Jurnal Pharmascience, Volume 13, Issue 1, March 2026, page: 255-271
ISSN-Print 2355 – 5386
ISSN-Online 2460-9560
https://dx.doi.org/10.20527/jps.v13i1.24762
Research Article

Acute Oral Toxicity of Methanolic Extracts from the Fruit and Leaf of Parijoto (*Medinilla speciosa* Blume) in Mice

Toksisitas Oral Akut Ekstrak Metanol dari Buah dan Daun Parijoto (Medinilla speciosa Blume) pada Mencit

Annisa Farida Muti*, Refian Ariesta Eka Maulana, Dhigna Luthfiyani Citra Pradana, Eldiza Puji Rahmi

Pharmacy Program, Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, South Jakarta, Jakarta, Indonesia
Email: afmuti@upnvj.ac.id

ABSTRACT

***Medinilla speciosa* Blume, or parijoto, contains flavonoids, saponins, alkaloids, and tannins with pharmacological potential. However, information regarding its safety profile, particularly acute toxicity data, is still very limited, so research is needed to evaluate its safety level. This study aims to evaluate the acute oral toxicity of *M. speciosa* leaf and fruit extracts in ddY mice. This study used a true experimental design. M. speciosa leaf and fruit extracts were obtained by ultrasonic-assisted extraction with methanol as a solvent. The acute oral toxicity test was conducted with a fixed-dose method at a single dose of 2000 mg/kgBW. Parameters observed for 14 days included mortality, clinical symptoms of toxicity, body weight changes, relative organ weights, and histopathological analysis of the liver and kidneys. The findings indicated an absence of fatalities across all treatment groups. The LD_{50} values for both extracts were determined to exceed 2000 mg/kgBW, categorising the fruit and leaf extracts as mildly toxic (category 5). Mild toxic symptoms were only observed in the treatment group receiving the fruit extract. Histopathological analysis of the liver and kidneys revealed mild cellular changes that were not statistically significant. Therefore, a single oral administration of *M. speciosa* fruit and leaf extracts at doses up to 2000 mg/kgBW is relatively safe.**

Keywords: Acute Toxicity, ddY Mice, Fixed-Dose Method, Oral, M. speciosa

ABSTRAK

Medinilla speciosa Blume, atau parijoto, mengandung berbagai senyawa bioaktif seperti flavonoid, saponin, alkaloid, dan tanin dengan potensi farmakologis. Namun, informasi mengenai profil keamanannya, khususnya data toksisitas akut, masih sangat terbatas sehingga perlu dilakukan penelitian untuk mengevaluasi tingkat keamanannya. Penelitian ini bertujuan untuk mengevaluasi toksisitas akut oral dari ekstrak daun dan buah M. speciosa pada mencit galur ddY. Penelitian ini menggunakan true experimental design. Ekstrak daun dan buah M. speciosa diperoleh secara ultrasonic-assisted extraction dengan pelarut metanol. Uji toksisitas akut oral dilakukan dengan fixed-dose method pada dosis tunggal 2000 mg/kgBB. Parameter yang diamati selama 14 hari meliputi mortalitas, gejala klinis toksisitas, perubahan berat badan, berat relatif organ, serta analisis makroskopik dan histopatologi organ hati dan ginjal. Hasil penelitian menunjukkan tidak ada mortalitas pada semua kelompok perlakuan. Nilai LD_{50} dari kedua ekstrak diestimasi lebih dari 2000 mg/kgBB, yang mengklasifikasikan ekstrak buah dan daun M. speciosa sebagai toksik ringan (kategori 5). Gejala toksik ringan hanya teramati pada kelompok perlakuan yang menerima ekstrak buah. Analisis histopatologi hati dan ginjal menunjukkan adanya perubahan seluler ringan yang tidak signifikan secara statistik. Dengan demikian, pemberian oral tunggal ekstrak buah dan daun M. speciosa pada dosis hingga 2000 mg/kgBB adalah relatif aman.

Kata Kunci: Fixed-dose Method, Mencit ddY, Oral, M. speciosa, Toksisitas Akut

I. INTRODUCTION

Preclinical trials are a requirement in the development of a drug, which are carried out to gather information related to pharmacology and toxicology so that efficacy and safety can be known scientifically (Pradono, J. et al., 2019). The acute oral toxicity test is an assessment designed to identify toxic effects that manifest within a brief period following the administration of a test substance, whether as a single dose or multiple doses administered within a 24-hour timeframe. The results and interpretation of data obtained from acute oral toxicity include a description of the LD_{50} range, the dose of a drug or substance that causes symptoms of toxicity, changes in the body weight of the test animal, and histopathological images of the test animal's vital organs (Umiyati et al., 2021).

Toxic compounds may be contained in a plant and can cause damage to the function of various organs, especially the liver and kidneys. The use of medicinal plants in specific doses and time intervals is thought to induce varying physiological responses in various organs. Extracts were administered daily for 8 days to determine the dose and time interval of each extract (Suhita et al., 2013; Riana et al., 2023).

The liver is susceptible to hepatotoxicity due to its role in the biotransformation of foreign substances (Aisyah et al., 2015), which can be identified through histological changes

(such as degeneration and necrosis) (Setyaputri, 2019). The kidneys are also a primary target (nephrotoxicity) because they excrete toxins (Jannah & Budijastuti, 2022), with damage also identified histologically (such as necrosis and edema) (Husin, 2018).

One plant species widespread in Indonesia with limited safety studies is *Medinilla speciosa* Blume, also known as parijoto. *M. speciosa* has attracted considerable attention as a health product. The secondary metabolites, including flavonoids, phenols, tannins, saponins, and alkaloids, provide diverse pharmacological effects, including increased breast milk supply, fertility, antifungal, antioxidant, cytotoxic, antibacterial, antibiofilm, antidiabetic, and anticholesterol effects (Agustina et al., 2022; Astutik et al., 2021; Fairuzzahra, 2021; Leksono, 2022; Luhurningtyas et al., 2020; Sugiarti et al., 2020; Vifta & Advistasari, 2018; Winanta et al., 2021), supporting its role as an orally consumed natural remedy based on local wisdom (Hanum et al., 2017; Lestari et al., 2021; Milanda et al., 2021).

A previous study reported that fresh *M. speciosa* juice exhibited low acute toxicity (>15 g/kg BW) (Megawati et al., 2017). However, this finding cannot be directly extrapolated to methanol extracts, as different preparation methods may alter the concentration and composition of bioactive compounds, potentially resulting in distinct toxicological profiles. To date, no study has specifically evaluated the acute oral toxicity of *M. speciosa* methanol extract in ddY mice. Therefore, the present study aimed (1) to assess the acute oral toxicity of *M. speciosa* methanol extract in ddY mice by observing behavioral changes, body weight, liver and kidney organ indices, and histopathological alterations, and (2) to determine its safety classification based on the obtained toxicity data.

II. METHOD

A. Materials

The materials used in this study consisted of adult female mice of the ddY strain, fresh *Medinilla speciosa* fruit and leaves, and distilled water. The chemicals included methanol (semiconductor grade), ethanol (analytical grade), hydrochloric acid (analytical grade), ferric chloride (analytical grade), Mayer's reagent (analytical grade), Dragendorff's reagent (analytical grade), carboxymethyl cellulose (analytical grade), and Tween 80, obtained from Merck KGaA and Sigma-Aldrich.

B. Plant Collection and Extraction

Fresh *M. speciosa* fruit and leaves were harvested from Kudus, Central Java, Indonesia and were authenticated by UPT Laboratorium Herbal Materia Medika Batu with number 000.9.3/1426/102/20/2025.

The fruit and leaf were rinsed under running tap water to eliminate soil particles and grime. Leaves were desiccated in an oven until fully dry, while fruits underwent freeze-drying for 60 hours at -40°C to eliminate moisture, followed by size reduction with a grinder.

The fruit and leaf of the *M. speciosa* were extracted using methanol solvent using the Ultrasonic Assisted Extraction (UAE) method at a material to solvent ratio of 1:20. Leaf extraction was performed at a temperature of 50°C for a duration of 40 minutes, whereas fruit extraction was conducted at 40°C for 30 minutes. The extraction process was repeated three times, with each filtrate filtered and the residue re-extracted. All filtrates were subsequently evaporated with a rotary vacuum evaporator at 50°C until a viscous extract was achieved, and the yield was determined based on the initial weight of the simplex.

C. Phytochemical Analysis

Preliminary phytochemical screening for flavonoids, alkaloids, saponins, and tannins was conducted following established protocols.

1. Test for flavonoids

Approximately 5 mL of extract was boiled and combined with ethanol, followed by the addition of an adequate amount of magnesium powder and several drops of strong hydrochloric acid. The emergence of a crimson hue in the solution signifies the existence of flavonoids. The formation of a red color in the solution indicates the presence of flavonoids (Rubianti et al., 2022).

2. Test for alkaloids

Approximately 5 mL of extract was dissolved in 10 mL of hydrochloric acid and thereafter distributed into three test tubes. The initial tube functioned as a control, whereas three drops of Dragendorff's reagent were introduced to the second tube and Mayer's reagent to the third tube, respectively. The emergence of an orange precipitate in the second tube and a white precipitate in the third tube signified the existence of alkaloid compounds (Rubianti et al., 2022).

3. Test for saponins

Approximately 2 mL of extract was combined with 15 mL of hot water and permitted to cool to room temperature. The mixture was subsequently agitated violently for 15 seconds, resulting in a stable foam measuring 1–15 cm in height that endured for a minimum of 15 minutes, signifying the presence of saponins (Rubianti et al., 2022).

4. Test for tannins

Five drops of 10% Ferric Chloride solution were applied to 1 mL of the extract in a test tube. A colour transition in the solution to dark blue, blue-black, or greenish-black signifies the presence of

polyphenol and tannin components (Rubianti et al., 2022).

D. Experimental Animals

Healthy female mice (*Mus musculus*), aged 8–12 weeks (with minimum weight 20 grams and weight variation not exceeding 20% of the average) were obtained from Animal Vet Laboratorium Services, Bogor. The animals were housed in plastic cages, maintained at a temperature of 28±2 °C, with relative humidity between 45% and 55%, under a 12-hour natural light/dark cycle. The mice were acclimatised for seven days with unrestricted access to regular pellets and clean drinking water ad libitum. Animals were individually marked for identification. The animal handling adhered to the protocols for the care and utilisation of laboratory animals and underwent an institutional ethical assessment by Komisi Etik Penelitian Universitas Pembangunan Nasional Veteran Jakarta was obtained before carrying out the study (with number 385/IX/2024/KEP).

E. Acute Oral Toxicity Study

The acute oral toxicity study on the fruits and leaves extracts of *M. speciosa* was conducted according to the 'fixed-dose method' described in Peraturan Badan Pengawas Obat dan Makanan Nomor 10 Tahun 2022 tentang Pedoman Uji Toksisitas Praklinik *In Vivo* (Badan Pengawas Obat dan Makanan Republik Indonesia, 2022). The study used a total of 40 female mice. The animals were divided into four groups (n=10 per group): fruit control, fruit extract (2000 mg/kg), leaf control, and leaf extract (2000 mg/kg). For each extract, the animals were randomly divided into two groups, with each group consisting of five mice. The extract was administered orally (per os) as a single dose of 2000 mg/kg body weight. The animals were monitored continuously for the initial 30 minutes following administration, subsequently every 4 hours for the first 24 hours, and then once daily for the following 14 days. Observations focused on signs of toxicity, including variations in body weight, alterations in skin, fur, and eyes, respiratory function, touch response, central nervous system activity, movement, salivation, faecal output, backward walking, stomach walking, and mortality. Body weights were recorded on days 0, 7, and 14. The LD_{50} was ultimately established.

F. Histopathological Analysis

The liver and kidney were rinsed with normal saline (NaCl 0.9%) and subsequently fixed in 10% buffered formalin. Tissue slices were produced by the paraffin process and stained use the Hematoxylin-Eosin technique. Each

preparations was examined under light microscope in 5 fields at 40x10 magnification. Each field were randomly counted 40 cells and scored by Manja-Roenigk (Table I) for liver and Venient for kidney (Table II).

Table I. Manja-Roenigk scoring

Rate of Change	Score
Normal	1
Parenchymatous degeneration	2
Ballooning degeneration	3
Necrosis	4

Table II. Venient scoring

Rate of Change	Score
Damage less than 25%	1
Damage 25% - 50%	2
Damage 50% - 75%	3
Damage more than 75%	4

G. Statistical Analysis

Analysis was conducted using IBM Statistical Package for the Social Sciences (SPSS) software version 26. The analysis summarised data as mean ± standard deviation (SD) through descriptive statistics. The Shapiro-Wilk test was performed to evaluate normality, subsequently leading to either One-way ANOVA or the Kruskal-Wallis test. Tukey's post hoc test was then performed for multiple comparisons. The differences in means between the treatment groups were considered significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Phytochemical analysis results

The results of the evaporation process showed that the yield value of the leaf extract was 9.3451%, higher than the fruit extract of 5.3719%. Phytochemical investigations showed the presence of flavonoids, alkaloids, saponins and tannins in the fruits and leaves extracts of *M. speciosa* (Table III).

Table III. Phytochemical groups present in *M. speciosa* extract

Phytochemical	Fruit Extract	Leaf Extract
Flavonoids	+	+
Alkaloids	-	-
Saponins	+	+
Tannins	+	+

Notes: +is traces present; - is traces absent

The bioactive constituents of *M. speciosa* are responsible for various pharmacological activities and certain toxic effects. The identified phytochemicals align with findings from other studies (Nabila et al., 2023; Vifta et al., 2022; Wijaya & Nisyak, 2020).

B. Acute oral toxicity results

The assessment of acute toxicity, particularly through the estimation of LD_{50}, plays a vital role in conducting toxicological studies on various chemicals, including plant extracts. The present study demonstrated that acute oral administration of single doses of fruit and leaf extracts, up to 2000 mg/kgBW, did not lead to

mortality. Only the *M. speciosa* leaf extract treatment group experienced mild behavioral changes. Therefore, the LD_{50} for both fruit and leaf extracts was above 2000 mg/kgBW. The value categorises the fruit and leaf extracts of *M. speciosa* as GHS Category 5 (Bulgheroni et al., 2009), indicating that the extracts exhibit relatively low acute toxicity.

1. Physical and behavioral manifestation of toxicity

There were no apparent behavioral changes in all animals treated with *M. speciosa* leaf extract. However, the mice that received 2000 mg/kgBW of *M. speciosa* fruit extract had mild behavioral changes such as eyes becoming droopy in the first 15 minutes and returning to normal afterward, then a weakened touch response in the first 30 minutes and returning to normal afterward, then there were changes in the movement activity of each test animal which became lethargic in the first 30 minutes and returned to normal afterward (Table IV).

The mild and reversible sedative effects observed in this study are plausibly associated with the flavonoid content of the *M. speciosa* fruit extract. Flavonoids are known modulators of GABA-A receptors, which regulate inhibitory neurotransmission and sedation (Ríos et al., 2022). Evidence from related *Passiflora* species indicates that apigenin exhibits significant sedative activity (Gazolaa et al., 2015), supporting the hypothesis that flavonoid constituents contributed to the transient decrease in motor activity and lethargy observed in mice.

The increased respiratory rate may be related to the presence of saponins, which at higher concentrations can irritate gastrointestinal mucosa and alter membrane permeability, potentially inducing physiological stress responses (Sharma et al., 2023). However, the absence of progressive or persistent clinical signs suggests that these effects were mild and not indicative of systemic toxicity under the tested conditions.

Importantly, some observed signs—such as drooping eyes, reduced responsiveness, and transient tachypnea—may also reflect confounding factors rather than direct extract toxicity. Mice are nocturnal animals, and observations conducted during their resting phase may naturally coincide with reduced activity (Rudini et al., 2021). Additionally, handling and oral gavage procedures are known to induce temporary behavioral changes (Jones et al., 2016). The rapid resolution of symptoms following single-dose exposure therefore indicates no sustained toxicological impact, although further repeated-dose and mechanistic studies are warranted to strengthen safety conclusions.

2. The bodyweight

No significant variations ($p > 0.05$) were observed in the mean body weights of all treated mice compared to the control group over the 14-day period (Figure 1). Although body weight increased over time in all groups, including the control, this pattern reflects normal physiological growth during the observation period rather than a treatment-related effect. The absence of weight loss, growth suppression, or other systemic toxicity signs indicates that the *M. speciosa* extract did not adversely affect body weight under the tested conditions.

3. The relative organ weight

Overall, there were no notable ($p > 0.05$) differences in the relative weights of the liver and kidney of the mice across the various groups when compared to the control (Table V). Nonetheless, there was a reduction in the relative kidney weight observed in mice administered with *M. speciosa* fruit and leaf extract. Furthermore, a reduction in relative liver weight was observed solely in mice that received treatment with *M. speciosa* fruit extract.

Table IV. Behavioral observations following single oral administration of *M. speciosa* extract

Group	Dose (mg/kgBB)	15 min	30 min	1 h	4 h	24 h
Control	-	Normal	Normal	Normal	Normal	Normal
Fruit extract	2000	Mild drooping eyes	Reduced touch response, lethargy	Recovered	Normal	Normal
Leaf extract	2000	Normal	Normal	Normal	Normal	Normal

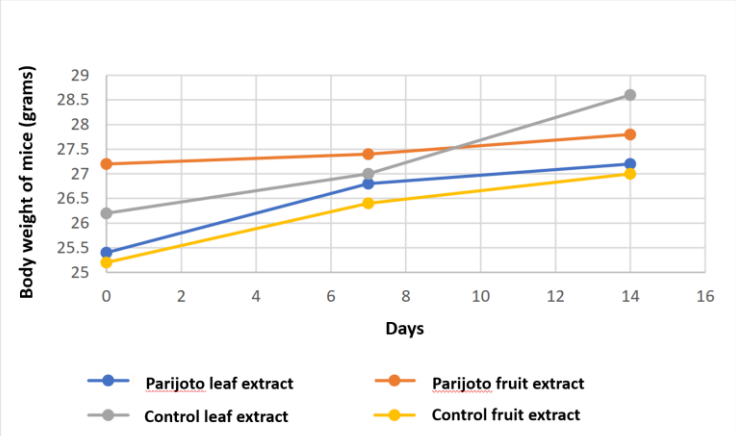

Figure 1. Mean body weight of mice after a single exposure to the *M. speciosa* extract for 14 days

Table V. Relative organ weight of the mice treated with *M. speciosa* extract for 14 days

Organ	Fruit Extract	Control fruit extract	p	Leaf Extract	Control leaf extract	p
Liver	6.030 ± 0.755%	5.963 ± 0.595%	0.881	6.421 ± 0.860%	6.590 ± 0.849%	0.762
Kidney	1.359 ± 0.154%	1.214 ± 0.174%	0.202	1.421 ± 0.118%	1.376 ± 0.073%	0.494

Measuring relative organ weight, particularly of the liver and kidneys, is an important indicator in in vivo toxicology to detect hypertrophy or atrophy as an early response to exposure to test compounds (Khalishah et al., 2021; Lazic et al., 2020). Changes in organ weight typically signify the potential toxicity of a substance. The decrease in relative kidney and liver weight may indicate that the extract exerted a harmful effect on these organs. Further research is required to investigate the impact of *M. speciosa* on kidney and hepatic function (Nalimu et al., 2022)

4. Histopathological analysis

Liver histopathology (Figure 2 and Figure 3) of the control and treatment fruit extract groups revealed tissue injury characterized by parenchymatous degeneration, hydropic degeneration, and necrosis. Conversely, histopathological analysis of the liver in the control leaf extract group demonstrated cellular injury, characterized by parenchymal degeneration and hydropic degeneration, but without evidence of necrosis. Nonetheless, given that analogous structural changes were noted in the control groups, it was concluded that these alterations may have resulted from environmental factors rather than the extracts themselves. The average liver histopathology scores of the control group and the 2000 mg/kgBW dose of *M. speciosa* fruit extract were 2.440 ± 0.697 and 2.400 ± 0.632, respectively; while in the control group and the 2000 mg/kgBW dose of *M. speciosa* leaf extract were 2.360 ± 0.625 and 2.280 ± 0.449, respectively. However, the liver cell damage that occurred was not significant between groups ($p > 0.05$).

The average kidney histopathology scores of the control group and the 2000 mg/kgBW dose of *M. speciosa* fruit extract were 1.220 ± 0.414 and 1.100 ± 0.300, respectively; while in the control group and the 2000 mg/kgBW dose of *M. speciosa* leaf extract were 1.200 ± 0.400 and 1.080 ± 0.271, respectively. However, the extent of kidney cell injury observed was not significantly different between groups ($p > 0.05$). Kidney histopathology analysis (Figure 4 and Figure 5) was performed by observing damage including tubular atrophy, tubular dilation, tubular necrosis, absence of the brush border, inflammation,

fibrosis, and enlargement of the glomerular diameter. Kidney histopathology in the fruit and leaf extract groups treated with the extracts showed tissue damage characterized by tubular dilation (yellow arrow), loss of brush border (red arrow), and inflammation (blue arrow). In contrast, all control groups experienced only brush border loss.

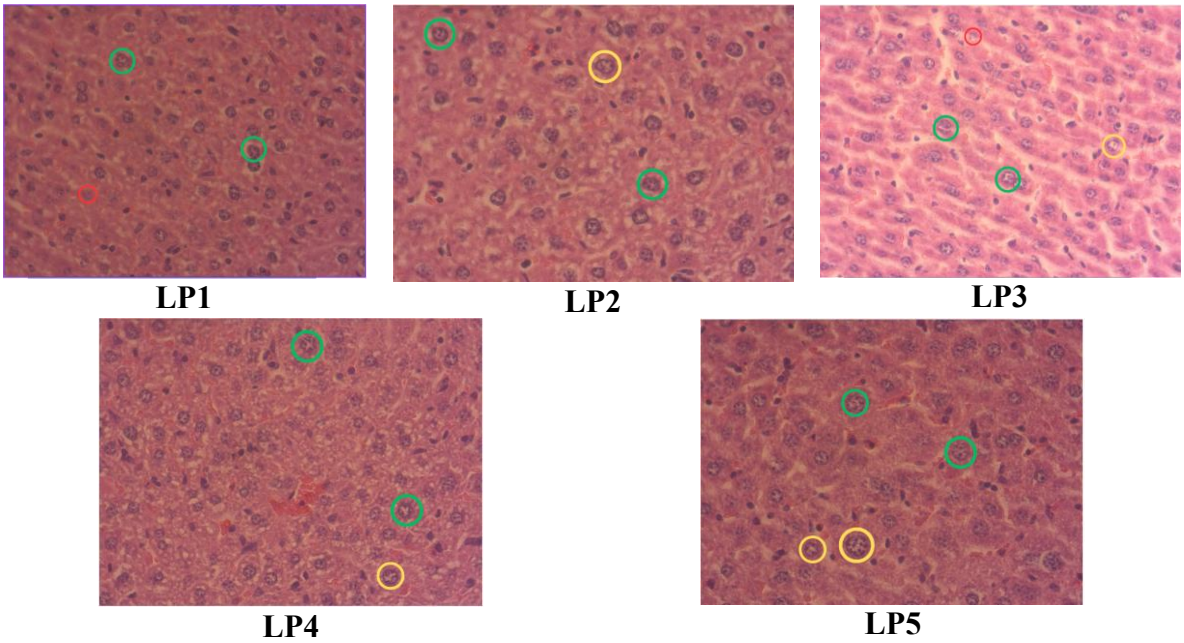

Figure 2. Histopathology of the liver organ with *M. speciosa* fruit extract (green = parenchymatous degeneration; yellow = hydropic degeneration; red = necrosis)

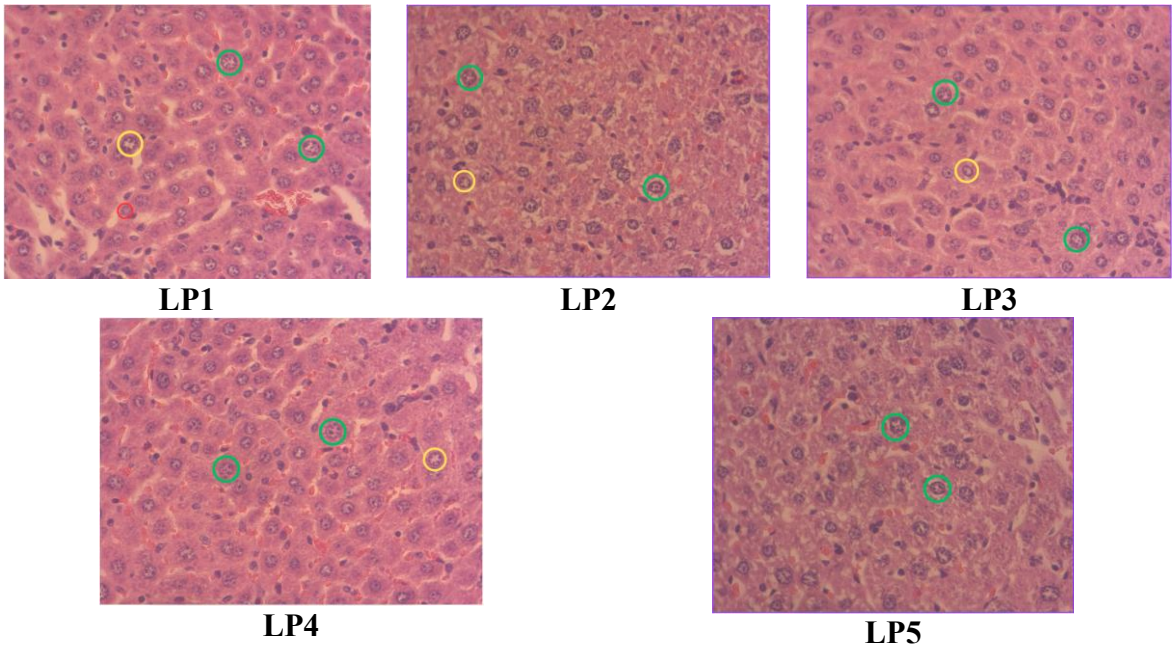

Figure 3. Histopathology of the liver organ with *M. speciosa* leaf extract (green = parenchymatous degeneration; yellow = hydropic degeneration; red = necrosis)

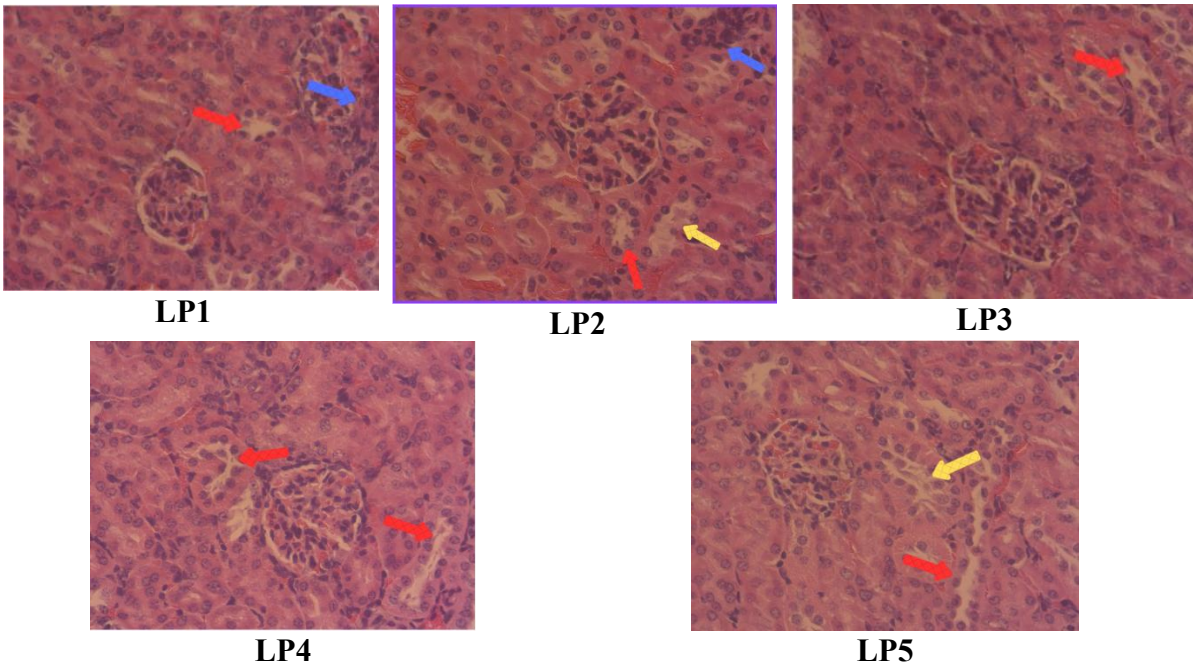

Figure 4. Histopathology of the kidney organ with *M. speciosa* fruit extract (red = loss of brush border; yellow = tubular dilation; blue = inflammation)

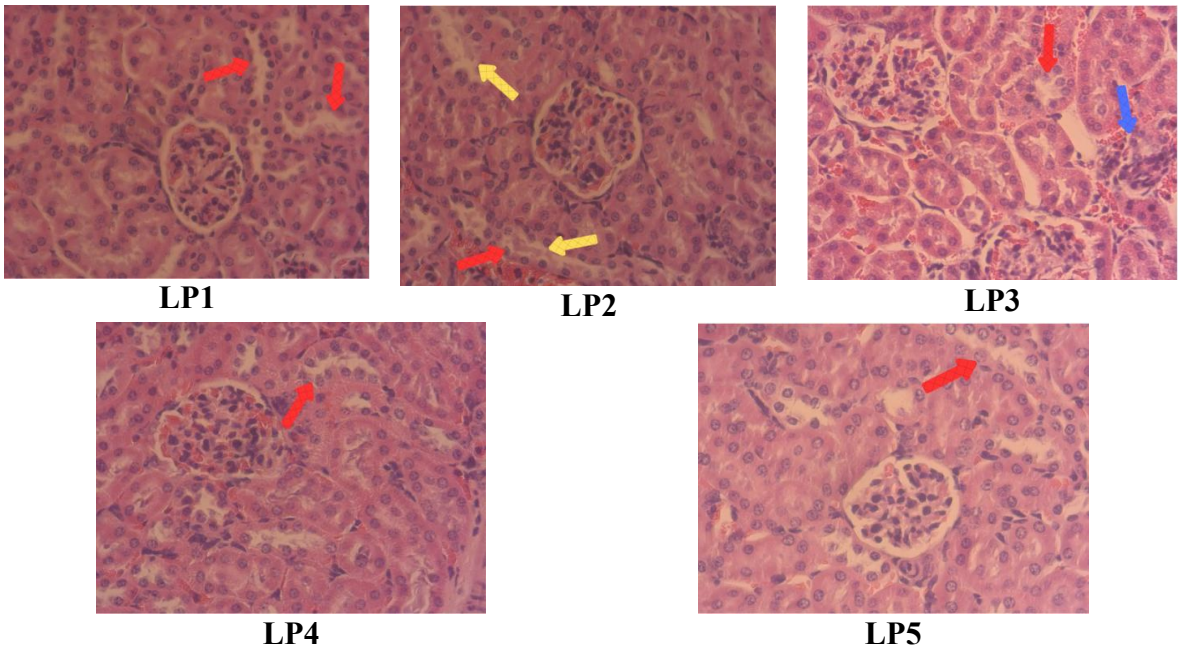

Figure 5. Histopathology of the kidney organ with *M. speciosa* leaf extract (red = loss of brush border; yellow = tubular dilation; blue = inflammation)

The adverse effects observed in liver and kidney tissues may also be associated with certain phytochemical constituents present in plant extracts. Previous phytochemical analyses of *M. speciosa* have identified flavonoids, tannins, and saponins, which may contribute to both protective antioxidant activity and potential toxicity when present at high concentrations.

Flavonoids are plant-derived polyphenolic compounds widely recognized for their antioxidant and anti-inflammatory properties, which contribute to cellular protection against oxidative stress–related damage in various organs, including the liver and kidneys. Their antioxidant activity is primarily attributed to their ability to scavenge reactive oxygen species (ROS), inhibit lipid peroxidation, and activate endogenous antioxidant defense systems such as the Nrf2-mediated pathway, thereby reducing oxidative stress, inflammation, and apoptosis in renal and hepatic tissues (Alsawaf et al., 2022). owever, despite these protective effects, excessive intake of flavonoids may also produce adverse biological outcomes. High concentrations of certain flavonoids, such as epigallocatechin gallate (EGCG) or quercetin, have been associated with hepatotoxicity and nephrotoxicity, possibly through mechanisms involving oxidative imbalance, inflammatory responses, and disruption of antioxidant enzyme systems. Experimental studies have reported elevated liver enzymes, hepatic necrosis, and renal dysfunction following high-dose flavonoid exposure, suggesting that while moderate levels of flavonoids may exert hepatoprotective and nephroprotective effects, excessive concentrations may contribute to liver and kidney injury due to dose-dependent pro-oxidant and metabolic disturbances (Singh et al., 2022; Tang & Zhang, 2022).

Tannins are polyphenolic compounds widely found in plant-based foods and beverages and are known for their strong antioxidant activity. Their hydroxyl-rich structure enables them to scavenge reactive oxygen species (ROS), inhibit lipid peroxidation, and chelate transition metals, thereby reducing oxidative stress and protecting tissues such as the liver and kidneys from oxidative damage. In addition, tannins may enhance antioxidant defense systems through the modulation of enzymes such as superoxide dismutase and glutathione peroxidase. owever, at high concentrations, tannins may also exert adverse effects due to their strong affinity for proteins and enzymes, leading to the formation of tannin–protein complexes that can disrupt metabolic processes and membrane functions. These interactions may contribute to hepatocellular stress and impair renal tubular function, indicating that while moderate levels of tannins may provide hepatoprotective and nephroprotective effects, excessive exposure may lead to potential liver and kidney toxicity (Cosme et al., 2025; Ozogul et al., 2025).

Saponins are amphipathic glycosides that can interact with membrane sterols such as cholesterol, leading to increased membrane permeability and

disruption of cellular integrity. This interaction may damage epithelial cell membranes and impair cellular transport processes. In renal tissue, membrane disruption in tubular epithelial cells may interfere with absorption and secretion mechanisms, resulting in structural alterations such as tubular epithelial degeneration, loss of the brush border, and tubular dilation. In addition, saponin exposure may induce oxidative stress through increased production of reactive oxygen species (ROS), which can damage cellular macromolecules and trigger inflammatory responses. In the liver, these mechanisms may disrupt hepatocyte membranes and metabolic functions, leading to hepatocellular injury. Therefore, saponin-induced toxicity in the liver and kidney is primarily associated with membrane disruption, oxidative stress, and subsequent inflammatory responses (Faizal & Geelen, 2013). Experimental studies have shown that saponin-containing extracts may cause proximal tubular brush border loss, glomerular bleeding, and cortical hemorrhage (Shafaei, 2012).

In toxicological investigations, mild histological alterations may occasionally be observed in control animals. These findings may arise from background lesions caused by natural biological variation, normal apoptotic turnover of cells, or mild physiological stress related to laboratory conditions. In addition, procedural artifacts during necropsy and histological preparation may contribute to certain cellular changes. Features such as hydropic degeneration or loss of cytoplasmic detail may indicate post-mortem autolysis, which can occur when there is a delay between euthanasia and tissue fixation (Chatterjee, 2014; Frazier et al., 2012; Thoolen et al., 2010). Therefore, mild histopathological changes in control groups should be interpreted cautiously and distinguished from treatment-related lesions.

IV. CONCLUSION

There were no deaths in any treatment group. The LD_{50} values of both extracts were estimated to be greater than 2000 mg/kgBW, which classifies as category 5 according to OECD standards. Mild toxic symptoms were observed only in the treatment group receiving the fruit extract. Histopathological analysis of the liver and kidneys revealed mild cellular changes that were not statistically significant. Therefore, with an estimated LD_{50} above 2000 mg/kgBW, *M. speciosa* fruit and leaf extracts can be regarded as nearly non-toxic for a single dose or brief administration.

CONFLICT OF INTEREST

All authors affirm the absence of any conflict of interest in this research.

REFERENCES

Agustina, N. I. A., Jeffrey, T., Hutauruk, W., Sulistyaningrum, N., Yudhanto, S. M., Liza, N. O. R., Kusumaningrum, L. I. A., Yasa, A., Saensouk, S., Naim, D., & Setyawan, A. D. W. I. (2022). Diversity of the medicinal plant in homegarden of local communities in the coastal area of Prigi Bay , Trenggalek , East Java , Indonesia. *Biodiversitas*, *23*(12), 6302–6312. https://doi.org/10.13057/biodiv/d231226

Aisyah, S., Budiman, H., Florenstina Br. G, D., Aliza, D., Salim, M. N., Balqis, U., & Armansyah, T. (2015). Efek Pemberian Minyak Jelantah Terhadap Gambaran Histopatologis Hati Tikus Putih (Rattus norvegicus). *Jurnal Medika Veterinaria*, *9*(1). https://doi.org/10.21157/j.med.vet..v9i1.2989

Alsawaf, S., Alnuaimi, F., Afzal, S., Thomas, R. M., Chelakkot, A. L., Ramadan, W. S., Hodeify, R., Matar, R., Merheb, M., Siddiqui, S. S., & Vazhappilly, C. G. (2022). Plant Flavonoids on Oxidative Stress-Mediated Kidney Inflammation. *Biology*, *11*(12), 1717. https://doi.org/10.3390/biology11121717

Astutik, P., Yuswantina, R., & Vifta, R. L. (2021). Perbandingan Aktivitas Antifungi Ekstrak Etanol 70% Dan 96% Buah Parijoto (Medinilla Speciosa) Terhadap Candida Albicans. *JHHS: Jurnal of Holistics and Health Scineces*, *3*(1), 32–40. https://doi.org/10.35473/jhhs.v3i1.66

Badan Pengawas Obat dan Makanan Republik Indonesia. (2022). *Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo*. Badan Pengawas Obat dan Makanan Republik Indonesia.

Bulgheroni, A., Kinsner-Ovaskainen, A., Hoffmann, S., Hartung, T., & Prieto, P. (2009). Estimation of acute oral toxicity using the No Observed Adverse Effect Level (NOAEL) from the 28 day repeated dose toxicity studies in rats. *Regulatory Toxicology and Pharmacology*, *53*(1), 16–19. https://doi.org/10.1016/j.yrtph.2008.10.001

Chatterjee, S. (2014). Artefacts in histopathology. *Journal of Oral and Maxillofacial Pathology*, *18*(5), 111–116. https://doi.org/10.4103/0973-029X.141346

Cosme, F., Aires, A., Pinto, T., Oliveira, I., Vilela, A., & Gonçalves, B. (2025). A Comprehensive Review of Bioactive Tannins in Foods and Beverages: Functional Properties, Health Benefits, and Sensory Qualities. *Molecules*, *30*(4), 800. https://doi.org/10.3390/molecules30040800

Fairuzzahra, J. A. (2021). *Uji Ekstrak Parijoto terhadap Enzim Alfa Glukosidase sebagai Antidiabetes dan Antibakteri* [Skripsi]. Universitas Wahid Hasyim.

Faizal, A., & Geelen, D. (2013). Saponins and their role in biological processes in plants. *Phytochemistry Reviews*, *12*(4), 877–893. https://doi.org/10.1007/s11101-013-9322-4

Frazier, K. S., Seely, J. C., Hard, G. C., Betton, G., Burnett, R., Nakatsuji, S., Nishikawa, A., Durchfeld-Meyer, B., & Bube, A. (2012). Proliferative and Nonproliferative Lesions of the Rat and Mouse Urinary System. In *Toxicologic Pathology* (Vol. 40, Issue 4). https://doi.org/10.1177/0192623312438736

Gazolaa, A. C., Costaa, G. M., Castellanos, L., Ramos, F. A., Reginatto, F. H., de Lima, T. C. M., & Schenkel, E. P. (2015). Involvement of GABAergic pathway in the sedative activity of apigenin, the main flavonoid from passiflora quadrangularis pericarp. *Revista Brasileira de Farmacognosia*, *25*(2), 158–163. https://doi.org/10.1016/j.bjp.2015.03.009

Hanum, A. S., Prihastanti, E., & Jumari. (2017). Ethnobotany of utilization, role, and philosopical meaning of parijoto (Medinilla, spp) on Mount Muria in Kudus Regency, Central Java. *AIP Conference Proceedings*, (August). https://doi.org/10.1063/1.4995210

Husin, A. P. (2018). *Uji Toksisitas Subkronik Ekstrak Jintan Hitam (Nigella Sativa) Terhadap Berat Dan Histologi Ginjal Tikus (Rattus Norvegicus) Galur Sprague Dawley* [Universitas Pembangunan Nasional Veteran Jakarta]. http://repository.upnvj.ac.id/id/eprint/2505

Jannah, D. R., & Budijastuti, W. (2022). Gambaran Histopatologi Toksisitas Ginjal Tikus Jantan (Rattus norvegicus) yang diberi Sirup Umbi Yakon (Smallanthus sonchifolius). *LenteraBio : Berkala Ilmiah Biologi*, *11*(2), 238–246. https://doi.org/10.26740/lenterabio.v11n2.p238-246

Jones, C. P., Boyd, K. L., & Wallace, J. M. (2016). Evaluation of mice undergoing serial oral gavage while awake or anesthetized. *Journal of the American Association for Laboratory Animal Science*, *55*(6), 805–810. https://aalas.kglmeridian.com/view/journals/72010024/55/6/article-p805.xml

Khalishah, H., Kurniawan, H., Nugraha, F., Nurbaeti, S. N., & Fajriaty, I. (2021). Pengaruh Pemberian Serbuk Cangkang Telur Terhadap Bobot Badan dan Indeks Organ Tikus Putih (Rattus norvegicus L.) Galur Wistar dalam 28 Hari. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, *5*(1), 1–6. https://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/49023

Lazic, S. E., Semenova, E., & Williams, D. P. (2020). Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. *Scientific Reports*, *10*(1), 6625. https://doi.org/10.1038/s41598-020-63465-y

Leksono, D. A. (2022). *Pengaruh Suhu Ekstraksi Teh Herbal Daun Parijoto (Medinilla speciosa) Metoda Maserasi Berbantu Gelombang Ultrasonik terhadap Aktivitas Antioksidan dan Organoleptiknya.* [Skripsi]. Universitas Semarang.

Lestari, I. B., Sudarmin, S., Ellianawati, E., & Sumarni, W. (2021). Development of Parijoto EthnoVlog Media to Explain the Scientific Reconstruction and Explanation of Parijoto (Medinilla javanensis) as Body Immunity. *Proceeding International Conference on Science, Education and Technology*, *7*(1), 260–266. https://proceedings.unnes.ac.id/ISET/article/view/1967

Luhurningtyas, F. P., Vifta, R. L., Syarohmawati, N., Adhi, M., Studi, P., Farmasi, S., Ngudi, U., No, J. D., & Timur, U. (2020). Cholesterol Lowering Effect of Chitosan Nanoparticles Using Parijoto Fruits Extracts. *Jurnal Farmasi Sains Dan Komunitas*, *17*(2), 102–111. https://dx.doi.org/10.24071/jpsc.002017

Megawati, A., Hastuti, E. D., & Sari, D. E. M. (2017). Uji Ketoksikan Akut Buah Parijoto Segar (Medinilla

Speciosa) terhadap Mencit Jantan Galur Swiss. *Cendekia Journal of Pharmacy*, *1*(1), 1–8. https://doi.org/10.31596/cjp.v1i1.1

Milanda, T., Lestari, K., & Tarina, N. T. I. (2021). Antibacterial Activity of Parijoto (Medinilla speciosa Blume) Fruit Against Serratia marcescens and Staphylococcus aureus Aktivitas Antibakteri Buah Parijoto (Medinilla speciosa Blume) terhadap Serratia marcescens dan Staphylococcus aureus. *Indonesian Journal of Pharmaceutical Science and Technology*, *8*(2), 76–85. https://doi.org/10.24198/ijpst.v8i2.32166

Nabila, I., Thadeus, M. S., & Herardi, R. (2023). Hepatoprotective Effect of Parijoto Fruit Extract (Medinilla speciosa Blume) on Male Mice Fed with High-Fat Diet. *Jurnal Gizi Pangan*, *18*(1), 21–30. https://doi.org/10.25182/jgp.2023.18.1.21-30

Nalimu, F., Oloro, J., Peter, E. L., & Ogwang, P. E. (2022). Acute and sub-acute oral toxicity of aqueous whole leaf and green rind extracts of Aloe vera in Wistar rats. *BMC Complementary Medicine and Therapies*, *22*(1), 1-14. https://doi.org/10.1186/s12906-021-03470-4

Ozogul, Y., Ucar, Y., Tadesse, E. E., Rathod, N., Kulawik, P., Trif, M., Esatbeyoglu, T., & Ozogul, F. (2025). Tannins for food preservation and human health: A review of current knowledge. *Applied Food Research*, *5*(1), 100738. https://doi.org/10.1016/j.afres.2025.100738

Pradono, J., Sampurno, O. D., Halim, F. X. S., Widowati, L., Imaningsih, N., Handayani, S., Isnawati, A., Delima, L., & C. W., R. (2019). *Bunga Rampai Uji Klinik*. Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan.

Riana, E. N., Ischak, N. I., Hrp, C. L. F., A., & E. I., K. (2023). *Toksikologi Dasar*. Yayasan Kita Menulis.

Ríos, J. L., Schinella, G. R., & Moragrega, I. (2022). Phenolics as GABAA Receptor Ligands: An Updated Review. *Molecules*, *27*(6), 1770. https://doi.org/10.3390/molecules27061770

Rubianti, I., Azmin, N., & Nasir, M. (2022). Analisis Skrining Fitokimia Ekstrak Etanol Daun Golka (Ageratum conyziodes) Sebagai Tumbuhan Obat Tradisional Masyarakat Bima. *JUSTER : Jurnal Sains Dan Terapan*, *1*(2), 7–12. https://doi.org/10.55784/juster.v1i2.67

Rudini, M., Kuswanto, E., & Yudistiro, M. K. (2021). Pengaruh Ekstrak Daun Tanaman Bidara (Ziziphus mauritiana) Terhadap Histopatology Hati Mencit (Mus musculus) yang Diberi Alkohol. *Organisms: Journal of Biosciences*, *1*(2), 109–118. https://doi.org/10.24042/organisms.v1i2.10927

Setyaputri, N. A. R. (2019). *Pengaruh Pemberian Ekstrak Bunga Rosela (Hibiscus sabdariffa L.) terhadap Perubahan Gambaran Histopatologi Hepar Tikus (Rattus novergicus) yang Diinduksi Etanol 20%* [Universitas Pembangunan Nasional Veteran Jakarta]. http://repository.upnvj.ac.id/id/eprint/1413

Shafaei, H. (2012). Citrullus colocynthis as a medicinal or poisonous plant: A revised fact. *Journal of Medicinal Plants Research*, *6*(35), 4922-4927. https://doi.org/10.5897/JMPR11.264

Sharma, K., Kaur, R., Kumar, S., Saini, R. K., Sharma, S., Pawde, S. V., & Kumar, V. (2023). Saponins: A concise review on food related

aspects, applications and health implications. *Food Chemistry Advances*, *2*, 100191. https://doi.org/10.1016/j.focha.2023.100191

Singh, P., Sharma, S., & Rath, S. K. (2022). A versatile flavonoid Quercetin: Study of its toxicity and differential gene expression in the liver of mice. *Phytomedicine Plus*, *2*(1), 100148. https://doi.org/10.1016/j.phyplu.2021.100148

Sugiarti, L., Andriyani, D. M., Pratitis, M. P., & Setyani, R. (2020). Aktivitas Antibakteri Fraksi n-Heksan, Etil Asetat dan Air Ekstrak Etanol Daun Parijoto (Medinilla speciosa Blume) terhadap Propionibacterium acnes dan Staphylococcus epidermidis. *Cendekia Journal of Pharmacy*, *4*(2), 120–130. https://doi.org/10.31596/cjp.v4i2.105

Suhita, N. L. P. R., Sudira, I. W., & Winaya, I. B. O. (2013). Histopatologi ginjal tikus putih akibat pemberian ekstrak pegagan (Centella asiatica) peroral. *Buletin Veteriner Udayana, 5*(1), 63-69.

Tang, Z., & Zhang, Q. (2022). The Potential Toxic Side Effects of Flavonoids. *BIOCELL*, *46*(2), 357–366. https://doi.org/10.32604/biocell.2022.015958

Thoolen, B., Maronpot, R. R., Harada, T., Nyska, A., Rousseaux, C., Nolte, T., Malarkey, D. E., Kaufmann, W., Küttler, K., Deschl, U., Nakae, D., Gregson, R., Vinlove, M. P., Brix, A. E., Singh, B., Belpoggi, F., & Ward, J. M. (2010). Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicologic Pathology*, *38*(7 SUPPL.), 5–81. https://doi.org/10.1177/0192623310386499

Umiyati, W., Pramesti, M. A., & Pujiastutik, E. (2021). Pest and Disease Identification in Parijoto Plant (Medinilla speciosa blume) at Nglurah Tawangmangu. *Jurnal Biologi Tropis*, *21*(3), 1073–1080. https://doi.org/10.29303/jbt.v21i3.2970

Vifta, R., & Advistasari, Y. D. (2018). Analisis penurunan kadar glukosa fraksi n-heksan buah parijoto (medinilla speciosa b) secara in vitro dengan metode spektrofotometri uv-vis. *Indonesian Journal of Chemical Science, 7*(3), 249-253. https://journal.unnes.ac.id/sju/ijcs/article/view/25045

Vifta, R. L., Nurlaila, D., & Yanti, S. (2022). Alginate-Based Nanoencapsulation on Ultrasonic-Assisted Extraction of Parijoto Fruit (Medinilla Speciosa Blume) and Its Antioxidant Activity. *Walisongo Jorunal of Chemistry*, *5*(2), 145–152. https://doi.org/10.21580/wjc.v5i2.12509

Wijaya, H. M., & Nisyak, M. (2020). Efektivitas Ekstrak Daun Parijoto (Medinila speciosa Blume) sebagai Penumbuh Rambut pada Hewan Uji Kelinci Jantan. *Jurnal Farmasi & Sains Indonesia*, *3*(2), 22–27. https://www.journal.stifera.ac.id/index.php/jfsi/article/view/51

Winanta, A., Hanik, L. S., & Febriansah, R. (2021). Antioxidant Activity and Cytotoxic Potential of Parijoto (Medinilla speciosa (Reinw ex BL)) Fruit Fractions on HeLa Cell Line. *Indonesian Journal of Cancer Chemoprevention*, *12*(2), 74–82. https://doi.org/10.14499/indonesianjcanchemoprev12iss2pp74-82