INTERNATIONAL CONFERENCE
ON LIFESTYLE DISEASES AND NATURAL MEDICINE (ICOLIFEMED)
Nomor ISSN: 3124-1115

The Effect of Goji Berry Extract (Lycium barbarum L.) on the Number of Staphylococcus aureus Colonies in Total Plate Count (TPC) in Wistar Rats (Rattus novergicus) with Conjunctivitis

Miranda Howen[1], Chrismis Novalinda Ginting [1], Linda Chiuman [1]
[1]Department of Biomedical, University of Prima Indonesia, (Medan) Indonesia

*E-mail : lindachiuman@unprimdn.ac.id

ABSTRACT
Background: Conjunctivitis is a common ocular infection that can be experimentally induced in animal models to evaluate potential therapeutic agents. This study investigated the antibacterial and healing effects of *Lycium barbarum L.* extract at varying concentrations compared with a standard antibiotic treatment in Wistar rats (*Rattus novergicus*) with induced conjunctivitis.
Methods: Twenty-five male Wistar rats were randomly assigned to five groups: (1) placebo control, (2) levofloxacin-treated, (3) 25% extract, (4) 35% extract, and (5) 45% extract. Conjunctivitis was induced using *Staphylococcus aureus*, followed by topical administration of the assigned treatments. Clinical signs of inflammation and total bacteria on plate count were assessed before and after treatment. Data were analyzed using mixed-design ANOVA with Tukey's post hoc test.
Results: Significant differences were found across groups and between pretest and posttest treatment conditions ($p < 0.05$). The levofloxacin group showed the greatest reduction in bacterial colonies and inflammation. The 45% extract groups demonstrated marked improvement compared to the placebo, 25%, and 35% extract groups, indicating a concentration-dependent response.
Conclusion: While levofloxacin exhibited superior antimicrobial efficacy, higher concentrations of *Lycium barbarum L.* extract also promoted recovery, supporting its potential as an adjunct therapy for bacterial conjunctivitis.

Keywords: *Conjunctivitis, Wistar rats, plant extract, antibiotic, ocular infection*

INTRODUCTION
Conjunctivitis is an inflammatory condition of the conjunctival mucosa, which may result from bacterial or viral infections, hypersensitivity reactions, mechanical trauma, or pharmacological agents. Among the various etiological agents, *Staphylococcus aureus* is recognized as a primary bacterial pathogen commonly implicated in bacterial conjunctivitis. *Staphylococcus aureus*, a Gram-positive coccus, exhibits multiple virulence determinants—such as protein A, exotoxins, and the ability to form biofilms—that enhance its adherence, colonization, and persistence on mucosal surfaces (Jasinka *et al.*, 2021). The rising prevalence of antimicrobial resistance among ocular pathogens has driven the investigation of alternative therapeutic strategies, particularly those involving natural bioactive compounds with broad-spectrum antimicrobial properties (Manente *et al.*, 2022).

Lycium barbarum L., commonly referred to as Goji berry, is a phytotherapeutic species extensively utilized in traditional East Asian medicine for its medicinal properties. Phytochemical investigations of *Lycium barbarum L.* have identified a diverse array of bioactive compounds, including polysaccharides—particularly *Lycium barbarum* polysaccharides (LBPs)—as well as flavonoids, phenolic acids, carotenoids such as zeaxanthin, and betaine. These bioactive constituents demonstrate a wide range of pharmacological effects, encompassing antioxidant, immunomodulatory, anti-inflammatory, and antimicrobial activities (Chiuman & Girsang, 2022). Several *in vitro* investigations have reported the antibacterial activity of *Lycium barbarum L.* extract against various pathogenic bacteria, indicating its potential as a natural source of antimicrobial agents (Yang *et al.*, 2022). Considering the limitations associated with conventional antibiotic therapy, especially in the treatment of superficial infections like conjunctivitis, the development of plant-derived therapeutics presents a viable and promising alternative approach (Sonoda & Takeuchi, 2023). Topical application of phytochemical-enriched extracts may confer localized antimicrobial activity while minimizing systemic toxicity. Within this therapeutic context, *Lycium barbarum L.* extract may function as a potential adjunct or alternative modality for managing bacterial conjunctivitis, effectively reducing microbial burden while minimizing ocular irritation and the risk of promoting antimicrobial resistance (Qin *et al.*, 2022).

The Total Plate Count (TPC) method provides a reliable quantitative microbiological assay for assessing the antimicrobial efficacy of *Lycium barbarum L.* extract by determining the number of viable bacterial cells. The use of Wistar rats (*Rattus norvegicus*) as an experimental animal model enables the simulation of conjunctival infection and facilitates the evaluation of bacterial load following therapeutic intervention (Pratama & Aldy, 2022). These experimental models provide a controlled and reproducible in vivo platform for investigating therapeutic interventions, enabling thorough evaluation of both clinical manifestations and microbiological parameters (Howen *et al.*, 2022).

This study is designed to evaluate the impact of *Lycium barbarum L.* extract on the colony-forming units of *Staphylococcus aureus* in conjunctival infections in Wistar rats, quantified using the Total Plate Count (TPC) method. The results of this study are anticipated to augment existing evidence supporting the therapeutic potential of natural compounds in bacterial infection management and may facilitate the incorporation of phytotherapeutic agents into conventional ocular pharmacological practices.

METHODS

An experimental with pretest and posttest control group was utilized for this research. This study was conducted at the Faculty of Pharmacy of the University of North Sumatra. Sample size was determined using Federer formula for this study and 25 wistar rats were used in this study. This study considered inclusion criteria such as male wistar rat, 160-200 gram of weight, and 11-12 weeks of age. The exclusion criteria such as female rats, weight out of the range of 160-200 gram, age out of range 11-12 weeks rats.

A total of 25 male Wistar rats (*Rattus norvegicus*) were used in this study and randomly divided into five groups (n = 5 per group) using a simple random sampling technique. The groups consisted of:

(1) a placebo group receiving Cendo Lyteers (sterile artificial tears) and *Staphylococcus aureus* inoculation (1.5×10^8 CFU/mL);
(2) a positive control group treated with levofloxacin 0.5% eye drops and *Staphylococcus aureus* (1.5×10^8 CFU/mL);
(3) three treatment groups administered *Lycium barbarum L.* extract eye drops at concentrations of 25%, 35%, and 45%, respectively, following inoculation with *S. aureus* (1.5×10^8 CFU/mL).

The *Lycium barbarum L.* extract eye drops were prepared by substituting the contents of sterile eye drop bottles with the plant extract at the designated concentrations (25%, 35%, and 45%), using the same excipient composition as standard ophthalmic preparations. The solutions were tested for sterility using Nutrient Agar (NA) media.

Prior to experimentation, all rats were acclimatized to laboratory conditions for seven days with free access to standard feed and water. On the eighth day, bacterial conjunctivitis was induced by inoculating both eyes with *S. aureus* suspension (1.5×10^8 CFU/mL), applied as one drop every 10 minutes for one hour. Treatments commenced on the twelfth day, with each group receiving one drops (0.05 ml) of their assigned formulation once daily until recovery or the completion of the observation period.

Bacterial quantification was performed using the Total Plate Count (TPC) method. Conjunctival swab samples were collected on day 11 (pretest) and day 17 (post-test, or day 5 after treatment). The samples were cultured on Mannitol Salt Agar (MSA) to determine the number of viable *S. aureus* colonies, enabling evaluation of the antimicrobial efficacy of the treatments.

RESULTS

All 25 Wistar rats completed the experimental procedures without mortality or notable adverse effects. Wistar rats obtained in this study derived from the Faculty of Pharmacy of North Sumatra University with age range from 11-12 weeks. These 25 rats were divided into 5 groups of treatment: control group receiving NaCl eye drops, antibiotic-treated group, group receiving 25% extract, group receiving 35% extract, and group receiving 45% extract. Induction of conjunctivitis was successful in all groups, as evidenced by redness, swelling, and discharge observed during the pretest evaluation. Each pretest and posttest groups showed significant difference ($p<0.05$) in the sum of total colony. Placebo group significancy interpreted as worsening after given the placebo treatment. On the other hand, levofloxacin and *Lycium barbarum L.* extract treatment shows a beneficial improvement. This ensured that any subsequent differences observed in the posttest phase could be attributed to the effects of the administered treatments.

Table 1. Mean Number of Colonies of Staphylococcus aureus

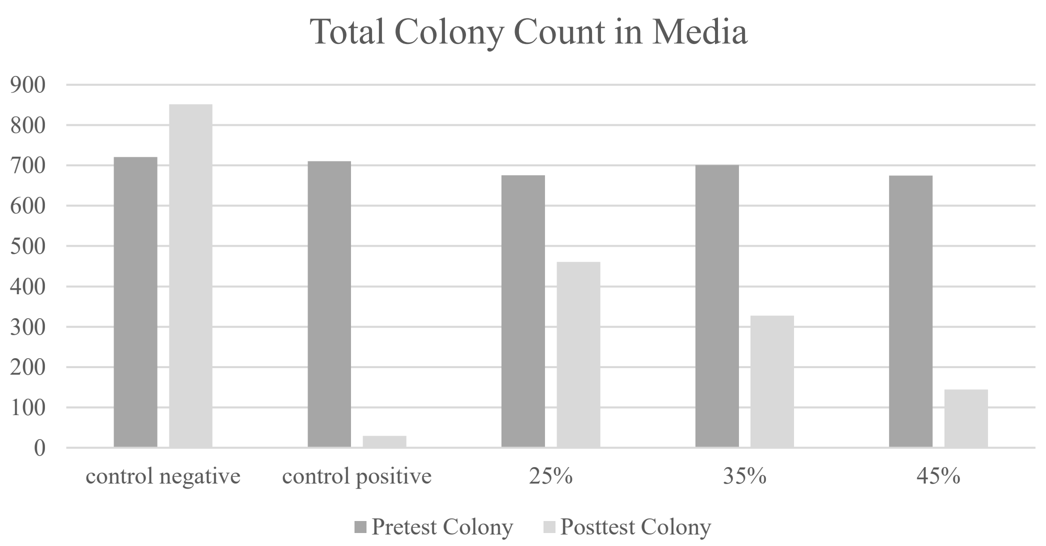
Group	n	Pretest Mean (SD)	Posttest Mean (SD)	Paired t-test (p)
Placebo (cendo lyteers)	5	720.4 (26,124)	851.6 (14,552)	0.022
Levofloxacin	5	710.20 (26,858)	29.40 (3,429)	0.008
Extract 25%	5	690.40 (21,960)	460.80 (12,718)	0.015
Extract 35%	5	701.0 (19,604)	327.40 (9,081)	0.011
Extract 45%	5	675.0 (20,182)	138.0 (7,925)	0.005

Following treatment, notable improvements in ocular condition were observed in most groups, with the magnitude of improvement varying according to the type of treatment administered. The placebo group showed no reduction in redness and swelling, while the antibiotic-treated group displayed the most rapid and complete recovery, characterized by clear conjunctival surfaces and absence of discharge by the end of the observation period. The extract-treated groups (25%, 35%, and 45%) also demonstrated gradual improvement, with higher concentrations showing greater recovery compared with lower concentrations and the placebo group.

The mixed-design ANOVA revealed significant main effects of time (pretest vs. posttest) and treatment group ($p < 0.05$), confirming that both the progression of time and the type of

treatment influenced recovery outcomes. Tukey's post hoc test indicated that the antibiotic group differed significantly from all other groups ($p < 0.05$) except the 45% extract group, exhibiting the highest mean reduction in conjunctival inflammation scores and bacterial load. Among the extract-treated groups, the 45% concentrations showed significantly greater improvement than the 25% and 35% extract and placebo.
Descriptive statistics supported these inferential findings. The placebo group displayed only a no mean score improvement, while the antibiotic group achieved the greatest reduction in inflammation and bacterial counts. The extract 25% group showed mild improvement, whereas the 35% and 45% extract groups showed substantial improvement, approaching but not matching the antibiotic group's efficacy. Graphical representation of the mean pretest and posttest scores illustrated a distinct downward trend in inflammation severity across all groups, with the steepest decline in the antibiotic and higher-concentration extract groups.

Figure 1. Trends in Colonies Number of Staphylococcus aureus



In summary, all treatment groups expect placebo group exhibited improvement from pretest to posttest, but the magnitude of recovery was significantly influenced by treatment type. The antibiotic group demonstrated the highest antibacterial and anti-inflammatory efficacy, while *Lycium barbarum L.* extract showed promising, dose-dependent therapeutic activity. The results confirm that the extract possesses measurable healing potential in conjunctivitis-induced Wistar rats, though it remains less potent than conventional antibiotic therapy. These findings support further investigation into optimizing extract concentration and formulation to enhance its therapeutic performance in ocular inflammation.

DISCUSSION

This study evaluated the therapeutic effects of varying concentrations of a plant extract (25%, 35%, and 45%) in comparison to placebo and levofloxacin (positive control) on conjunctivitis induced in Wistar rats. A total of 25 rats were divided equally into five treatment groups and assessed at both pretest and posttest time points. Overall, all treatment groups showed some degree of improvement compared to baseline, but the antibiotic-treated group demonstrated the most pronounced reduction in bacterial count and clinical signs of conjunctivitis. The higher extract concentrations (35% and 45%) also produced measurable improvements, although their effects did not surpass those of the antibiotic group.

The superior performance of the antibiotic group aligns with the expected pharmacological action of broad-spectrum ocular antibiotics, which rapidly reduce bacterial load by directly inhibiting bacterial growth and replication. Antibiotics remain the gold standard for treating bacterial conjunctivitis because of their fast-acting and targeted antimicrobial properties, resulting in decreased bacterial colonization and inflammation within a short period. The rapid improvement observed in the antibiotic-treated rats reinforces the effectiveness of conventional antibiotic therapy in acute ocular infections.

Although the plant extract groups did not outperform the antibiotic treatment, the 35% and 45% concentrations produced moderate and statistically significant improvements compared with the placebo group. This suggests that the extract contains bioactive compounds with antimicrobial and anti-inflammatory potential, but the potency may be lower or the onset of action slower than that of the antibiotic. It is possible that the extract works more indirectly—through modulation of inflammatory mediators and local immune responses—rather than immediate bactericidal action. This distinction is important for understanding its therapeutic role as an adjunct or supportive treatment, rather than as a replacement for antibiotics.

The observed activity of the extract at higher concentrations can be attributed to phytochemicals such as flavonoids, phenolics, alkaloids, and tannins, which are commonly found in medicinal plants with antimicrobial properties. These compounds can disrupt bacterial cell membranes, inhibit biofilm formation, and modulate host inflammatory responses. However, their concentration-dependent nature and lower potency compared to synthetic antibiotics may explain the smaller effect size observed. In contrast, antibiotics act directly and efficiently on bacterial metabolic pathways, leading to a faster reduction in bacterial load (Sharma *et al.*, 2025).

These findings highlight the continued clinical relevance of antibiotics as first-line therapy for bacterial conjunctivitis, especially in acute cases (Honkila *et al.*, 2022). However, the plant extract's moderate antimicrobial and anti-inflammatory effects may still be clinically useful. Plant-based preparations could be developed as complementary or maintenance therapies, potentially reducing the required antibiotic dose or duration, thereby helping to minimize the risk of antibiotic resistance. Moreover, in regions with limited access to pharmaceuticals, standardized extracts could offer a cost-effective alternative or preventive measure, though not a substitute for antibiotic therapy in severe infections (Jia *et al.*, 2025).

This study has several limitations that should be addressed in future research. The sample size was relatively small (n = 25), and only short-term effects were measured between pretest and posttest. A longer follow-up period would help determine whether the plant extract has sustained effects or simply delays bacterial regrowth. Additionally, histopathological and molecular analyses could clarify the specific mechanisms by which the extract influences inflammation and healing. Future studies should also explore synergistic effects between the extract and antibiotics to evaluate their potential in combination therapy.

CONCLUSION

In conclusion, the results of this study demonstrate that antibiotic treatment remains the most effective approach for rapidly reducing bacterial load in experimentally induced conjunctivitis in Wistar rats. Nonetheless, the plant extract at 35% and 45% concentrations also produced measurable therapeutic effects, supporting its potential as an adjunct treatment. While it cannot replace conventional antibiotics in acute bacterial conjunctivitis, it may serve as a valuable complementary agent to enhance treatment outcomes, reduce antibiotic use, or provide alternative options in resource-limited settings. Further investigation into its active compounds, mechanism of action, and long-term efficacy is warranted.

ACKNOWLEDGEMENT

Special appreciation is extended to the animal care staff for their assistance in maintaining the Wistar rats throughout the experimental period. The authors also thank their colleagues and mentors for their valuable input, guidance, and encouragement during the course of this study.

This research was conducted as part of the fulfillment of academic requirements at Universitas Prima Indonesia. The authors declare that no external funding was received for this study.

REFERENCES

Jasińska, E., Bogut, A., Magryś, A., & Olender, A. (2021). Evaluation of the role of staphylococci in the pathomechanism of conjunctivitis. *International ophthalmology*, *41*(7), 2585-2600.

Manente, R., Santella, B., Pagliano, P., Santoro, E., Casolaro, V., Borrelli, A., ... & Boccia, G. (2022). Prevalence and antimicrobial resistance of causative agents to ocular infections. *Antibiotics*, *11*(4), 463.

Chiuman, L., & Girsang, E. (2022). Antibacterial Effectiveness Of Banana Fruit Extract (Musa Paradisiaca Cv. Awak) Against Staphylococcus Aureus And Propionibacterium Acne Bacteria With Disc Defusion Method. *Journal Health & Science: Gorontalo Journal Health and Science Community*, *6*(2), 202-212.

Yang, T., Hu, Y., Yan, Y., Zhou, W., Chen, G., Zeng, X., & Cao, Y. (2022). Characterization and evaluation of antioxidant and anti-inflammatory activities of flavonoids from the fruits of Lycium barbarum. *Foods*, *11*(3), 306.

Sonoda, K., & Takeuchi, Y. (2023). Antibiotics for acute bacterial conjunctivitis. *American Family Physician*, *107*(6), 581A-581B.

Qin, D., Deng, Y., Wang, L., & Yin, H. (2022). Therapeutic effects of topical application of Lycium barbarum polysaccharide in a murine model of dry eye. *Frontiers in Medicine*, *9*, 827594.

Pratama, Y., & Fithria, A. (2022). The effect of *Piper betle* L. extract on the growth of *Streptococcus pneumoniae* colonies in Wistar strain rat model bacterial conjunctivitis. *European Modern Society Journal, 6*(3).

Howen, M., Balatif, R., Lubis, N. D. A., Amelia, S., & Yusrani, E. (2022). The Number of Bacteria Colonies in Carp Fish (Cyprinus carpio) After Administration of Lime (Citrus aurantifolia) and Orange Extract (Citrus jambhiri Lush.). *Journal of Saintech Transfer*, *5*(1), 29-33.

Sharma, V., Sharma, D., Saini, M., Jain, A., Chaudhary, J., Kaur, N., ... & Gupta, G. (2025). Flavonoids as antimicrobial agents: a comprehensive review of mechanisms and therapeutic potential. *Current Pharmaceutical Biotechnology*.

Honkila, M., Koskela, U., Kontiokari, T., Mattila, M. L., Kristo, A., Valtonen, R., ... & Tapiainen, T. (2022). Effect of topical antibiotics on duration of acute infective conjunctivitis in children: a randomized clinical trial and a systematic review and meta-analysis. *JAMA network open*, *5*(10), e2234459-e2234459.

Jia, D., Arbab, S., Ullah, H., Alzahrani, K. J., Alzahrani, F. M., Alsharif, K. F., ... & Li, K. (2025). Antibacterial Activity of Traditional Medicinal Plants: Combating Antibiotics Resistance in Animal Wound Infections. *Veterinary Medicine and Science*, *11*(3), e70361.