Journal Syifa Sciences and Clinical Research (JSSCR)
Volume 7 Number 3, 2025
Journal Homepage: http://ejurnal.uniga.ac.id/index.php/jsscr, E-ISSN: 2656-9612 P-ISSN:2656-8187
DOI: https://doi.org/10.37311/jsscr.v7i3.34643

Flavonoid Profile of *Peperomia pellucida* Extract and Its Potential for Ophthalmic Therapy

Anis Rosida[1] , Noor Hujjatusnaini[2]*, Ayatusa' adah[3]

[1,2,3] *Department of Mathematics and Natural Sciences, Faculty of Tarbiyah and Teacher Training, IAIN Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia*

** Corresponding Author. Email:noor.hujjatusnaini@iain-palangkaraya.ac.id*

ABSTRACT

Peperomia pellucida is a tropical plant known for antioxidant, anti-inflammatory, and antibacterial activities attributed mainly to its flavonoid content. This study aimed to determine the total flavonoid content of the ethanolic leaf extract of *P. pellucida* and to evaluate its potential to reduce intraocular pressure (IOP) in Balb/C mice with *Staphylococcus aureus*–induced ocular infection. Total flavonoids were quantified spectrophotometrically using the aluminium chloride ($AlCl_3$) method at 415 nm with quercetin as the reference standard. An in vivo assay was conducted on 25 mice randomly allocated into five groups, comprising a negative control (distilled water), a positive control (0.5% timolol), and three groups receiving *P. pellucida* extract eye drops at different concentrations. IOP was measured with a Schiotz tonometer before infection, after infection, and after therapy. The ethanolic extract contained 66.96 mg quercetin equivalents (QE)/g dry extract. The group receiving a 60% extract concentration showed the greatest mean IOP reduction (9.35 mmHg), whereas lower and higher concentrations produced smaller decreases. However, one-way ANOVA showed no statistically significant differences in IOP reduction among treatment groups ($p = 0.822$). These findings indicate a biological tendency toward IOP lowering by *P. pellucida* extract, but the current evidence is preliminary; studies with larger sample sizes, optimised dosing regimens, and more refined outcome measures are required to confirm its therapeutic significance in infection-related ophthalmotonus.

Keywords:
Peperomia pellucida; Flavonoids; Intraocular pressure; Ophthalmotonus; *Staphylococcus aureus*

Received:	*Accepted:*	*Online:*
2025-09-30	*2025-12-17*	*2025-12-23*

1. Introduction

Increased intraocular pressure (IOP) or ocular hypertension is one of the most important eye health problems, because persistent elevation of IOP may cause progressive optic nerve damage leading to visual impairment and even permanent blindness [1]. Besides glaucoma, infectious and inflammatory conditions of the ocular surface, such as conjunctivitis and keratitis, can also disturb aqueous humour dynamics and contribute to ophthalmotonus disorders. Epidemiological data show that conjunctivitis is highly prevalent: in the United States the incidence reaches 135 per 10,000 population across age groups [2], while in Indonesia the prevalence is reported at 9.7%, making it the second most common eye disease. Several hospital-based studies in Yogyakarta, Bantul, and Manado have reported that conjunctivitis affects both sexes and

a wide range of age groups, with a slight predominance among women in some regions [3].

Conventional management of elevated IOP commonly relies on topical antibiotics and synthetic anti-inflammatory or antiglaucoma drugs. Although effective, these agents may cause undesirable adverse effects, including ocular irritation, iris discolouration, systemic reactions, and the development of bacterial resistance. Long-term dependence on synthetic compound–based therapies can therefore pose safety concerns and reduce patient adherence. For this reason, there is growing interest in exploring medicinal plants with anti-infective and anti-inflammatory properties as safer adjuncts or alternatives in ophthalmic therapy.

One plant with promising potential is *Peperomia pellucida* (Piperaceae), which has long been used in traditional medicine to treat inflammation, infections, and metabolic disorders [4]. Phytochemical studies indicate that *P. pellucida* contains alkaloids, flavonoids, tannins, saponins, phenols, and steroids/triterpenoids, many of which exhibit antioxidant, anti-inflammatory, and antimicrobial activities [5]–[7]. Flavonoids, in particular, have been reported to protect ocular tissues through antioxidative and vasodilatory mechanisms, potentially contributing to IOP stabilisation [9], whereas tannins and other polyphenols may support ocular surface integrity and modulate vascular permeability [10]. Previous in vitro studies have also shown that extracts of *P. pellucida* can inhibit the growth of *Staphylococcus aureus*, one of the major pathogens causing ocular infections [11].

Despite these traditional uses and preliminary pharmacological data, scientific evidence regarding the effect of *P. pellucida* on IOP remains very limited. More specific investigations are needed to clarify whether its bioactive components, particularly flavonoids, can contribute to the management of infection-related ophthalmotonus. Therefore, this study aimed to identify and quantify the total flavonoid content of the ethanolic leaf extract of *P. pellucida* and to evaluate its potential as a supportive therapeutic agent for IOP abnormalities in a Balb/C mouse model of *S. aureus*–induced ocular infection. It is expected that this research will provide an initial scientific basis for future development of *P. pellucida*–based ophthalmic preparations.

2. Method

Study design

This study was conducted at the Microbiology Laboratory, Biology Education Department, State Islamic Institute of Palangka Raya, using a laboratory experimental design with a post-test-only control group. A completely randomised design (CRD) was applied to evaluate the effectiveness of *Peperomia pellucida* extract as ophthalmotonus therapy in mice with *Staphylococcus aureus*–induced ocular infection.

Materials

The materials used in this study included male Balb/C mice, powdered *Peperomia pellucida* leaf extract, pure *Staphylococcus aureus* culture, 70% and 96% ethanol, 0.5% timolol eye drops, 0.5% pantocaine, 0.1% peptone solution, and sterile distilled water.

Equipment

The equipment used in this study comprised an autoclave, laminar airflow cabinet, incubator, hot plate, magnetic stirrer, beakers, Erlenmeyer flasks, Petri dishes, micropipettes, microtubes, glass droppers, digital balance, refrigerator, animal cages with feeding and drinking facilities, and a Schiotz tonometer for intraocular pressure (IOP) measurement.

Extraction of *Peperomia pellucida*

Fresh *P. pellucida* leaves obtained from Situbondo Regency, East Java, were washed, cut into small pieces, and shade-dried for approximately seven days. The dried simplicia were ground into powder and stored in an airtight container. Extraction was performed by maceration with 95% ethanol, followed by filtration and concentration of the filtrate using a rotary evaporator under reduced pressure to obtain a viscous ethanolic extract [11].

Phytochemical screening

Phytochemical screening of the *P. pellucida* ethanolic extract was conducted to identify major classes of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids. For alkaloids, the extract was mixed with 25% ammonia solution and chloroform; the chloroform layer was then reacted with Mayer's reagent, and the formation of a cream-coloured precipitate indicated a positive result. Flavonoids were tested by dissolving the extract in methanol, followed by the addition of NaOH, concentrated H_2SO_4, and magnesium and HCl powders; a change in colour to red, purple, or orange-yellow indicated the presence of flavonoids. Saponins were detected by vigorously shaking the extract vertically for about 10 seconds; the formation of stable foam that persisted after the addition of 1% HCl indicated a positive result. Tannins were identified by reacting the extract with 1% $FeCl_3$ solution; a dark blue or blackish green colour indicated the presence of tannins. Steroids and triterpenoids were detected using the Liebermann–Burchard reaction, by adding chloroform, acetic anhydride, and concentrated H_2SO_4 to the extract residue; a greenish-blue colour indicated steroids, whereas a purplish-red colour indicated triterpenoids.

Determination of total flavonoid content

Total flavonoid content of the *P. pellucida* ethanolic leaf extract was determined spectrophotometrically using the aluminium chloride ($AlCl_3$) complexation method with quercetin as the reference standard. A series of quercetin standard solutions (20–100 µg/mL) was prepared in methanol, and 1 mL of each standard was mixed with $AlCl_3$ reagent according to the established protocol; after incubation, absorbance was measured at 415 nm using a UV–Vis spectrophotometer. A calibration curve was constructed by plotting absorbance versus quercetin concentration, and the linear regression equation and coefficient of determination (R^2) were obtained.

For the extract, an appropriate aliquot of the ethanolic extract was diluted in methanol and treated with $AlCl_3$ reagent under the same conditions as the standards. The absorbance of three replicate samples was measured at 415 nm, and the mean absorbance value was inserted into the quercetin calibration equation to calculate the equivalent flavonoid concentration. Total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of dry extract.

Experimental animals and infection model

The animal model used in this study consisted of male Balb/C mice infected to induce ophthalmia due to *S. aureus* infection. A total of 25 mice aged approximately 3–4 weeks and weighing about 28 g were used [12]. Mice were acclimatised under standard laboratory conditions with ad libitum access to food and water.

Ocular infection was induced using a suspension of *S. aureus* in 0.1% peptone solution. The suspension was instilled topically as one drop into the left eye three times daily for three consecutive days [13]. Mice were observed for the development of clinical signs of infection, including conjunctival hyperaemia, swelling, and increased IOP. Baseline IOP (pre-infection) was measured prior to inoculation.

Treatment groups and interventions

After confirmation of infection and measurement of post-infection IOP, the 25 mice were randomly allocated into five treatment groups (P1–P5), with five animals per group. Group P1 served as the negative control and received sterile distilled water, while Group P2 served as the positive control and was treated with 0.5% timolol eye drops. Groups P3, P4, and P5 received *P. pellucida* extract eye drops at concentrations of 60%, 80%, and 100%, respectively. All treatments were administered topically as one drop (approximately 20–30 µL) to the left eye three times daily for seven consecutive days. Clinical signs and IOP were monitored daily to evaluate the ability of the extract to reduce infection-induced IOP elevation.

Measurement of intraocular pressure

IOP measurements were performed using a Schiotz tonometer after instillation of one drop of 0.5% pantocaine as a local anaesthetic. The mouse was positioned laterally so that the corneal surface was horizontal, and the tonometer footplate was gently placed on the cornea to avoid excessive pressure. Once the needle position stabilised, the reading was recorded. IOP was measured at three time points: before infection (normal IOP), after infection (elevated IOP), and after seven days of therapy, following the guidelines of the American Academy of Ophthalmology [14].

Data analysis

Normality of IOP-reduction data in each treatment group (P1–P5) was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Because all groups showed p-values greater than 0.05, the data were considered normally distributed and were analysed using one-way analysis of variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). Differences were regarded as statistically significant when $p < 0.05$.

Ethical approval

All experimental procedures involving animals were carried out in accordance with applicable ethical guidelines and were approved by the Ethics Committee of Palangka Raya University (No. 143/UN24.9/LL/2025).

3. Results and Discussion

Phytochemical profile of *Peperomia pellucida* extract

The phytochemical screening results in **Table 1** show that the ethanolic extract of *Peperomia pellucida* leaves contains a wide range of secondary metabolites, including flavonoids, tannins, alkaloids, saponins, phenols, and steroids/triterpenoids. All tested classes yielded positive reactions, as indicated by characteristic colour changes or precipitates. This pattern is consistent with previous phytochemical studies on *P. pellucida*, which also reported the presence of flavonoids, tannins, alkaloids, saponins, and other phenolic constituents in the leaf extract [5]–[7]. The presence of these compounds supports the traditional use of *P. pellucida* as a medicinal plant and suggests multiple potential mechanisms relevant to ocular therapy. Flavonoids and other phenolic compounds are widely recognised for their antioxidant and anti-inflammatory activities, tannins and saponins may contribute astringent and membrane-modulating effects, while steroids/triterpenoids and alkaloids can exert additional anti-inflammatory and antimicrobial actions [8]–[10]. Collectively, this phytochemical profile provides a rational basis for further investigation of *P. pellucida* as a candidate in managing eye infections and associated intraocular pressure (IOP) disturbances.

Table 1. Phytochemical screening of *Peperomia pellucida* ethanolic leaf extract

Phytochemical group	Test result	Indicator reaction
Flavonoids	+	Yellow–orange to red colour
Tannins	+	Green to dark blue colour
Alkaloids	+	Brown precipitate
Saponins	+	Stable, persistent foam
Phenols	+	Green to dark blue colour
Steroids/triterpenoids	+	Reddish-brown precipitate

Note: (+) present; (–) not detected.

Total flavonoid content of the ethanolic extract

Quantitative analysis of total flavonoids in the ethanolic extract of *P. pellucida* leaves is summarised in **Table 2**. Flavonoid content was determined spectrophotometrically using the aluminium chloride ($AlCl_3$) complexation method with a maximum absorption wavelength of 415 nm. The quercetin standard curve produced the linear regression equation $y = 0.0092x - 0.002$with a coefficient of determination $R^2 = 0.999$, indicating an excellent linear relationship between concentration and absorbance. Inserting the mean absorbance of three extract replicates (0.614) into this equation yielded a flavonoid concentration of 66.96 µg/mL, equivalent to 66.96 mg quercetin equivalents (QE) per gram of dry extract.

Table 2. Calibration data and total flavonoid content of *Peperomia pellucida* ethanolic leaf extract

Section	Quercetin concentration (µg/mL)	Absorbance at 415 nm	Sample absorbance	Information
Calibration curve	20	0.181	–	
	40	0.365	–	
	60	0.548	–	
	80	0.733	–	
	100	0.918	–	
Sample readings	–	–	0.612	
	–	–	0.621	
	–	–	0.608	
Summary	–	–	Mean = 0.614	Regression: (y = 0.0092x - 0.002); R^2 = 0.999); total flavonoid = 66.96 mg QE/g dry extract

Figure 1 depicts the relationship between quercetin concentration and absorbance at 415 nm. The data points cluster closely around the regression line and the R^2 value of 0.999 confirms that the colourimetric $AlCl_3$ method provides high accuracy and precision for quantifying total flavonoids in the extract. The relatively high flavonoid content observed in this study reinforces the potential of *P. pellucida* as a rich source of bioactive flavonoid compounds, which are known to possess antioxidant, anti-inflammatory, and antimicrobial properties that may contribute to ocular protection and IOP stabilisation [15].

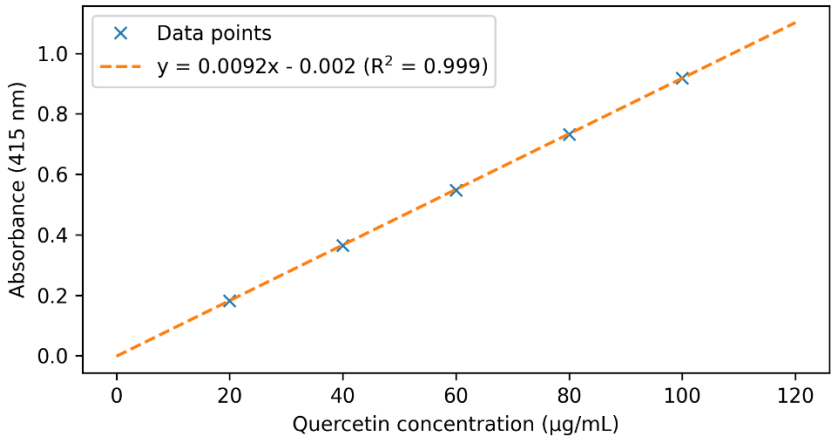

Figure 1. Calibration curve of quercetin standard for determination of total flavonoid content in *Peperomia pellucida* ethanolic leaf extract ($AlCl_3$ method, 415 nm)

Effect of *Peperomia pellucida* extract on intraocular pressure

The effect of *P. pellucida* extract on IOP in mice with *Staphylococcus aureus*–induced ocular infection is illustrated in **Figure 2**, which compares IOP values before infection, after infection, and after seven days of therapy. In the negative-control group (P1), which received only distilled water, IOP decreased from 24.3 mmHg to 18.3 mmHg (reduction of 6.0 mmHg). Although no pharmacologically active agent was administered, this reduction is likely related to the natural resolution of inflammation after the acute infectious phase. Endogenous pro-resolving mediators such as resolvins and lipoxins have been reported to alleviate ocular inflammation and improve aqueous humour outflow, leading to a gradual decline in IOP [16]. In addition, topical saline irrigation can provide a mild cooling and cleansing effect that improves ocular surface comfort and may indirectly facilitate trabecular meshwork relaxation and IOP regulation [17].

In the positive-control group (P2), which received 0.5% timolol eye drops, IOP decreased from 24.5 mmHg to 18.0 mmHg (reduction of 6.5 mmHg). Timolol is a β-adrenergic blocker that reduces aqueous humour production by inhibiting β_2 receptors in the ciliary body and remains a standard treatment for glaucoma [18]. The magnitude of IOP reduction in this group was only slightly greater than that observed in the negative control, suggesting that in the context of acute infection, endogenous inflammatory-resolution mechanisms still contribute substantially to IOP normalisation.

Among the extract-treated groups, the greatest mean IOP reduction was observed in Group P3, which received 60% *P. pellucida* extract. In this group, IOP decreased from 24.95 mmHg to 15.6 mmHg, corresponding to a reduction of 9.35 mmHg. This relatively large decrease suggests that the 60% concentration may provide an optimal balance between efficacy and tolerability. The effect can plausibly be attributed to the combined actions of flavonoids, tannins, and saponins present in the extract, which have been reported to modulate COX-2 and NF-κB signalling pathways, reduce prostaglandin-mediated inflammation, and exert antioxidant and antibacterial effects that help control the underlying infection [20].

Groups P4 and P5, treated with 80% and 100% extract, respectively, also showed decreases in IOP—from 24.8 to 17.2 mmHg (reduction of 7.6 mmHg) in P4 and from 23.4 to 19.8 mmHg (reduction of 3.6 mmHg) in P5. Although these findings indicate a biological response, the magnitude of IOP reduction was smaller than in the 60% group.

This pattern suggests a possible non-linear dose–response relationship, in which further increases in extract concentration do not proportionally enhance the IOP-lowering effect and may even attenuate it. At higher concentrations, receptor or transporter systems involved in the response may become saturated, local tissue irritation may occur, or the stability and solubility of active constituents may decrease, thereby limiting their bioavailability and pharmacodynamic impact [21],[22].

Statistical analysis of IOP reduction and interpretation

Normality testing of IOP-reduction data in all treatment groups using the Kolmogorov–Smirnov and Shapiro–Wilk tests yielded p-values greater than 0.05 (Kolmogorov–Smirnov: P1 = 0.200, P2 = 0.200, P3 = 0.200, P4 = 0.200, P5 = 0.135; Shapiro–Wilk: P1 = 0.122, P2 = 0.259, P3 = 0.117, P4 = 0.225, P5 = 0.093). Thus, the IOP-reduction data were considered normally distributed at the 95% confidence level, fulfilling the assumptions required for parametric analysis using one-way ANOVA. The mean reduction in IOP in each group is summarised in **Table 3**.

Table 3. Mean reduction in intraocular pressure (IOP) after 7 days of treatment in mice with *Staphylococcus aureus*–induced ocular infection

Group	Treatment	n	Mean IOP reduction (mmHg)
P1	Distilled water (negative control)	5	6.0
P2	Timolol 0.5% (positive control)	5	6.5
P3	*Peperomia pellucida* extract 60%	5	9.35
P4	*Peperomia pellucida* extract 80%	5	7.6
P5	*Peperomia pellucida* extract 100%	5	3.6

Note: Values are expressed as mean IOP reduction per group. One-way ANOVA showed no statistically significant difference among groups (F(4,20) = 0.378; p = 0.822, ns).

Although the 60% extract group (P3) showed the largest numerical reduction in IOP (9.35 mmHg), followed by the 80% extract group (P4) and the timolol group (P2), one-way ANOVA indicated that these differences were not statistically significant (F(4,20) = 0.378; p = 0.822). Statistically, this finding means that the variation between groups is not greater than the variation within groups, so the observed differences in mean IOP reduction cannot be distinguished from random fluctuations. In other words, based on this dataset, none of the treatments can be concluded to be superior to the others in terms of IOP reduction at the 5% significance level.

The lack of statistical significance is likely influenced by several methodological and biological factors. The relatively small sample size (n = 5 per group) limits the statistical power to detect small to moderate effect sizes, especially in the presence of inter-individual variability in IOP responses. In addition, the seven-day treatment duration and the dosing regimen used in this study may not have been sufficient for the pharmacological effects of the extract to reach their full potential. Previous studies have shown that antihypertensive and neuroprotective actions of natural flavonoids often require adequate exposure time and effective concentrations to produce measurable clinical impact [24]. Although flavonoids and other constituents of *P. pellucida* possess antioxidant and anti-inflammatory activities that could indirectly favour IOP reduction [25], they may not directly target the main regulatory mechanisms of aqueous humour production and outflow. Classical IOP-lowering drugs typically act on carbonic anhydrase, prostaglandin receptors, or trabecular meshwork contractility, leading to more pronounced and specific effects on aqueous humour dynamics, whereas the extract may exert broader, less targeted actions that translate into only moderate changes in IOP within the time frame of this experiment.

Despite the absence of statistically significant differences among groups, the consistent downward trend in IOP across all extract-treated groups, particularly at the 60% concentration, still suggests a potential biological response to *P. pellucida*

administration. As highlighted by Wasserstein and Lazar [26], the p-value does not quantify the magnitude or clinical relevance of an effect, but rather the degree to which the observed data are compatible with the null hypothesis. The non-significant result in this study may therefore reflect a combination of limited sample size, relatively high within-group variability, suboptimal dosing, and a moderate true effect size of the extract. These factors cannot be confirmed as definitive causes, but they provide plausible explanations for the current findings and indicate important aspects that should be optimised in future research.

Limitations of the study

This study has several limitations that should be considered when interpreting the findings. First, the sample size was relatively small (n = 5 per group), which reduces the statistical power to detect small to moderate differences in intraocular pressure (IOP) reduction between treatment groups. Second, the treatment duration of seven days may not have been sufficient for the pharmacological effects of *Peperomia pellucida* extract to reach their full potential, particularly for natural products whose antihypertensive and neuroprotective actions often require longer exposure [24]. Third, only a crude ethanolic extract was used, without standardisation of specific active constituents or fractionation, so the contribution of individual flavonoids or other compounds to the observed effects could not be delineated. Fourth, the study employed a single animal species and a single model of *Staphylococcus aureus*–induced ocular infection, which limits the generalisability of the results to other types of ocular hypertension or to human clinical conditions. Future studies with larger sample sizes, longer treatment periods, standardised or fractionated extracts, and additional outcome measures such as bacterial load, histopathology, and aqueous humour dynamics will be necessary to confirm and extend these preliminary findings.

4. Conclusion

Administration of *Peperomia pellucida* extract showed a biological tendency to reduce intraocular pressure in mice with *Staphylococcus aureus*–induced ocular infection. The greatest mean reduction in IOP was observed at the 60% extract concentration, whereas both lower and higher concentrations produced smaller decreases. However, one-way ANOVA ($p = 0.822$) indicated that the differences among treatment groups were not statistically significant. Accordingly, these findings should be interpreted as preliminary and are not sufficient to establish a definitive therapeutic effect of the extract. Further studies with larger sample sizes, optimised dosing regimens, and additional outcome measures are required to strengthen the evidence base and clarify the mechanisms underlying the observed IOP-lowering trend.

Acknowledgements:
The authors would like to thank the Microbiology Laboratory of IAIN Palangka Raya for all forms of support, provision of facilities and infrastructure, and the facilities that have enabled this research to be carried out successfully. The authors also express their gratitude for the cooperation, assistance, and guidance provided by all laboratory staff during the research process.

Conflicts of Interest:
The authors declare no conflict of interest regarding the publication of this paper.

References

[1] A. M. Awaluddin., "Study of the Phytochemical Content and Antioxidant Effects of Istoma longiflora and Clitornia ternatea Extracts as Ophthalmotonus Therapy Agents," *Indonesian Journal of Phytopharmaceuticals,* vol. 11, no. 2, p. 55, 2024, [Online]. Available: https://doi: 10.33096/jffi.v11i2.1283

[2] R. A. Hudaiva, *Overview of knowledge about conjunctivitis among Mechanical Engineering students at the Faculty of Engineering, University of Jember*. Undergraduate thesis, Universitas Jember, 2020. [Online]. Available: https://repository.unej.ac.id/jspui/bitstream/123456789/104147/1/Rhevy%20Asril%20Hudaiva%20-%20162310101070%20Sdh.pdf

[3] V. Yulianti, *Prevalence and profile of conjunctivitis patients at Dr. Mohammad Hoesin General Hospital Palembang in 2019–2021*. Undergraduate thesis, Universitas Sriwijaya, 2022. [Online]. Available: https://repository.unsri.ac.id/85099/3/RAMA_11201_04011181924045_8805330017_0026127404_01_front_ref.pdf

[4] M. de F. Arrigoni-Blank., "Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae)," *Journal of Ethnopharmacology*, vol. 91, no. 2–3, pp. 215–218, 2004. [Online]. Available: https://doi.org/10.1016/j.jep.2003.12.030

[5] G. K. Oloyede, P. A. Onocha, and B. B. Olaniran, "Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomia pellucida* from Nigeria," *Advances in Environmental Biology*, vol. 5, no. 12, pp. 3700–3706, 2011. [Online]. Available: https://www.aensiweb.com/old/aeb/2011/3700-3709.pdf

[6] C. R. Nwokocha., "Possible mechanism of action of the hypotensive effect of *Peperomia pellucida* and interactions between human cytochrome P450 enzymes," *Medicinal & Aromatic Plants*, vol. 1, p. 105, 2012. [Online]. Available: https://www.longdom.org/open-access/possible-mechanism-of-action-of-the-hypotensive-effect-of-empeperomia-pellucidaem-and-interactions-between-human-cytochr-26397.html

[7] M. Hidayati, "The therapeutic potential of combined *Piper crocatum* and *Tinospora crispa* extracts in repairing the skin barrier of hyperglycemic mice," *Jurnal Ilmiah Biologi Unsoed*, vol. 7, no. 1, 2025. [Online]. Available: https://jurnalonline.unsoed.ac.id/index.php/bioe/article/14812/

[8] R. Fatmalia and R. Efi, "Antibacterial activity of Chinese betel leaf (*Peperomia pellucida*) decoction against the growth of *Staphylococcus aureus* and *Escherichia coli*," *Jurnal Kesehatan Andalas*, vol. 7, no. 2, pp. 295–300, 2018. [Online]. Available: https://doi.org/10.25077/jka.v6i3.756

[9] P. Érdelyi, T. Bakondi, A. Gergely, and C. Virág, "The protective effects of flavonoids in ocular diseases: Role in oxidative stress and vascular regulation," *Journal of Nutritional Biochemistry*, vol. 84, 108453, 2020. [Online]. Available: https://doi.org/10.1016/j.jnutbio.2020.108453

[10] A. Farooq and M. Ashraf, "Role of plant polyphenols in ocular health: Focus on tannins as potential therapeutic agents," *Phytotherapy Research*, vol. 34, no. 6, pp. 1245–1258, 2020. [Online]. Available: https://doi.org/10.1002/ptr.6615

[11] M. Lolowang, "Patterns of aerobic bacteria causing conjunctivitis in outpatients at the Manado Community Eye Health Centre," *Jurnal e-Biomedik (eBM)*, vol. 2, no. 1, 2014. [Online]. Available: https://doi.org/10.35790/ebm.2.1.2014.3760

[12] S. Karlina et al., "Therapeutic effects of *Myrmecodia* sp. on the morphology of fallopian tube epithelial cells in mice with pelvic inflammatory disease," *Jurnal*

Biologi Lingkungan, Industri, Kesehatan, vol. 11, no. 2, 2025. [Online]. Available: https://doi.org/10.31289/biolink.v11i2.14012

[13] Yulianto, *The effect of eye-drop suspension on intraocular pressure in Balb/C mice infected with Staphylococcus aureus*. Undergraduate thesis, Universitas Muhammadiyah Yogyakarta, 2023. [Online]. Available: https://etd.umy.ac.id/view/year/2023.default.html

[14] H. P. Hastuti and A. P. Nirwana, "Inhibitory activity of kitolod (*Hippobroma longiflora*) leaf decoction against the growth of *Staphylococcus aureus*," *Jurnal Farmasi (Journal of Pharmacy)*, vol. 10, no. 1, Apr. 2021. [Online]. Available: https://ojs.stikesnas.ac.id/jf/id/article/view/80

[15] R. Andrianto., "Combination of *Piper crocatum* and *Tinospora crispa* extracts on blood glucose levels in hyperglycemic mice," *Jurnal Kajian Teori dan Praktik Tadris IPA*, vol. 6, no. 1, p. 205, 2025. [Online]. Available: https://ejurnals.com/ojs/index.php/jktpp/article/view/1580

[16] C. N. Serhan and N. Chiang, "Endogenous pro-resolving and anti-inflammatory lipid mediators: A new pharmacologic genus," *British Journal of Pharmacology*, vol. 153, Suppl. 1, pp. S200–S215, 2008. [Online]. Available: https://doi.org/10.1038/sj.bjp.0707489

[17] G. N. Patton and H. J. Lee, "Chemical insights into topical agents in intraocular pressure management: From glaucoma etiopathology to therapeutic approaches," *Pharmaceutics*, vol. 16, no. 2, p. 274, Feb. 2024. [Online]. Available: https://doi.org/10.3390/pharmaceutics16020274

[18] American Academy of Ophthalmology, "Primary Open-Angle Glaucoma Preferred Practice Pattern®," Jan. 2021. [Online]. Available: https://emedicine.medscape.com/article/1206147-guideline

[19] A. Kapoor and Priya Chandel, "Mechanism of action of timolol in reducing aqueous humour production via β_2-receptor blockade in the ciliary body," *Journal of Ocular Pharmacology and Therapeutics*, vol. 11, no. 3, pp. 150–155, 2019.

[20] P. Bellavite, "Neuroprotective potentials of flavonoids: Experimental studies and mechanisms of action," *Antioxidants*, vol. 12, no. 2, 280, 2023. [Online]. Available: https://doi.org/10.3390/antiox12020280

[21] N. Ramadhani et al "Anti-inflammatory Activity of Various Plants Suspected to Originate from Flavonoids," *Farmaka*, 2017.

[22] Beausoleil, C., M. Rousselle, A. T. Cravedi, J. S. Debrauwer, G. H. V. V. Fini, M. M. Nesslany, A. Budzinski, and J. Houdeau, "Evaluating the evidence for non-monotonic dose–response relationships in mammalian in vivo studies," *Toxicology*, vol. 384, pp. 28–41, 2017. [Online]. Available: https://doi.org/10.1177/1559325818798282

[23] N. Hujjatusnaini and R. Nirmalasari, "Antihyperglycemic effect of *Musa paradisiaca* extract in vivo," *Jurnal Ilmiah Ilmu-Ilmu Kesehatan*, 2024. [Online]. Available: https://doi.org/10.30595/medisains.v0i0.18814

[24] E. J. Calabrese, "Hormesis: The dose–response for the 21st century: The future has arrived," *Toxicology*, vol. 425, art. 152249, 2019. [Online]. Available: https://doi.org/10.1016/j.tox.2019.152249

[25] J. F. Hair, W. C. Black, B. J. Babin, and R. E. Anderson, *Multivariate Data Analysis*, 8th ed. Andover, UK: Cengage, 2019/2022. [Online]. Available (catalog): https://www.cengage.co.uk/books/9781473756540/

[26] Zahra, M., Abrahamse, H., and George, B. P., "Flavonoids: Antioxidant powerhouses and their role in nanomedicine," Antioxidants, vol. 13, no. 8, p. 922, 2024. [Online]. Available: https://doi.org/10.3390/antiox13080922