stagnation and blood stasis type AM, especially in reducing abnormal uterine bleeding caused by Dienogest^[23]. Jia Haijin used Shaofu Zhuyu Decoction combined with Leuprolide to treat AM postoperative patients, achieving higher overall efficacy and reducing dysmenorrhea scores, disease indicators, and adverse

reaction rates compared to using Leuprolide alone^[60]. Wang Liyuan et al. showed that the combination of Sanjie Zhen Tong Capsule and Dienogest in treating AM could exert synergistic effects through multi-target mechanisms, significantly improving hormonal balance, uterine morphological indicators, optimizing menstrual cycle characteristics, and ensuring medication safety^[61]. Research by Qiao Xiaoli et al. demonstrated that the combined application of Guizhi Fuling Wan and Ethinyl Estradiol/Progesterone Tablets could enhance clinical efficacy in AM through multi-target synergistic mechanisms, significantly alleviating dysmenorrhea symptoms, reducing uterine volume, improving coagulation function, and downregulating serum-related factor levels^[62]. Research by Li Juan et al. showed that Mifepristone combined with Xuefu Zhuyu Decoction has a definite therapeutic effect on AM, outperforming the single Mifepristone regimen^[63]. The combination of traditional and Western medicine leverages their respective advantages, forming complementary and synergistic effects, providing new ideas and methods for the comprehensive treatment of AM.

The team has long been dedicated to research on AM treatment, validating the therapeutic effects of compound traditional Chinese medicine based on Danshen and Huangqi in ICR mouse models of adenomyosis over a decade ago^[24]. This compound traditional Chinese medicine is the same as the “Danhuang Quyu Capsule/ Tablets” (referred to as Danhuang) formula (20 medicinal materials), with the main functions being: invigorating blood circulation and relieving pain, softening and resolving masses, suggesting that Danhuang may have a therapeutic effect on AM (this indication is not listed in the Danhuang instructions). In subsequent exploratory and non-systematic clinical observations using Danhuang to treat moderate to severe patients, the team found that using Danhuang alone for treatment took a long time and had poor efficacy. Therefore, the team combined the rich theoretical and practical experience accumulated from the previous “Mirena Three Plan” grading treatment of AM and the principles of pharmacoeconomics maximizing cost-effectiveness and efficacy, adopting a combination of traditional and Western medicine and grading treatment approach, gradually forming a methodology based on “Danhuang,” used alone or in combination with Mifepristone or Leuprolide/Triptorelin to treat mild, moderate, and severe AM, achieving satisfactory treatment outcomes through prospective and systematic clinical research^[25]. The “Danhuang Three Plan” consists of: 1. Danhuang Plan: using Danhuang alone, suitable for mild AM; 2. Danmi Plan: Danhuang + Mifepristone, suitable

for moderate AM; 3. Danrui Plan: Danhuang + Leuprolide (Leuprolide/Triptorelin), suitable for severe AM^[64].

Clinical research results of the “Danhuang Three Plan” indicate that the grading treatment of mild, moderate, and severe AM is very significant. Considering efficacy alone, the “Danrui Plan” shows the fastest effect and best efficacy, making it worthy of priority consideration for patients with severe AM who need to rapidly reduce uterine lesions to meet fertility needs. However, under the premise of ensuring efficacy, the three principles of pharmacoeconomics must be considered in clinical practice. The “grading and adjustment” treatment model of the Danhuang Three Plan not only reduces treatment costs but also minimizes potential toxic side effects of drugs, reflecting the maximization of cost-effectiveness and efficacy, and is the reason and charm of “grading” treatment. The “Danhuang Three Plan” provides a systematic, complete, and non-invasive treatment concept and methodology for AM, representing another innovation and breakthrough by the team following the “Mirena Three Plan” grading treatment of AM.

六、 Conclusion and Outloo

AM is prevalent among women of reproductive age, with an incidence rate of 5% to 70% in the female population^[65-66]. In the context of fertility preservation, treatments for AM that protect fertility should receive widespread attention. Among the existing methods for preserving fertility, the combination of traditional and Western medicine has shown unique advantages. Its core advantage lies in the synergistic effects of traditional and Western medicines, as seen in the “Danhuang Three Plan,” where Western medicine (Mifepristone, GnRHa) suppresses/block the gonadal axis and prevents the formation of new lesions; while Danhuang’s “invigorating blood circulation and resolving stasis” eliminates already formed lesions, thus having a significant effect on reversing signs (shrinking lesions) and restoring uterine morphology and volume, which undoubtedly has clear practical significance in the context of fertility preservation. The concept of blending traditional and Western medicine should become the foundation for drug treatment of AM to achieve non-invasive and rapid elimination of lesions.

Furthermore, AM is a disease primarily characterized by changes in the uterine myometrium, with varying degrees of lesions, akin to cancer staging treatment, which should be treated differently based on “staging” or “grading” with different treatment plans. The team’s treatment model for AM combining traditional and Western medicine, grading, and adjustment is conducive

to non-invasive, precise, and standardized disease treatment, also aligning with the three principles of health economics and clinical medication habits. The idea of “grading” treatment should become the cornerstone of AM treatment.

Although the combination treatment concept represented by the “Danhuang Three Plan” has shown satisfactory therapeutic effects in preserving fertility in AM, there are also corresponding issues, such as the lack of AM indications in the Danhuang guidelines, which hinders clinical application under strict insurance controls; gastrointestinal reactions caused by oral Danhuang may obstruct treatment continuity and affect efficacy; AM is a chronic disease requiring long-term management, all of which are clinical challenges. Therefore, increasing the new indications for existing traditional Chinese medicines (such as Danhuang) or developing new formulations (such as injections); conducting in-depth research on new traditional Chinese medicines that can effectively shrink ectopic lesions and establishing long-term chronic disease management systems should be the direction for non-invasive and fertility-preserving treatment of AM.

With reason and belief, as we learn more about AM and develop new treatments and formulations, we can expect more personalized and effective drug therapies. The combination of traditional and Western medicine treatment model is expected to become mainstream, providing AM patients with more comprehensive and efficient non-invasive treatment options.

References:

1. Shang Wenxia, Kang Zhiyuan, Liu Hongqi. Clinical experience of Professor Liu Hongqi in treating uterine adenomyosis [J]. *Guangxi Journal of Traditional Chinese Medicine*, 2023, 46(06): 55-7+62.
2. Lang Jinghe. In-depth research and development of endometriosis [J]. *Chinese Journal of Obstetrics and Gynecology*, 2010, 45(4): 241-2.
3. Li Ning. Comparison of the effects of Acetate Leuprolide and Drospirenone Ethinyl Estradiol Tablets in treating postoperative patients with adenomyosis [J]. *Chinese Journal of Practical Medicine*, 2025, 37(16): 155-8.
4. Peng Chao, Zhou Yingfang. Application and selection of drug treatment in the long-term management of endometriosis [J]. *Chinese Journal of Practical Gynecology and Obstetrics*, 2021, 37(03): 303-8.
5. Lang Jinghe. Historical, current, and developmental understanding of endometriosis [J]. *Chinese Journal of Practical Gynecology and Obstetrics*, 2020, 36(03): 193-6.

6. Wang Li, Li Fangmei, Zhang Yi, et al. Expert consensus on the diagnosis and treatment of malignant transformation of adenomyosis with integrated traditional and Western medicine (2024 edition) [J]. *Shandong Journal of Traditional Chinese Medicine*, 2025, 44(02): 130-40.
7. Cao Yanhua. Thoughts and suggestions on fertility preservation against the backdrop of ultra-low fertility rates [J]. *Chinese Journal of Public Health Management*, 2022, 38(06): 733-6.
8. Cao Yingying, Xu Hongbin. Research progress on Dienogest in the treatment of adenomyosis [J]. *Practical Journal of Obstetrics and Gynecology*, 2025, 41(08): 645-8.
9. Zhen Xiaohong. Observation of the treatment of adenomyosis with low-dose Mifepristone, LNG-IUS system, and GnRH α [J]. *Chinese Practical Medical Journal*, 2012, (14): 18-21.
10. Zhang Ying. Comparative study of clinical efficacy between laparoscopic surgery and HIFU treatment for uterine adenomyosis [J]. *Chinese and Foreign Medical Research*, 2021, 40(13): 30-2.
11. Dong Xin. Multifactorial study on the improvement of reproductive health by HIFU treatment for uterine fibroids and adenomyosis [M].
12. Zheng S, Rong Y, Zhu H, et al. Role of magnetic resonance-high intensity focused ultrasound (MR-HIFU) in uterine fibroids management: an updated systematic review and meta-analysis [J]. *Wideochir Inne Tech Maloinwazyjne*, 2022, 17(1): 83-94.
13. Wang K, Xing G, Yang P, et al. High-Bandwidth Heterodyne Laser Interferometer for the Measurement of High-Intensity Focused Ultrasound Pressure [J]. *Micromachines (Basel)*, 2023, 14(12).
14. Lang Jinghe. Several issues regarding adenomyosis [J]. *Chinese Journal of Practical Gynecology and Obstetrics*, 2017, 33(02): 129-33.
15. Pontis A, D'Alterio M N, Pirarba S, et al. Adenomyosis: a systematic review of medical treatment [J]. *Gynecol Endocrinol*, 2016, 32(9): 696-700.
16. Donnez J, Stratopoulou C A, Dolmans M M. Uterine Adenomyosis: From Disease Pathogenesis to a New Medical Approach Using GnRH Antagonists [J]. *Int J Environ Res Public Health*, 2021, 18(19).
17. Etrusco A, Barra F, Chiantera V, et al. Current Medical Therapy for Adenomyosis: From Bench to Bedside [J]. *Journal of Drugs*, 2023, 83(17): 1595-611.
18. Zhang S, Duan H. Risk factors and countermeasures for abnormal uterine bleeding during dienogest therapy for adenomyosis: a review [J]. *Front Reprod Health*, 2025, 7: 1550814.
19. Liang Rong, Li Aihua, Zhang Shiqian. Review of the 2023 SOGC Clinical Practice Guidelines for the Diagnosis and Treatment of Adenomyosis [J]. *Journal of Obstetrics, Gynecology, and Genetics (Electronic Edition)*, 2023, 13(03): 1-6.
20. Huang Xiaoyuan. Progress in Drug and Surgical Treatment of Adenomyosis [J]. *Journal of Maternal and Child Health*, 2025, 4(16): 22-5+33.
21. Wu Zhongkai, Cao Min, Hong Wenshun, et al. Clinical benefits of Yiqi Yangxue Zhihe Decoction (a traditional Chinese medicine formula) for treating patients with excessive menstrual bleeding due to adenomyosis [J]. *Liaoning Journal of Traditional Chinese Medicine*: 1-11.
22. Zhang Xiyuan, Li Lei. Clinical effects of promoting blood circulation and removing stasis combined with acupoint application in treating adenomyosis [J]. *Clinical Rational Drug Use*, 2025, 18(23): 129-31.
23. Wei Xinjun, Huang Meihua, Ma Xiaoping, et al. Efficacy of Xuandan Sanjie Decoction combined with Dienogest for treating qi stagnation and blood stasis type adenomyosis [J]. *Journal of Modern Integrated Chinese and Western Medicine*, 2025, 34(04): 489-92+535.
24. Ren Xiaoyan, Sun Cui, Gu Hengfang, et al. Experimental study on the treatment of adenomyosis in ICR mice using compound traditional Chinese medicine [J]. *Journal of Zunyi Medical University*, 2016, 39(06): 597-601.
25. Su Yuanhua, Liang Zhigang, Ren Xiaoyan, et al. Clinical study on the "Dan Huang San Fang" graded treatment of mild, moderate, and severe adenomyosis [J]. *Journal of Zunyi Medical University*, 2024, 47(12): 1178-83+94.
26. Xu Wenjian, Zhou Shun, Huang Chengyi, et al. Clinical comparative analysis of uterine artery embolization and oral Danazol for treating adenomyosis [J]. *Practical Journal of Obstetrics and Gynecology*, 2012, 28(04): 304-7.
27. Zheng Y, Ma R, Xu H, et al. Efficacy and safety of different subsequent therapies after fertility preserving surgery for endometriosis: A systematic review and network meta-analysis [J]. *Medicine (Baltimore)*, 2023, 102(31): e34496.
28. Veth V B, van de Kar M M, Duffy J M, et al. Gonadotropin-releasing hormone analogues for endometriosis [J]. *Cochrane Database Syst Rev*, 2023, 6(6): Cd014788.
29. Squillace A L A, Simonian D S, Allegro M C, et al. Adenomyosis and in vitro fertilization impacts - A literature review [J]. *JBRA Assist Reprod*, 2021,

- 25(2): 303-9.
30. Liao Weizhong, Yang Hongbo. Value assessment of Mifepristone and Enantone after surgery for severe pelvic endometriosis [J]. *China Practical Medicine*, 2020, 15(14): 133-5.
 31. Huang Mi, Ye Zhifa, Liu Caixia. Comparison of the efficacy and safety of Medroxyprogesterone Acetate and Enantone for treating endometriosis [J]. *Practical Medical Journal*, 2006, (16): 1935-7.
 32. Li R R, Xi Q, Tao L, et al. A systematic review and Bayesian analysis of the adverse effects of Dienogest [J]. *BMC Pharmacol Toxicol*, 2024, 25(1): 43.
 33. Chinese Medical Association Obstetrics and Gynecology Physician Branch Endometriosis Study Group. Consensus on the clinical application of Dienogest among Chinese experts [J]. *Chinese Journal of Obstetrics and Gynecology*, 2024, 59(7): 505-12.
 34. Hassanin A I, Youssef A A, Yousef A M, et al. Comparison of Dienogest versus combined oral contraceptive pills for treating women with adenomyosis: A randomized clinical trial [J]. *Int J Gynaecol Obstet*, 2021, 154(2): 263-9.
 35. Harada T, Ota I, Kitawaki J, et al. Real-world outcomes of the levonorgestrel-releasing IUD for heavy menstrual bleeding or dysmenorrhea in Japanese patients: A prospective observational study (J-MIRAI) [J]. *Contraception*, 2022, 116: 22-8.
 36. Fang Deyong. Clinical study on the treatment of adenomyosis and threshold screening of lesions using Mirena [D], 2014.
 37. Meng Yinyu, Jiang Chengsu, You Mengyuan, et al. Experimental study on the diffusion pattern of Levonorgestrel in a transparent silicone gel model [J]. *Journal of Zunyi Medical University*, 2019, 42(04): 416-21.
 38. Zhao Jing, Li Qinghan, Fang Deyong, et al. Efficacy of myometrial resection and uterine reconstruction combined with Mirena in treating moderate to severe adenomyosis [J]. *China Maternal and Child Health*, 2013, 28(15): 2464-6.
 39. Tu Jiao, Fang Deyong, Xiao Yanbing. Clinical study on threshold screening of lesions in adenomyosis treatment using Mirena [J]. *China Pharmaceutical Industry*, 2015, 24(19): 19-20.
 40. Fang Deyong, Zhao Jing, Li Qinghan, et al. Preliminary study on threshold screening of lesions in adenomyosis treatment using Mirena [J]. *Journal of Zunyi Medical University*, 2014, 37(04): 418-21.
 41. Lee K H, Kim J K, Lee M A, et al. Relationship between uterine volume and discontinuation of treatment with levonorgestrel-releasing intrauterine devices in patients with adenomyosis [J]. *Arch Gynecol Obstet*, 2016, 294(3): 561-6.
 42. Yang Li, Xiao Yanbing, Han Lei. Research progress on adenomyosis treatment focused on preserving the uterus [J]. *Journal of Surgical Anatomy*, 2020, 29(12): 1019-23.
 43. Capezzuoli T, Rossi M, La Torre F, et al. Hormonal drugs for the treatment of endometriosis [J]. *Curr Opin Pharmacol*, 2022, 67: 102311.
 44. Zhang Di. Study on the phenomenon and mechanism of autophagy for treating adenomyosis with GnRH agonists [J]. *Chinese Journal of Fertility Health*, 2019, 30(04): 342-7+62+401.
 45. Wu Shufang, Wei Dixia. Systematic review of GnRHa application in patients with endometriosis after conservative surgery [J]. *Hainan Medical Journal*, 2014, 25(08): 1127-9.
 46. Pang L L, Mei J, Fan L X, et al. Efficacy of High-Intensity Focused Ultrasound Combined With GnRH-a for Adenomyosis: A Systematic Review and Meta-Analysis [J]. *Front Public Health*, 2021, 9: 688264.
 47. Golan A. GnRH analogues in the treatment of uterine fibroids [J]. *Hum Reprod*, 1996, 11 Suppl 3: 33-41.
 48. Xing Yan. Observation of the efficacy of Mifepristone in treating 35 cases of uterine fibroids [J]. *Jiangsu Clinical Medicine Journal*, 2001, 5(1): 64.
 49. Li Ya. Research progress on the regulatory mechanism of RU486 on endometrial hyperplasia [J]. *Foreign Medicine (Obstetrics and Gynecology Volume)*, 2001, 28(1).
 50. Che X, Wang J, He J, et al. A new trick for an old dog: The application of Mifepristone in the treatment of adenomyosis [J]. *J Cell Mol Med*, 2020, 24(2): 1724-37.
 51. Che X, Wang J, He J, et al. The new application of Mifepristone in the relief of adenomyosis-caused dysmenorrhea [J]. *Int J Med Sci*, 2020, 17(2): 224-33.
 52. Che X, Wang J, Sun W, et al. Effect of Mifepristone vs Placebo for Treatment of Adenomyosis With Pain Symptoms: A Randomized Clinical Trial [J]. *JAMA Netw Open*, 2023, 6(6): e2317860.
 53. Mei Xueying. Comparison of the efficacy of GnRH agonists and Mifepristone in the treatment of uterine fibroids [J]. *China Pharmaceutical Industry*, 2016, 25(4): 40-1.
 54. ACOG Committee Opinion No. 760: Dysmenorrhea and Endometriosis in the Adolescent [J]. *Obstet Gynecol*, 2018, 132(6): e249-e58.
 55. Cai Xiaohui, He Yanlan, Ye Yiting. Effects of modified Gui Zhi Fu Ling Wan serum on immune factors

- and the expression of PTEN, TIMP-2, and VEGF proteins in rats with adenomyosis [J]. *Xinjiang Journal of Traditional Chinese Medicine*, 2024, 42(05): 82-5.
56. Luo Xuejuan, Chen Qiong, Yuan Jianhuan. Clinical efficacy of Xuefu Zhuyu Decoction for treating adenomyosis [J]. *Shenzhen Journal of Integrated Traditional Chinese and Western Medicine*, 2024, 34(16): 47-50.
 57. Jiang Xinchán, Fan Weizhi, Wang Shuai, et al. Effects of modified Shaoyao Gancao Decoction on cell proliferation and migration in adenomyosis [J]. *Chinese Journal of Traditional Chinese Medicine*, 2018, 36(02): 292-5.
 58. Jiang Xinchán, Li Kunyin, Guan Yongge, et al. Effects of modified Shaoyao Gancao Decoction on P53-273H regulation of adenomyosis cell proliferation [J]. *Journal of Beijing University of Traditional Chinese Medicine*, 2018, 41(07): 547-52.
 59. Gao Jueya. Effects of modified Shaoyao Gancao Decoction on endometrial thickness and serum hormone levels in patients with adenomyosis [J]. *China Maternal and Child Health*, 2019, 34(22): 5125-8.
 60. Jia Haijin. Effects of Shaofu Zhuyu Decoction combined with Leuprolide for treating postoperative patients with adenomyosis [J]. *China Min Kang Medicine*, 2024, 36(23): 94-6.
 61. Wang Liyuan, Sun Xiaona. Clinical effects of Sanjie Zhen Tong Capsule combined with Dienogest for treating adenomyosis [J]. *Shenzhen Journal of Integrated Traditional Chinese and Western Medicine*, 2025, 35(05): 48-50+4.
 62. Wang Sisi, Liu Yunchun, Jiao Guiqing, et al. Clinical study on the treatment of adenomyosis with modified Gui Zhi Fu Ling Wan combined with Dienogest [J]. *Modern Drugs and Clinics*, 2024, 39(02): 456-60.
 63. Li Juan, Hu Xin, Zhang Xiaomei. Clinical observation of Mifepristone combined with Xuefu Zhuyu Decoction for treating adenomyosis [J]. *Medical Information*, 2021, 34(23): 168-71.
 64. Xiao Yanbing. Theory and practice of graded therapies for adenomyosis [J]. *Journal of Zunyi Medical University*, 2025, 48(03): 213-8.
 65. Kolovos G, Dedes I, Imboden S, et al. Adenomyosis: A Call for Awareness, Early Detection, and Effective Treatment Strategies: A Narrative Review [J]. *Healthcare (Basel)*, 2024, 12(16).
 66. Taran F A, Stewart E A, Brucker S. Adenomyosis: Epidemiology, Risk Factors, Clinical Phenotype and Surgical and Interventional Alternatives to Hysterectomy [J]. *Geburtshilfe Frauenheilkd*, 2013, 73(9): 924-31.

ARTICLE

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

Alajar, Alexandra Ysabel D., Babani, Jenisha Prakash, Evangelista, Chylyn Jane F., Ibasco, Shereen Rose C., Uy, Jomar Christian V., Gariando, Carol Fe A.

CENTRO ESCOLAR UNIVERSITY - SCHOOL OF OPTOMETRY MANILA CITY, PHILIPPINES 1005

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 guajava* 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

**Corresponding Author:*

Name: ALEXANDRA YSABEL D. ALAJAR

Date of Birth: February 23, 2000

Gender: Female

Ethnicity: FILIPINO / PHILIPPINES

Education: DOCTOR OF OPTOMETRY

Major: DOCTOR OF OPTOMETRY

Title/Professional Position: FACULTY

Research Field: OPTOMETRY RESEARCH

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 pneumoniae*, *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,

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.

1.1 The 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.

1.2 The 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.

1.3 Antimicrobial 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.

1.4 Antimicrobial 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 >3 logs 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

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

2.1 Authentication 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,

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

4.1 Secure Permission for Assessment at the Microbiology Laboratory

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

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

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: ± 10 min and $24 \text{h} \pm 1 \text{h}$. 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 $37^\circ\text{C} \pm 1^\circ\text{C}$.

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

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 ($>> 3.0 \times 10^5$ 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 - 1.5×10^5 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 Górnica 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

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:

6.1 A 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.

6.2 The 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* strain due to its multidrug resistant categorization, and its ability to release beta-lactamase, as a potent gram-negative bacterium.

6.3 In 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

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.

REFERENCES

- [1] Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson D., & David A. (2017). Lightfoot phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* 6:42

- [2] Amraei, S., & Ahmadi, S. (2022). Recent studies on antimicrobial and anticancer activities of saponins: a mini-review. *Nano Micro Biosystems*, 1(1), 22-26. doi: 10.22034/nmbj.2022.160182
- [3] American Optometric Association. (n.d.). Care System for Rigid Gas Permeable Contact Lenses. Retrieved from <https://www.aoa.org/patients-and-public/caring-for-your-vision/contact-lenses/care-system-for-rigid-gas-permeable-contact-lenses>
- [4] Arshinoff, S., Hofmann, I., & Hemi, N. (2021). Role of rheology in tears and artificial tears, *Journal of Cataract & Refractive Surgery: Volume 47 - Issue 5 - p. 655-661*.
- [5] Asiedu-Gykye, I. J., Mahmood, A. S., Awortwe, C., & Nyarko, A. K. (2015). Toxicological assessment of polyhexamethylene biguanide for water treatment. *Interdisciplinary Toxicology*, 8(4), 193–202. <https://doi.org/10.1515/intox-2015-0029>
- [6] Astley, R., Miller, F. C., Mursalin, M. H., Coburn, P. S., & Callegan, M. C. (2019). An Eye on Staphylococcus aureus Toxins: Roles in Ocular Damage and Inflammation. *Toxins*, 11(6), 356. <https://doi.org/10.3390/toxins11060356>
- [7] Avato, P., Bucci, R., Tava, A., Vitali, C., Rosato, A., Bialy, Z., & Jurzysta, M. (2006). Antimicrobial activity of saponins from *Medicago* sp.: structure-activity relationship. *Phytotherapy Research*, 20(6), 454–457. <https://doi.org/10.1002/ptr.1876>
- [8] Bartlett, J., Karpecki, P.M., Melton, R., & Thomas, R.K. (2011). Keratitis: new paradigms in the understanding and management of keratitis. *Rev Optom*.
- [9] Bennett, L., Y. Hsu, H., Tai, S., Ernst, B., Schmidt, E. J., Parihar, R., Horwood, C., & Edelstein, S. L. (2019). Contact lens versus non-contact lens-related corneal ulcers at an academic center. *Eye & Contact Lens: Science & Clinical Practice*, 45(5), 301–305. <https://doi.org/10.1097/icl.0000000000000568>
- [10] Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., & Yadav, A. (2013). Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) on Two Gram-Negative and Gram-Positive Bacteria. *International Journal of Microbiology*, 2013, 1–7. <https://doi.org/10.1155/2013/746165>
- [11] Boyd, K. (2022, April 22). How to take care of contact lenses. *American Academy of Ophthalmology*. Retrieved from: <https://www.aaao.org/eye-health/glasses-contacts/contact-lens-care>
- [12] British Pharmacopoeia Commission (2016). *British Pharmacopoeia*. Great Britain: Stationery Office.
- [13] Capanoglu, E., Jassbi, A. R., Miron, A., & Xiao, J. (2016). Advance on the Flavonoid C Glycosides and Health Benefits. *Dietary Phytochemicals Nutrition and Health*, S29- S45.
- [14] Centers for Disease Control and Prevention (2021). Contact Lens Care Systems & Solutions. Retrieved August 10, 2021. <https://www.cdc.gov/contactlenses/care-systems.html>
- [15] Center for World University Rankings. (2022). University of the Philippines Manila ranking: 2021-2022. CWUR. Retrieved November 8, 2022, from <https://cwur.org/2021-22/University-of-the-Philippines-Manila.php>
- [16] Chambers, H. F., & DeLeo, F. R. (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Reviews Microbiology*, 7(9), 629-641.
- [17] Christensen, G. D., Bispo, P. J., Bell, R. A., & Janzon, A. (2012). Biofilm formation by *Staphylococcus aureus* OCULAR PATHOGENESIS. *Journal of Clinical Microbiology*, 50(1), 56-60.
- [18] Clarke, S. R., & Foster, S. J. (2006). Surface adhesins of *Staphylococcus aureus*. *Advances in Microbial Physiology*, 51, 187-224.
- [19] Cooney, S., O'Brien, S., Iversen, C., & Fanning, S. (2014). Bacteria: other pathogenic enterobacteriaceae – enterobacter and other genera. In Elsevier eBooks (pp. 433–441). <https://doi.org/10.1016/b978-0-12-378612-8.00104-9>
- [20] Correa, P. C., Lui, A. C. F., Silva, C. B., Gracitelli, C. P. B., Mimica, L. M., Sasagawa, S. M., & Netto, A. L. (2018). Study of the Effectiveness of Multipurpose Solutions on the Bacterial Disinfection of Silicone Hydrogel Contact Lenses In Vitro. *Eye & Contact Lens*, 44, S24. <https://doi.org/10.1097/ICL.0000000000000428>
- [21] Cowan, M.M. (1999). “Plant products as antimicrobial agents,” *Clinical Microbiology Reviews*, vol. 12, no. 4, pp. 564–582
- [22] Cushnie, T. P., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, 26(5), 343–356. <https://doi.org/10.1016/j.ijantimicag.2005.09.002>
- [23] Dahiya, P., & Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian J Pharm Sci*. 2012;74:443–50. doi: 10.4103/0250-474X.108420.
- [24] Dakappa, S.S., Adhikarim R., Timilsina, S.S., Sajjekhan, S. (2013). A review on the medicinal plant *Psidium Guajava* Linn. (Myrtaceae). *J Drug Deliv Ther*. 3(2):162–8.
- [25] Dalton, K., Rogers, R., & Jones, L. (2006). Physical Properties of Multi-Purpose Contact Lens Solutions).

- [26] Dalton, K., Subbaraman, L.N., Rogers, R., & Jones L. (2008). Physical properties of soft contact lens solutions. *Optom Vis Sci*; 85: 122–128.
- [27] Das, M. & Goswami, S. (2019) Antifungal and Antibacterial Property of Guava (*Psidium guajava* L.) Leaf Extract: Role of Phytochemicals. *International Journal of Health Sciences and Research*; 9(2): 39-45. ISSN: 2249-9571
- [28] Different types of contact lens solutions | Vision Direct UK. (n.d.). Retrieved September 24, 2022, from <https://www.visiondirect.co.uk/wearing-lenses/types-of-contact-lens-solutions>
- [29] Diggle, S. P., & Whiteley, M. (2020). Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. *Microbiology (Reading, England)*, 166(1), 30–33. <https://doi.org/10.1099/mic.0.000860>
- [30] Dionisio, D. [Mang Danny]. (n.d.). Mang Danny's Organic & Natural Farm. Retrieved January 11, 2023, from <https://www.facebook.com/people/Mang-Dannys-Organic-Natural-Farm/100064751139032/>
- [31] Doms (London), F. M. M. H. S. A., Frcs(C), M. R. S. M., & Facs, F. M. M., I. (2012, August 29). *The Ophthalmic Assistant: A Text for Allied and Associated Ophthalmic Personnel: Expert Consult - Online and Print (9th ed.)*. Saunders.
- [32] Emina, M. & Idu, F. (2022). Bacteria and parasites in contact lenses of asymptomatic wearers in Nigeria. *J Optom*. 2022 Apr;4(2):69–74. doi: 10.1016/S1888-4296(11)70044-9. Epub 2022. PMID: PMC3974398.
- [33] Eryılmaz, M., et al. (2018). Antimicrobial efficacies of four multi-purpose contact lens care solutions. *Ankara Üniversitesi Eczacılık Fakültesi Dergisi*. 42. 15-22. 10.1501/Eczfak-0000000603.
- [34] Falahati-Marvast, F., Alipour, F., Farokhzadian, J. et al. (2021) Determining the information needs of contact lens wearers for better education and more support: a qualitative study. *BMC Ophthalmol* 21, 325. <https://doi.org/10.1186/s12886-021-02085-0>
- [35] Feskanich, D., Ziegler, R., Michaud, D., Giovannucci, E., Speizer, F., Willett, W., & Colditz GA. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *J Natl Cancer Inst*. 92:1812–23.
- [36] Fleiszig, S.M. & Efron, N. (1992). conjunctival flora in extended wear of rigid gas permeable contact lenses. *Optom Vis Sci*. 69:354–357.
- [37] Food and Drug Administration (2018). Contact Lens Care. Retrieved from: <https://www.fda.gov/consumers/free-publications-women/contact-lens-care>
- [38] Foster, T. (1996). *Staphylococcus*. *Medical Microbiology* - NCBI Bookshelf. <https://www.ncbi.nlm.nih.gov/books/NBK8448/>
- [39] Fustor, M., Nuevo, J., & Oro, R. (2008). Comparative Antibacterial Efficacy of Multi-Purpose Solutions and Hydrogen Peroxide Lens Care Systems
- [40] Gabriel, M. M., McAnally, C., & Bartell, J. (2018). Antimicrobial efficacy of multipurpose disinfecting solutions in the presence of contact lenses and lens cases. *Eye & contact lens*, 44(2), 125.
- [41] Gjerde, H., & Mishra, A. (2018). Contact lens-related *Pseudomonas aeruginosa* keratitis in a 49-year-old woman. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*, 190(2), E54. <https://doi.org/10.1503/cmaj.171165>
- [42] Gnanamani, A., Periasamy, H., & Paul-Satyaseela, M. (2017). *Staphylococcus aureus*: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach. In *InTech eBooks*. <https://doi.org/10.5772/67338>
- [43] Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019) Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochem Rev* 18, 241–272. <https://doi.org/10.1007/s11101-018-9591-z>
- [44] Grant, T. & Tang, A. (2020). A survey of contact lens wearers and eye care professionals on satisfaction with a new smart-surface silicone hydrogel daily disposable contact lens. *Clinical Optometry*. 12:9-15
- [45] Green, M., Sara, S., Hughes, I., Apel, A., & Stapleton, F. (2019). Trends in contact lens microbial keratitis 1999 to 2015: A retrospective clinical review. *Clinical & Experimental Ophthalmology*, 47(6), 726–732. <https://doi.org/10.1111/ceo.13484>
- [46] Grover I. & Bala S. (1993). Studies on antimutagenic effect of guava (*Psidium guajava*) in *Salmonella typhimurium*. *Mut Res*. 300:1–3.
- [47] Gutiérrez-Grijalva E., Picos S., Leyva L., Criollo M., Vazquez O., & Heredia J. (2017). Flavonoids and phenolic acids from *Oregano*: occurrence, biological activity and health benefits. *Plants* 7:2
- [48] Hatami, H., Ghaffari Jolfayi, A., Ebrahimi, A., Golmohammadi, S., Zangiabadian, M., & Nasiri, M.J. (2021). Contact Lens Associated Bacterial Keratitis: Common Organisms, Antibiotic Therapy, and Global Resistance Trends: A Systematic Review. *Frontiers in Ophthalmology*.
- [49] He, Q. & Venant, N. (2004). Antioxidant power of phytochemicals from *Psidium guajava* leaf. *J Zhejiang Univ Sci A*. 5(6):676–83.
- [50] Hinojosa, J., Patel, N., Zhu, M., & Robertson, D (2017). Antimicrobial Efficacy of Contact Lens Care Solutions Against Neutrophil-Enhanced Bacterial

- Biofilms. *Trans. Vis. Sci. Tech*;6(2):11.
doi: <https://doi.org/10.1167/tvst.6.2.11>
- [50] Hudson, J. (2014). MICROBIOLOGICAL SAFETY OF MEAT | *Staphylococcus aureus*. In Elsevier eBooks (pp. 376–381). <https://doi.org/10.1016/b978-0-12-384731-7.00041-6>
- [51] Iglewski, B. H. (1996). *Pseudomonas*. Medical Microbiology - NCBI Bookshelf. [https://www.ncbi.nlm.nih.gov/books/NBK8326/#:~:text=The%20cell%20envelope%20of%20P,%2C%20and%20lipopolysaccharide%20\(LPS\).](https://www.ncbi.nlm.nih.gov/books/NBK8326/#:~:text=The%20cell%20envelope%20of%20P,%2C%20and%20lipopolysaccharide%20(LPS).)
- [52] Iguban, E., Nañagas, J., & De Mesa-Rodriguez, R. (2013). The Antimicrobial Efficacy of Multipurpose Contact Lens Solutions on Standard Strains of Common Ocular Pathogens. *Philipp J Ophthalmol*, 38, 35–42. <https://paojournal.com/wp-content/uploads/2019/02/Antimicrobial.pdf>
- [53] Iguban, E., Nañagas, J.P., & De Mesa-Rodriguez, R.F. (2013, June). The Antimicrobial Efficacy of Multipurpose Contact Lens Solutions on Standard Strains of Common Ocular Pathogens. *Philippine Journal of Ophthalmology*. Retrieved September 24, 2022, from <https://paojournal.com/article/the-antimicrobial-efficacy-of-multipurpose-contact-lens-solutions-on-standard-strains-of-common-ocular-pathogens/>
- [54] ISO 14729:2001/A1:2010. Ophthalmic Optics—Contact Lens Care Products—Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses. International Organization for Standardization; Geneva, Switzerland: 2010.
- [55] ISO 18259:2001/2014. Ophthalmic Optics—Contact Lens Care Products—Method to Assess Contact Lens Care Products with Contact Lenses in a Lens Case, Challenged with Bacterial and Fungal Organisms. International Organization for Standardization; Geneva, Switzerland: 2014.
- [56] Jaiarj, P., Khoohaswan P., Wongkrajang Y., Peungvicha P., Suriyawong P., Sumal Saraya M.L., et al. (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. Leaf extract. *J Ethnopharmacol*. 67(2):203–12.
- [57] Jeong S., Cho S.K., Ahn K.S., Lee J.H., Yang D.C., & Kim J. (2004). Anti-inflammatory effects of an ethanolic extract of guava (*Psidium guajava* L.) leaves in vitro and in vivo. *J Med Food*. 17(6):678–85.
- [58] Jitendra, P. K., Banik, A., & Dixit, S. (2011). A new trend: ocular drug delivery system. *International Journal of Pharmaceutical. Sciences*, 2(3), 720-744.
- [59] Johnson & Johnson Medical Ltd. (n.d.). Contact Lens Care. The Vision Care Institute. Retrieved from: https://www.jnjvisioncare.ac/sites/default/files/public/ae/documents/42109_tvci_txtbk_chptr_11_snglpgs_hr.pdf
- [60] Joseph B. & Priya R.M. (2011). Phytochemical and biopharmaceutical aspects of *Psidium guajava* (L.) essential oil: a review. *Res J Med Plant*. 5(4):432–42.
- Kaczmarek, B. (2020) Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials—A Minireview. *Materials*.; 13(14):3224. <https://doi.org/10.3390/ma13143224>
- [61] Karaca, I., Barut Selver, O., Palamar, M., Egrilmez, S., Aydemir, S., & Yagci, A. (2020). Contact lens-associated microbial keratitis in a Tertiary Eye Care Center in Turkey. *Eye & Contact Lens: Science & Clinical Practice*, 46(2), 110–115. <https://doi.org/10.1097/icl.0000000000000617>
- [62] Khadka, B., Mahato, M., Tuladhar, R., & Singh, A. (2019). Effect of *Psidium guajava* L on Biofilm Forming Multidrug Resistant Extended Spectrum Beta Lactamase (ESBL) Producing *Pseudomonas aeruginosa*. *Tribhuvan University Journal of Microbiology*. 6. 19-25. 10.3126/tujm.v6i0.26574.
- [63] Khanna, A., Khanna, M., & Aggarwal, A. (2013). *Serratia marcescens*- a rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients. *Journal of clinical and diagnostic research: JCDR*, 7(2), 243–246. <https://doi.org/10.7860/JCDR/2013/5010.2737>
- [64] Kreger A. S. (1983). Pathogenesis of *Pseudomonas aeruginosa* ocular diseases. *Reviews of infectious diseases*, 5 Suppl 5, S931–S935. https://doi.org/10.1093/clinids/5.supplement_5.s931
- [65] Kumar, M. (2021). Guava (*Psidium guajava* L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. MDPI. Retrieved October 11, 2022, from <https://www.mdpi.com/2304-8158/10/4/752>
- [66] Lamb, A. J. (2005, November). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5), 343–356. <https://doi.org/10.1016/j.ijantimicag.2005.09.002>
- [67] Lazarus, R. (2021, November 4). A Guide to Contact Lens Solutions. *Optometrists.org*. Retrieved October 11, 2022, from <https://www.optometrists.org/general-practice-optometry/optical/guide-to-contact-lenses/a-guide-to-contact-lens-solutions/>
- [68] Mailoa M., Mahendradatta, M., Laga, A., & Djide, N., (2014). Antimicrobial activities of tannins extract from guava leaves (*Psidium guajava* L.) on pathogens microbial. *International Journal of Scientific and Technology Research*, 3: 236-241.

- [69] Mansour, E., Khaled, A. B., Lachiheb, B., Abid, M., Bachar, Kh., & Ferchichi, A. (2018). Phenolic compounds, antioxidant, and antibacterial activities of peel extract from Tunisian Pomegranate. JKUAT Repository Home. <http://ir.jkuat.ac.ke/handle/123456789/4350>
- [70] Matsuzaki K, Ishii R, & Kobiyama K. (2010). New benzophenone and quercetin galloyl glycosides from *Psidium guajava* L. *J Nat Med.* 64:252–6.
- [71] Mayo Foundation for Medical Education and Research. (2022, September 1). Chlorhexidine (topical application route) side effects. Mayo Clinic. Retrieved September 20, 2022, from <https://www.mayoclinic.org/drugs-supplements/chlorhexidine-topical-application-route/side-effects/drug/20070874>
- [72] McAnally, C., Walters, R., Campolo, A., Harris, V., King, J., Thomas, M., Gabriel, M.M., Shannon, P., & Cray, M. (2021). Antimicrobial Efficacy of Contact Lens Solutions Assessed by ISO Standards. *Microorganisms.* 9(10):2173. doi: 10.3390/microorganisms9102173
- [73] Mukhtar, H.M., Ansari, S.H., Bhat, Z.A., Naved, T., & Singh P. (2004). Antidiabetic activity of an ethanol extract obtained from the stem bark of *Psidium guajava* (Myrtaceae). *Die Pharmazie*;61:725–7.
- [74] Narayana, B., Pooja, R., Sevitha, B., & Vidyalakshmi, K. (2018). Comparison of the Antimicrobial Efficacy of Various Contact Lens Solutions to Inhibit the Growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *International Journal of Microbiology*, vol. 2018, Article ID 5916712, <https://doi.org/10.1155/2018/5916712>
- [75] Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., & Rahman, M. (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clinical Phytoscience.* 4. 10.1186/s40816-018-0093-8.
- [76] Ngene, A.C., Aguiyi, J.C., Chibuike, C.J., Ifeanyi, V.O., Ukaegbu-Obi, K.M., Kim, E.G., Ohaeri, U.C. and Onyemegbulem, B.O. (2019) Antibacterial Activity of *Psidium guajava* Leaf Extract against Selected Pathogenic Bacteria. *Advances in Microbiology*, 9, 1012-1022. <https://doi.org/10.4236/aim.2019.912066>
- [77] Osonga, F. J., Akgül, A., Miller, R. M., Eshun, G. B., Yazgan, İ., Akgul, A., & Sadik, O. A. (2019). Antimicrobial activity of a new class of phosphorylated and modified flavonoids. *ACS Omega*, 4(7), 12865–12871. <https://doi.org/10.1021/acsomega.9b00077>
- [78] Patel, P., Joshi, C., Birdi, T., & Kothari, V. (2019). Anti-infective efficacy of *Psidium guajava* L. leaves against certain pathogenic bacteria. *F1000Research*, 8, 12. <https://doi.org/10.12688/f1000research.17500.2>
- [79] Pucker, A.D. & Tichenor, A.A. (2020). A review of contact lens dropout. *Clinical Optometry.* 12:85–94
- [80] Raj, A., Menon, V. & Sharma, N. (2020) Phytochemical screening, antimicrobial, antioxidant and cytotoxic potential of different extracts of *Psidium guajava* leaves. *Vegetos* 33, 750–758. <https://doi.org/10.1007/s42535-020-00151-4>
- [81] Raksha L, Gangashettappa N, Shantala GB, Nandan BR, Sinha D. Study of biofilm formation in bacterial isolates from contact lens wearers. *Indian J Ophthalmol.* 2020 Jan;68(1):23-28. doi: 10.4103/ijo.IJO_947_19. PMID: 31856459; PMCID: PMC6951123.
- [82] Ramirez Contreras, J.M., Mariezcurrena Berasain, M.D., Pinzon Martinez, D.L., Archundia Velarde, E.D., & Roque Otero, A.M. (2021). Ethanol and aqueous extracts characterization from guava (*Psidium guajava* L.) and avocado (*Persea americana* Mill.) residues. *Ciência e Tecnologia de Alimentos.* 41. 10.1590/fst.11620.
- [83] Robertson, D.M. & Cavanagh, H.D. (2011). Non-compliance with contact lens wear and care practices: a comparative analysis. *Optom Vis Sci* 88:1402–1408.
- [84] Saleh, B., & Al-Mariri, A. (2020). Antibacterial Activity Evaluation of *Psidium guajava* L. (Myrtaceae) Crude Extracts Against Selected Bacterial Pathogens: Antibacterial Activity Evaluation of *Psidium guajava* L. *Biological Sciences - PJSIR*, 63(2), 119–126. <https://doi.org/10.52763/PJSIR.BIOL.SCI.63.2.2020.119.126>
- [85] Sanches, N.R., Cortez, D.A.G., Schiavini, M.S., Nakamura, C.V., & Filho, B.P.D. (2005). An evaluation of antibacterial activities of *Psidium guajava* (L.) *Brazilian Archives of Biology and Technology.* 48(3):429–436
- [86] Seo, J., Lee, S., Elam, M.L., Johnson, S.A., Kang, J., & Arjmandi, B.H. (2014). Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Sci Nutr*;2(2):174-80. doi: 10.1002/fsn3.91
- [87] Situ, P., Simpson, T., Jones, L., & Fonn, D. (2010). Effects of silicone hydrogel contact lens wear on ocular surface sensitivity to tactile, pneumatic mechanical, and chemical stimulation. *Investigative Ophthalmology & Visual Science*
- [88] Sizar, O., Leslie, S.W., & Unakal, C.G. (2023, May 30). Gram-Positive bacteria. *StatPearls - NCBI Bookshelf.* <https://www.ncbi.nlm.nih.gov/books/>

- NBK470553/#:~:text=Gram%2Dpositive%20cocci%3A,arthritis%2C%20osteomyelitis%2C%20and%20abscesses
- [89] Shaheena, S., Chintagunta, A., Dirisala, V., & Kumar N. (2019). Extraction of bioactive compounds from *Psidium guajava* and their application in dentistry. *AMB Express* 9(1):208
- [90] Sharma, A., Biharee, A., Kumar, A., & Jaitak, V. (2020, October 21). Antimicrobial Terpenoids as a Potential Substitute in Overcoming Antimicrobial Resistance. *Current Drug Targets*, 21(14), 1476–1494. <https://doi.org/10.2174/1389450121666200520103427>
- [91] Sing, R., Bansal, S., & Mishra, M.K. (2020). Formulation and Evaluation of Herbal Oral Gel Containing Extracts of Powdered *Psidium guajava* Linn Leaves with *Curcuma longa* Linn Rhizomes to Treat Mouth Ulcer. *Int J Drug Dev & Res Vol.12 No.2*: 150.
- [92] Singh, R., Chintagunta, A., Agarwal, D., Kureel, R., & Kumar, S. (2019). Varietal replacement rate: prospects and challenges for global food security. *Glob Food Secur* 2019:100324
- [93] Smith, R.M. & Siwatibau, S. (1975). Sesquiterpene hydrocarbons of fujian guavas. *Phytochemistry*;14(9):2013–5.
- [94] Slonczewski, J., Foster, J.W., Zinser, E.R., (2009). *Microbiology: An Evolving Science*. | W. W. Norton & Company. <https://wwnorton.com/books/9781324033523>
- [95] Szczotka-Flynn, L.B., Pearlman, E., & Ghannoum, M. (2010). Microbial contamination of contact lenses, lens care solutions, and their accessories: a literature review. *Eye Contact Lens*; 36(2):116-29. doi: 10.1097/ICL.0b013e3181d20cae
- [96] Tagousop, C. N., Tamokou, J.-D., Ekom, S. E., Ng-nokam, D., & Voutquenne-Nazabadioko, L. (2018). Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. *BMC Complementary and Alternative Medicine*, 18(1). <https://doi.org/10.1186/s12906-018-2321-7>
- [97] Tanaka, Y., Yuasa, J., Baba, M., Tanikawa, T., Nakagawa, Y., & Matsuyama, T. (2004). Temperature-Dependent Bacteriostatic Activity of *Serratia marcescens*. *Microbes and Environments*, 19(3), 236–240. <https://doi.org/10.1264/jsme2.19.236>
- [98] Tannins.org. 2022. Tannin, natural antibacterial | Tannins.org. [online] Available at: <<https://www.tannins.org/the-antimicrobial-activity-of-tannins/>> [Accessed 24 September 2022].
- [99] Tatli Cankaya, I. I., & Somuncuoglu, E. I. (2021). Potential and Prophylactic Use of Plants Containing Saponin-Type Compounds as Antibiofilm Agents against Respiratory Tract Infections. Evidence-based complementary and alternative medicine: eCAM, 2021, 6814215. <https://doi.org/10.1155/2021/6814215>
- [100] Teixeira, R., Camparoto, M., & Mantovani, M. (2003). Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L., in in vitro and in vivo assays. *Genet Mol Biol*. 555:551–5.
- [101] Tena, D., Rodríguez, N., Toribio, L., & González-Praetorius, A. (2019). Infectious keratitis: Microbiological review of 297 cases. *Japanese Journal of Infectious Diseases*, 72(2), 121–123. <https://doi.org/10.7883/yoken.jjid.2018.269>
- [102] Thammavongsa, V., Kim, H. K., & Missiakas, D. (2015). Schneewind, O. Staphylococcal manipulation of host immune responses. *Nature Reviews Microbiology*, 13(9), 529-543.
- [103] Trease, G. & Evans, W. (1989). *Pharmacognosy*, Macmillan, London, UK, 11th edition.
- [104] Tzima, K., Brunton, N.P., Rai, D. (2020). *Food Biochem*. 44:e13148. doi: 10.1111/jfbc.13148
- [105] United States Pharmacopeial Convention (2016). *United States Pharmacopoeia 39-National Formulary 34*. USA: Stationery Office.
- [106] University of the Philippines, Manila. (n.d.). History of UP Manila. History of UP Manila | University of the Philippines Manila. Retrieved November 8, 2022, from <https://www.upm.edu.ph/node/36>
- [107] Vieira, R.H., Rodrigues, D.P., Gonçalves, F.A., Menezes, F.G., Aragão, J.S., & Sousa, O.V. (2001). Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Rev Inst Med Trop Sao Paulo*;43(3):145-8. doi: 10.1590/s0036-46652001000300005. PMID: 11452322.
- [108] Wang, R., Braughton, K. R., Kretschmer, D., Bach, T. H. L., Queck, S. Y., Li, M., ... & Otto, M. (2007). Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nature Medicine*, 13(12), 1510-1514.
- [109] What is in contact lens solution? | Lenstore.co.uk. (n.d.). Retrieved September 24, 2022, from <https://www.lenstore.co.uk/eyecare/what-contact-lens-solution>
- [110] Woods, C., Ruston, D., Hough, T., & Efron, N. (1999, July). Clinical performance of an innovative back surface: eye & contact lens. *LWW*. <https://jour>

- nals.lww.com/claojournal/Abstract/1999/07000/Disinfection,_Cleaning,_and_Storage_Solutions_for.11.aspx
- [111] Wu, Y. T., Zhu, L. S., Tam, K. P., Evans, D. J., & Fleiszig, S. M. (2015). *Pseudomonas aeruginosa* Survival at Posterior Contact Lens Surfaces after Daily Wear. *Optometry and vision science: official publication of the American Academy of Optometry*, 92(6), 659–664. <https://doi.org/10.1097/OPX.0000000000000597>
- [112] Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., & Yu, X. (2020). Advances in Pharmacological Activities of Terpenoids. *Natural Product Communications*, 15(3). doi:10.1177/1934578X20903555
- [113] Zastrow, M. A. (2021, June 11). What You Need To Know About Contact Lens Solution. *Downtown Eyes*. Retrieved October 11, 2022, from <https://www.downtowneyes.com/blog/eye-care/what-you-need-to-know-about-contact-lens-solution/>
- [114] Zhu, Q., Yang, B., Deng, N., Li, Y., Wang, T., Qi, H., & Liu, L. (2018). The use of contact lenses among university students in Chengdu: Knowledge and practice of contact lens wearers. *Contact Lens and Anterior Eye*, 41(2), 229–233. <https://doi.org/10.1016/j.clae.2017.12.008>

About the Publisher

Synergy Publishing Pte. Ltd. (Syn) is an international publisher of online, open access and scholarly peer-reviewed journals covering a wide range of academic disciplines including science, technology, medicine, engineering, education and social science. Reflecting the latest research from a broad sweep of subjects, our content is accessible world-wide—both in print and online.

Syn aims to provide an analytics as well as platform for information exchange and discussion that help organizations and professionals in advancing society for the betterment of mankind. Syn hopes to be indexed by well-known databases in order to expand its reach to the science community, and eventually grow to be a reputable publisher recognized by scholars and researchers around the world.

Database Inclusion



Asia & Pacific Science
Citation Index



Creative Commons



China National Knowledge
Infrastructure



Google Scholar



Crossref



MyScienceWork