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ORIGINAL ARTICLE

EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF PIPER BETLE L. EXTRACT ON BACTERIAL CONJUNCTIVITIS MODEL OF WISTAR RAT CAUSED BY STREPTOCOCCUS PNEUMONIAE

Yudhi Pratama[1], Fithria Aldy[2]
[1] Ophthalmology Resident, University of Sumatera Utara, Medan, Indonesia
[2] Ophthalmologist, University of Sumatera Utara, Medan, Indonesia
Email: yudhiinyiak@gmail.com

ABSTRACT

Introduction: *Conjunctivitis is an eye infection accounted as the third leading ophthalmology cases after cataract and glaucoma globally, with Streptococcus pneumoniae as the main causes in children. Several studies had indicated that Piper betle L. extract inhibited Streptococcus pneumoniae growth.*

Methods: *This study was an analytical study with true experimental design and pre-test-post-test control group. Streptococcus pneumoniae colony growth media was analyzed using Mueller Hinton agar. Streptococcus pneumoniae colony was calculated using Total Plate Count methods.*

Results: *Sample of this study involved 30 male wistar rat aged 4 – 9 minggu. Kruskal Wallis test reveald a significant differences of Streptococcus pneumoniae colony based on the treatment group (p=0,023). Subsequent test using Posthoc revealed that there was a significant differences in Streptococcus pneumoniae colony between placebo group and group using 25% (p = 0,039) and 30% (p=0,006) piper betle. Test results also revealed that no significant difference was found between placebo group and groups using 20% piper betle (p=0,159) and ofloxacin (p=1,000).*

Conclusion: *There was a significant difference in Streptococcus pneumoniae colony among placebo group and piper betle eyedrops 25 % and 30 % group. However, significant result among placebo group and piper betle L. 20 % and ofloxacin group were absent.*

Keywords: *Bacterial conjungtivitis, Streptococcus pneumoniae, Piper betle L.*

INTRODUCTION

Bacterial conjunctivitis can be transmitted directly from an infected individual or it can be caused by abnormal proliferation of native conjunctival flora. The most common pathogen for bacterial conjunctivitis in adults is Staphylococcus sp., followed by Streptococcus pneumoniae and Haemophilus influenzae.[1,2,3] In a study, the incidence of bacterial conjunctivitis was estimated to be 135 in 10,000.[4] Conjunctivitis was included in the top 10 diseases encountered in outpatients in hospitals in Indonesia in 2010.[3.5]

Bacterial conjunctivitis is generally self-limiting, but treatment with topical antibiotics is associated with earlier clinical and microbiological remission. Since the introduction of antibiotics, bacterial resistance continues to be an ongoing problem across infectious diseases, and eye infection pathogens are no exception. The Ocular Tracking Resistance in US Today (TRUST) study reported in vitro resistance among isolates of S. aureus, H. influenzae, and S. pneumoniae to a number of commonly used topical antibiotics.[6] The Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) study showed most Staphylococcus showed resistance to Oxacillin and Azithromycin, while S. pneumoniae, P. aeruginosa, and H. influenzae showed low resistance to most antibiotics. Antibiotic resistance was also common among Staphylococcus and S. pneumoniae isolates collected from pediatric patients with eye infections. Streptococcus pneumoniae isolates, 35% and 38%, respectively, showed resistance to Azithromycin and Penicillin.[7]

S. pneumoniae, the most pathogenic and infectious Streptococcus is one of the common causes in patients with conjunctivitis and dacryocystitis.[8] S. pneumoniae has many virulence factors, including capsule polysaccharides, pneumolysin, neuraminidase, and zinc metalloproteinase, all of which contribute to the severity of eye infections. Nonencapsulated S. pneumoniae (NESp) is the cause of most cases of conjunctivitis.[9,10] NESp conjunctivitis strains and encapsulated strains secrete zinc metalloproteinase (ZmpC) which causes increased bacterial internalization by removing mucin 16 and cleaving human matrix metalloproteinase 9 (MMP-9).[11]

Chemical analysis carried out on Piper betle L. (betel) revealed the presence of various phytochemicals. The phytochemicals present in Piper betle L. have antimicrobial, antiseptic, antioxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-genotoxic, wound healing, anti-hemolytic, anti-dermatophytic, anti-hypercholesterolemic, immunomodulatory and anti-asthmatic properties.[12,13] Phenol can be bacteriostatic or bactericidal depending on the concentration level. Euganol can increase the permeability of bacterial membranes, which causes its bactericidal properties. Bactericidal properties are also found in kavikol.[14] Saponins in betel plants can work as antimicrobials and damage the cytoplasmic membrane, causing cell death. Meanwhile, the mechanism of action of flavonoid is thought to be by denaturing bacterial cell proteins and irreparably destroying cell membranes.[15,16,17] The purpose of this study was to determine the effect of Piper betle L. extract. on the growth of Streptococcus pneumoniae colonies in Wistar strain rats model of bacterial conjunctivitis.

METHODS

This research is an analytic study with a true experimental design with a pretest-posttest control group design approach; which aims to determine the effect of Piper betle L. leaf extract on the growth of Streptococcus pneumoniae colonies in Wistar strain rats with bacterial conjunctivitis model. This research was conducted in several places, the Laboratory of Pharmacy Biology, Faculty of Pharmacy, University of North Sumatra for the identification of plants and their active ingredients and for the manufacture of Piper betle L. extract, Laboratory of Clinical Pharmacology, Department of Pharmacology and Therapy, Faculty of Medicine, University of North Sumatra for the treatment of experimental animals, and laboratories Microbiology, Faculty of Medicine, University of North Sumatra for the calculation of the number of Streptococcus pneumoniae colonies. The number of samples used were 30 male wistar rats with simple random sampling technique which were then divided into 5 groups, including one negative control group (K(-)) (water/aquabides + Streptococcus pneumoniae 1.5 x 108 CFU /ml), one positive control group (K(+)) (Ofloxacin 0.3% eye drops + Streptococcus pneumoniae 1.5 x 108 CFU/ml), and three groups of Piper betle leaf extract eye drops + Streptococcus pneumoniae 1.5 x 108 CFU/ ml (P1, P2, and P3) (20%, 25%, and 30%), with repetition to 6 mice in each group.

Green betel leaf extract eye drops were made by replacing 0.3% Ofloxacin with green betel leaf extract with concentrations of 20%, 25% and 30%. The additional composition is equivalent to the additives of 1 bottle of 0.3% Ofloxacin eye drops. After the composition was adjusted, the sterilization process was carried out by boiling the solution at a temperature of 98-100℃ for 30 minutes. After the sterilization process, a sterilization test was carried out by adding green betel leaf eye drops to the Nutrient Agar (NA) medium.

Before being given treatment, experimental rats (Rattus norvegicus) were adapted to the laboratory environment for 7 days by giving standard feed and drinking to all rats. On the 8th day, the mice's eyes were inoculated with Streptococcus pneumoniae 1.5 x 10^8 CFU/ml as much as 1 drop every 10 minutes for 1 hour in both eyes. On the 12th day, negative control, positive control and Piper betle L. eye drops were given with a concentration of 20%, 25% and 30%. Mice received 4 drops of green betel leaf eye drops (0.3 ml) in the rat's eye until it healed. The number of S. pneumoniae bacterial colonies was counted in the pretest and post-test groups on the 11th and 17th days or H+5 after Piper betle L. eye drops were administered. The data obtained were the number of S. pneumoniae colonies (Total Plate Count [TPC]) on Mueller Hinton agar media from rat conjunctival swabs on day 11 and day 17 after mouse adaptation.

After all the data has been collected, the data is tabulated in the form of tables and dataprocessing is carried out. The collected data (ratio scale) was analyzed statistically with the One Way Anova test and the Post Hoc test if it met the requirements, including normal data distribution and unequal data variance results. If it does not meet the requirements, then the collected data is analyzed using the Kruskal-Wallis test (nonparametric) to replace the One Way

Anova test (parametric). Data processing was continued with the Mann Whitney test (nonparametric), replacing the Post Hoc test analysis.

RESULTS

Table 1 shows the number of Streptococcus pneumoniae colonies in Wistar Strain rats before treatment in each treatment group.

Group	n	Mean (SD)	Median (Min – Max)	p
Placebo	6	1780,33 (469,77)	1764 (1127 – 2342)	0,094
DS 20 %	6	1329,67 (496,77)	1259 (740,33 – 2206,7)	
DS 25 %	6	1937,62 (953,37)	1644,85 (918,67 – 3352,3)	
DS 30 %	6	1353,72 (330,52)	1283,35 (945,33 – 1863,3)	
Ofloxacin	6	1096,82 (630,08)	831,83 (710,33 – 2356,3)	

The highest number of *Streptococcus pneumoniae* colonies was seen in the group of rats given DS 25% with a mean of 1937 CFU (SD = 953.37 CFU). The lowest number of colonies was seen in the group of rats given ofloxacin with a mean of 1096.82 CFU (SD = 630.08 CFU). Kruskal Wallis test showed that there was no significant difference in the number of *Streptococcus pneumoniae* colonies based on the treatment group (p=0.094) before treatment. The number of *Streptococcus pneumoniae* colonies in Wistar Strain rats after treatment in each treatment group can be seen in table 2.

This study showed that most of unilateral cataract were congenital or infantile (60%), with 53.3% idiopathic, 5% had congenital infection and 1.7% had down syndrome. Another cause of cataract we found in this study were traumatic cataract (36.6%), uveitis (1.7%) and iatrogenic (1.7%).

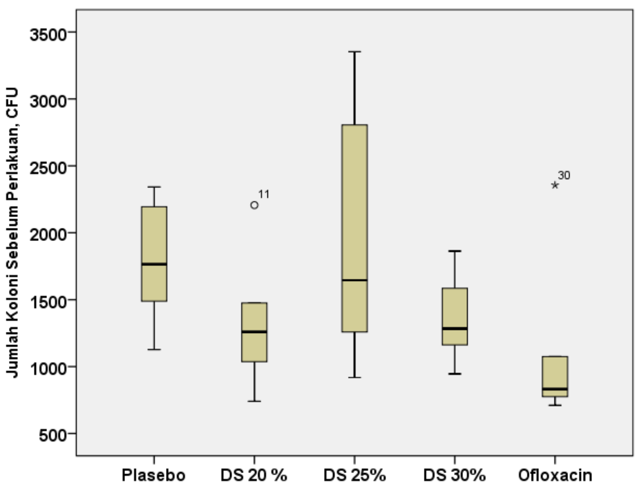

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Figure 1. Boxplot Graph of Streptococcus pneumoniae Colonies in Wistar Strain Mice before Treatment.

Table 2. Number of Streptococcus pneumoniae colonies in Wistar Strain Mice with bacterial conjunctivitis after treatment

Group	n	Mean (SD)	Median (Min – Max)	p
Placebo	6	1623,89 (324,55)	1629 (1185,33 – 1975,33)	<0,001*
DS 20 %	6	836,67 (53,44)	854 (762,33 – 898)	
DS 25 %	6	697,28 (43,9)	705,83 (613 – 734,33)	
DS 30 %	6	562,94 (52,65)	548,17 (498 – 638,33)	
Ofloxacin	6	752,11 (82,61)	763,17 (640,33 – 852,67)	

The highest number of Streptococcus pneumoniae colonies was seen in the group of rats given placebo with a mean of 1623.89 CFU (SD = 324.55 CFU). The lowest number of colonies was seen in the group of mice given 30% betel leaf with an average of 562.94 CFU (SD = 52.65 CFU). In the Oneway Anova test, there was a significant difference in the number of Streptococcus pneumoniae colonies based on the treatment group (p<0.001) after treatment.

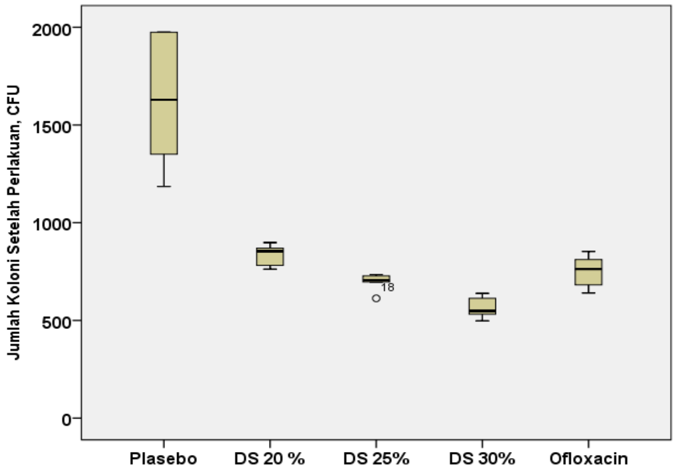

Figure 2. Boxplot Graph of Streptococcus pneumoniae Colonies in Wistar Strain Mice After Treatment

The follow-up test (Postoc) in table 3 shows a significant difference in the number of Streptococcus pneumoniae colonies between the group of rats given placebo and the whole group of rats given betel leaf and the group of rats given ofloxacin. There was no significant difference in the number of Streptococcus pneumoniae colonies between the group of rats given 20% and 25% betel leaf and the group of rats given ofloxacin (p>0.05). However, there was a significant difference in the number of Streptococcus pneumoniae colonies between groups of rats given 30% betel leaf and ofloxacin (p=0.013).

Table 4 shows the difference in the number of Streptococcus pneumoniae colonies in Wistar Strain rats between before and after treatment in each group. The results of the analysis showed that in the group of rats given placebo (p=0.557) and ofloxacin (p=0.173) there was no significant difference in the number of colonies before and after treatment. In the group of rats given 20% betel leaf (p=0.047), 25% betel leaf (p=0.025), and 30% betel leaf (p=0.003), there was a significant difference in the number of colonies. Changes in the number of Streptococcus pneumoniae colonies in Wistar rats between before and after treatment in each group are shown in table 5.

Table 3. Results of Advanced Test Analysis (Postoc) Number of *Streptococcus pneumoniae* Colonies in Wistar Strain Rats After Treatment

Group	n	Posthoc*			
		DS 20%	DS 25%	DS 30%	Ofloxacin
Placebo	6	0,017	0,008	0,004	0,009
DS 20 %	6		0,007	<0,001	0,496
DS 25 %	6			0,008	0,880
DS 30 %	6				0,013
Ofloxacin	6				

*Tamhane

Table 4. Differences in the number of *Streptococcus pneumoniae colonies* in Wistar Strain rats with bacterial conjunctivitis between before and after treatment

Group	n	Before Treatment	After Treatment	p
		Mean (SD)	Mean (SD)	
Placebo	6	1780,33 (469,77)	1623,89 (324,55)	0,557[a]
DS 20 %	6	1329,67 (496,77)	836,67 (53,44)	0,047[a]
DS 25 %	6	1937,62 (953,37)	697,28 (43,9)	0,025[a]
DS 30 %	6	1353,72 (330,52)	562,94 (52,65)	0,003[a]
Ofloxacin	6	1096,82 (630,08)	752,11 (82,61)	0,173[b]

[a]T Dependent, [b] Wilcoxon

Table 5. Changes (Delta) of *Streptococcus pneumoniae* Colonies in Wistar Strain Mice with Bacterial Conjunctivitis between Before and After Treatment

Group	n	Mean (SD)	Median (Min – Max)	p
Placebo	6	156,45 (285,1)	262,02 (-223,03 – 445)	0,023*
DS 20 %	6	493,01 (461,38)	436 (-22 – 1308,7)	
DS 25 %	6	1240,33 (964,37)	971,18 (207 – 2652,3)	
DS 30 %	6	790,78 (351,41)	744,35 (395,33 – 1365,3)	
Ofloxacin	6	344,71 (645,01)	135,33 (-77,34 – 1630,3)	

*KruskalWallis

The highest change in the number of *Streptococcus pneumoniae* colonies was seen in the group of rats given 25% betel leaf with an average of 1240.33 CFU (SD = 964.37 CFU), followed by the group of rats given 30% betel leaf with an average of 790.78 CFU (SD). = 351.41 CFU). The lowest change (delta) in the number of *Streptococcus pneumoniae* colonies was seen in the group of rats given placebo with a mean of 156.45 CFU (SD = 28.52 CFU). In the Kruskal Wallis test, there was a significant difference in the change (delta) of the number of Streptococcus pneumoniae colonies based on the treatment group (p=0.023).

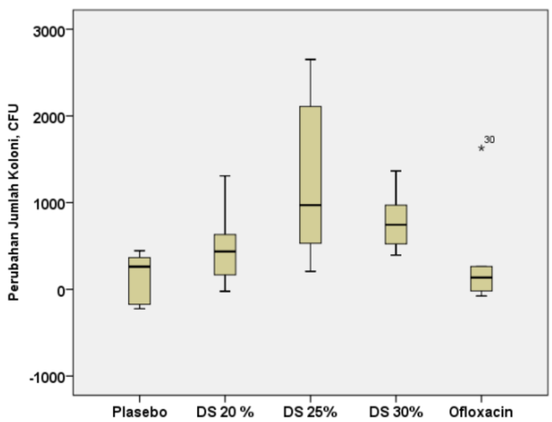

Figure 3. Boxplot Graph of Change (Delta) Number of *Streptococcus pneumoniae* Colonies in Wistar Strain Rats

The results of the Posthoc test in table 6 show a significant difference in the number of Streptococcus pneumoniae colonies between the group of rats given placebo and the group of rats given betel leaf 25% (p = 0.039) and 30% (p = 0.006). However, there was no significant difference in changes in the number of Streptococcus pneumoniae colonies between the groups of rats given placebo and those given 20% betel leaf (p=0.159) and those given ofloxacin (p=1,000). Changes in the number of Streptococcus pneumoniae colonies between the group of rats given 25% betel leaf and the group of rats given 30% betel leaf did not show a significant difference.(p=0.323)

Table 6. Results of Advanced Test Analysis (Postoc) Changes in the Number of Colonies of Streptococcus pneumoniae in Wistar Strain Mice between See before and After Treatment

Group	n	Posthoc			
		DS 20%	DS 25%	DS 30%	Ofloxacin
Placebo	6	0,159[a]	0,039[a]	0,006[a]	1,000[b]
DS 20 %	6		0,118[a]	0,237[a]	0,337[b]
DS 25 %	6			0,323[a]	0,055[b]
DS 30 %	6				0,055[b]
Ofloxacin	6				

[a]T Independent, [b]Mann Whitney

DISCUSSION

This research is an analytic study with a true experimental design and a pretest-posttest control group design approach; which aims to determine the effect of *Piper betle* L. leaf extract on the growth of *Streptococcus pneumoniae* colonies in Wistar strain rats with bacterial conjunctivitis model. This research was conducted on 30 white rats (*Rattus norvegicus*) wistar strain aged 4-9 weeks. Mice that had been adapted for a week were divided into 5 treatment groups and each treatment group contained 6 rats. All mice were inoculated with 1 drop of *Streptococcus pneumoniae* every 10 minutes for 1 hour in both eyes. Mice were left for 3 days to develop conjunctivitis and pretest samples were taken using the rat conjunctival swab method to count the number of *Streptococcus pneumoniae* colonies. The placebo group was given aquabidest eye drops 4 times 1 drop per day for 5 days. The treatment group was given *Piper betle* L. 20%, 25%, and 30% eye drops, each given 4 times 1 drop per day for 5 days. The control group was given ofloxacin 0.3% eye drops 4 times 1 drop per day for 5 days. Posttest sampling was then carried out using the rat conjunctival swab method to calculate the number of *Streptococcus pneumoniae* colonies.

Piper betle L. is a plant that has several phytochemicals in it. The main phytochemical constituent of betel nut is the essential oil which is responsible for its characteristic pungent aroma. The phytochemicals present in Piper betle L. have antimicrobial, antiseptic, antioxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-genotoxic, wound healing, anti-

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hemolytic, anti-dermatophytic, anti-hypercholesterolemic, immunomodulatory and anti-asthmatic properties.[12,13]

The results showed a change in the number of *Streptococcus pneumoniae* colonies before and after treatment, the highest was seen in the group of rats given betel leaf 25% with an average of 1240.33 CFU (SD = 964.37 CFU), and the second was seen in the group of rats given betel leaf with concentration of 30% with a mean of 790.78 CFU (SD = 351.4 CFU). The lowest number of *Streptococcus pneumoniae* colonies was seen in the group of rats given placebo with a mean of 156.45 CFU (SD = 28.52 CFU). In the Kruskal Wallis test, there was a significant difference in the number of *Streptococcus pneumoniae* colonies based on the treatment group (p=0.023), which was probably due to differences in the concentration of chemical compounds, such as polyphenols, saponins, and flavonoids contained in eye drops of *Piper betle* L. extract which played a role in bacteriostatic and bactericidal processes on the number of *Streptococcus pneumoniae* colonies in experimental animals. This study is in line with the research of Kaveti B et. al. which stated that the ethanol extract of *Piper betle* L. leaves was more effective than aqueous extract with a larger inhibition zone.[18]

This study showed that there was a significant difference in the number of Streptococcus pneumoniae colonies between the group of rats given placebo and the group of rats given betel leaf 25% (p = 0.039) and 30% (p = 0.006). However, there was no significant difference in the change in the number of *Streptococcus pneumoniae* colonies between the rat group given placebo and the rat group given 20% betel leaf (p=0.159) and the rat group given ofloxacin (p=1,000). Changes in the number of *Streptococcus pneumoniae* colonies between the group of rats given 25% betel leaf and the group of rats given 30% betel leaf did not show a significant difference (p=0.323). This research is in line with the research of Aisyah et. al. which showed a decrease in the number of gram-positive bacteria (*Staphylococcus aureus*) on eye drops of *Piper betle* L. leaf extract with concentrations of 50%, 75%, and 100% in experimental animal models of bacterial conjunctivitis.[19] This study is also in line with research by Rajeshbabu P et. al. which showed that Piper betle L. leaf extract could inhibit the growth of *Streptococcus pneumoniae* bacteria.[20] Different results were found in the study of Sujono et. al. which stated that 10%, 20%, 30%, and 40% of *Piper betle* L. leaf extract could inhibit the growth of gram-positive bacteria, including *Streptococcus pyogenes* and *Streptococcus aureus*, because from this study only *Piper betle* L. leaf extract with a concentration of 30% showed a significant result.[21]

In this study, there was also no significant difference in the change in the number of *Streptococcus pneumoniae* colonies between the group of rats given placebo and the group of rats given ofloxacin (p=1,000). This is not in accordance with the recommendations for antibiotic therapy in bacterial conjunctivitis which recommends administration of ofloxacin 1-2 drops, 4 times a day for a week with evidence level A.[4]

The results of this study are also inconsistent with the ARMOR Surveillance Study which stated that *Streptococcus pneumoniae* isolates, 35% and 38%, respectively, showed resistance to Azithromycin and Penicillin, indicating that antibiotic resistance was common among *Staphylococcus* and *Streptococcus pneumoniae* isolates collected from pediatric patients with eye infections.[7]

This study is also not in line with the statement of the International Council of Ophthalmology which states that ofloxacin has in vitro activity against a variety of aerobic and anaerobic bacteria, both gram-positive and gram-negative. Ofloxacin is thought to exert a bactericidal effect on susceptible bacterial cells by inhibiting DNA gyrase. Ofloxacin has been shown to be active against most strains of the organism in both in vitro and clinical trials in conjunctival and/or corneal infections.[22]

CONCLUSION

Based on the results of the study, it was concluded that betel leaf extract (Piper betle L.) could reduce Streptococcus pneumoniae colonies in the group of wistar strain rats. The best concentration of betel leaf extract to reduce Streptococcus pneumoniae colonies in the group of wistar strain rats with bacterial conjunctivitis model was a concentration of 25%.

REFERENCES

1. Garcia-Ferrer FJ, Augsburger JJ, Corrêa ZM. Conjunctiva & Tears. In: Riordan-Eva P, Augsburger JJ, editors. Vaughan & Asbury's GENERAL OPHTHALMOLOGY. 19th ed. New York: McGraw-Hill Education; 2018. p. 200–81.
2. MM P, JK L. Bacterial Conjunctivitis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
3. Azari AA, Arabi A. Conjunctivitis: A Systematic Review. Journal of Ophthalmic and Vision Research. 2020;15(3):372–95.
4. Smith AF, Waycaster C. Estimate of the Direct and Indirect Annual Cost of Bacterial Conjunctivitis in the United States. 2009;11:1–11.
5. Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia Tahun 2019. Kementerian Kesehatan Republik Indonesia. 2020.
6. Asbell PA, Colby KA, Deng S, McDonnell P, Meisler DM, Raizman MB, et al. Ocular TRUST: Nationwide Antimicrobial Susceptibility Patterns in Ocular Isolates. American Journal of Ophthalmology. 2008;145(6).
7. Alter SJ, Sanfilippo CM, Asbell PA, Decory HH. Antibiotic Resistance Among Pediatric-Sourced Ocular Pathogens: 8-Year Findings from The Antibiotic Resistance Monitoring in Ocular Microorganisms (Armor) Surveillance Study. Pediatric Infectious Disease Journal. 2019;38(2):138–45.
8. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M. Bacterial Profile of Ocular Infections: A Systematic Review. BMC Ophthalmology. 2017;17(1):1–9.
9. Jedrzejas MJ. Pneumococcal Virulence Factors: Structure and Function. Microbiology and Molecular Biology Reviews. 2001;65(2):187–207.
10. Valentino MD, McGuire AM, Rosch JW, Bispo PJM, Burnham C, Sanfilippo CM, et al. Unencapsulated Streptococcus pneumoniae from conjunctivitis encode variant traits and belong to a distinct phylogenetic cluster. Nature Communications. 2014;5(May):1–12.

11. Govindarajan B, Menon BB, Spurr-Michaud S, Rastogi K, Gilmore MS, Argüeso P, et al. A Metalloproteinase Secreted by Streptococcus pneumoniae Removes Membrane Mucin MUC16 from the Epithelial Glycocalyx Barrier. PLoS ONE. 2012;7(3):1–12.
12. Chowdhury U, Baruah PK. Betelvine (Piper betle L .): A Potential Source for Oral Care. Current Botany. 2020;11:87–92.
13. Dasgupta N, De B. Antioxidant activity of Piper betle L. leaf extract in vitro. Food Chemistry. 2004;88(2):219–24.
14. Begam KMF, Ravichandran P, Manimekalai V. Phytochemical Analysis of Some Selected Varieties of Piper Betle L. International Journal of Current Pharmaceutical Research. 2018;10(2):89.
15. Fazal F, Mane PP, Rai MP, Thilakchand KR, Bhat HP. The Phytochemistry, Traditional Uses and Pharmacology of Piper Betel Linn (Betel Leaf): A Pan-Asiatic Medicinal Plant. The Chinese Journal of Integrated Traditional and Western Medicine. 2014;8(26):1–11.
16. Made N, Mara D, Nayaka W, Malida M, Sasadara V, Sanjaya DA, et al. Piper betle (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications. Molecules. 2021;26(2321):1–21.
17. Salehi B, Zakaria ZA, Gyawali R, Ibrahim SA, Rajkovic J, Shinwari ZK, et al. Piper species: A Comprehensive Review on Their Phytochemistry, Biological Activities and Applications. Vol. 24, Molecules. 2019.
18. Balaji K, Rafiullah Baig M, Kaveti B, Tan L, Sin Kuan T, Baig M. Antibacterial Activity Of Piper Betel Leaves Healthcare providers' knowledge, attitude and behaviour towards breast cancer diagnosis and treatment in Malaysia-A mini systematic review Article Barriers to colorectal cancer screening in Asia: A systematic review View project Antibacterial Activity Of Piper Betel Leaves [Internet]. Vol. 2, International Journal of Pharmacy Teaching & Practices. 2011. Available from: https://www.researchgate.net/publication/236592977
19. Nur MAP, Sudaryanto S, Lestari ES, Kresnoadi E. The Effects of Green Betel Leaf (Piper betle) Extract Eye Drops on the Number of Staphylococcus aureus Colonies in Conjunctivitis Wistar Rats Model (Rattus novergicus). Majalah Obat Tradisional. 2021 Aug 31;26(2):123.
20. Sekar T. In vitro anti bacterial activity of Piper betle L and black betel CV. Kammar leaves against Staphylococcus aureus and Streptococcus pneumonia. Journal of Pharmacy Research [Internet]. 2011;4(7):2223–5. Available from: www.jpronline.info
21. Sujono H, Rizal S, Purbaya S, Jasmansyah J. UJI AKTIVITAS ANTIBAKTERI MINYAK ATSIRI DAUN SIRIH HIJAU (Piper betle L.) TERHADAP BAKTERI Streptococcus pyogenes dan Staphylococcus aureus. Jurnal Kartika Kimia. 2019 May 31;2(1)
22. Palis A, Barrio-Barrio J, Mayorga E, Mili-Boussen I, Noche C, Swaminathan M, et al. The International Council of Ophthalmology Ophthalmic clinical evaluation exercise. Indian Journal of Ophthalmology. 2021;69(1):43