

Protective effects of *Centella asiatica* (L.) Urb. on pulmonary histomorphology in cigarette smoke-exposed Wistar rats

Mulyati Sri Rahayu^{1*}, Juwita Sahputri², Daffa Faruq Arrazzaq Albin³

¹Department of Anatomical Pathology, Faculty of Medicine, Universitas Malikussaleh. Jalan Cot Teungku Nie, Muara Baru Regency 24355, Aceh Province, Indonesia

²Department of Microbiology, Faculty of Medicine, Universitas Malikussaleh. Jalan Cot Teungku Nie, Muara Baru Regency 24355, Aceh Province, Indonesia

³Undergraduate Medical Student, Faculty of Medicine, Universitas Malikussaleh. Jalan Cot Teungku Nie, Muara Baru Regency 24355, Aceh Province, Indonesia

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Abstract: Cigarette smoke contains harmful chemicals that cause acute and chronic lung inflammation and oxidative stress. *Centella asiatica* contains secondary metabolites with potential antioxidant properties for phytotherapy or phytoprevention. This study examined the effects of varying doses of *Centella asiatica* extract on lung damage in Wistar rats exposed to cigarette smoke. Twenty-four adult male Wistar rats were divided into six groups (n = 4): normal control, negative control (three cigarettes daily without extract), positive control (vitamin E) and three treatment groups that received three cigarettes per day and extract at 120, 180, and 240 mg/kg body weight for 28 days. Histopathological examination of lung tissues was performed using the modified Manja–Roegnik scoring method. Data were analysed using the Kruskal–Wallis test and a Bonferroni correction post hoc test. The lung tissue scores for the normal, negative control and positive groups were 1.00 ± 0.00 , 2.67 ± 0.25 , and 1.67 ± 0.25 , respectively. In the treatment group, the scores were 2.16 ± 0.59 (120 mg/kg), 1.83 ± 0.33 (180 mg/kg), and 2.65 ± 0.66 (240 mg/kg). Extract of *Centella asiatica* (180 mg/kg) significantly reduced lung damage caused by exposure to cigarette smoke ($p = 0.002$) and was as effective as vitamin E ($p = 1.000$). *Centella asiatica* extract reduced lung tissue damage in rats exposed to cigarette smoke due to its antioxidant properties.

1. INTRODUCTION

Cigarettes contain over 4,000 harmful toxic substances, including tar, nicotine, carbon monoxide, radioactive materials (polonium-210), acetone, ammonia, naphthalene, arsenic, and hydrogen cyanide (American Cancer Society, 2020; Cheng *et al.*, 2022). Exposure to cigarette smoke (CS) can generate free radicals in humans, which initiate lipid peroxidation. This oxidative process in cell membrane components, then, can lead to the formation of toxic compounds, such as reactive oxygen species (ROS) (Herdiani & Putri, 2018), which are known to be primarily associated with chronic obstructive pulmonary disease (COPD).

*CONTACT Mulyati Sri RAHAYU ✉ mulyati.srirahayu@unimal.ac.id 🏢 Department of Anatomical Pathology, Faculty of Medicine, Universitas Malikussaleh. Jalan Cot Teungku Nie, Muara Baru Regency 24355, Aceh Province, Indonesia.

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COPD is the third leading cause of death globally, accounting for 3.23 million fatalities in 2019. By 2023, COPD constituted 3.6% of the overall burden of the disease, with 50% of these deaths attributed to respiratory diseases. Almost all COPD-related deaths among individuals aged under 70 years occur in low- and middle-income countries (LMICs). Cigarette smoking and air pollution are typically responsible for these cases of COPD. In high-income countries, smoking is the leading cause of COPD in over 70% of instances. In contrast, smoking accounts for 30-40% of COPD cases in LMICs. The reported prevalence of COPD in Indonesia was 3.7%, and the highest prevalence was observed among male smokers and ex-smokers (Badan Penelitian and Pengembangan Kesehatan, 2018; WHO, 2023). In line with that, a previous study by Rohmani *et al.*, (2018) reported that exposure to conventional cigarette smoke caused moderate to severe alveolar damage in rats.

Centella asiatica (L.) Urb. is a plant indigenous to the tropical regions of Asia and is widely used in traditional medicine (Susetyarini *et al.*, 2020). Several studies have documented the presence of active metabolites, such as alkaloids, saponins, tannins, flavonoids, triterpenoid saponins, and glycosides. Among these, tannins, saponins, and flavonoids have been shown to possess antioxidant properties that effectively combat free radicals in the body (Maisetta *et al.*, 2019). *Centella asiatica* is known to contain the active metabolite compound asiaticoside. The ethanol extract of *Centella asiatica* reduced the expression of MMP-1, thus preventing alveolar collagen damage in tuberculosis patients. In addition, asiaticoside, an active metabolite, can enhance lung tissue resolution by increasing collagen synthesis (Griana, 2019; Idris & Mohd Nadzir, 2021). Another study revealed that asiaticoside, extracted from *Centella asiatica*, can significantly reduce inflammation in several organs, including the lungs, in a rat model (Yuyun *et al.*, 2018). Mustika *et al.*, 2021 further demonstrated that the ethanol extract of *Centella asiatica* contains components that can promote apoptosis in rats' alveolar macrophages. Moreover, secondary metabolites derived from plants underscore the promising potential of secondary metabolites from plants as phytotherapeutic agents. This study, therefore, aimed to examine the effects of different doses of *Centella asiatica* extract on lung damage in Wistar rats exposed to cigarette smoke, providing crucial insights into this area of research.

2. MATERIAL and METHODS

2.1. Preparation of *Centella asiatica* Extract

To obtain the plant extract, three kilograms of *Centella asiatica* leaves were first thoroughly cleaned and air-dried for seven days. The dried *Centella asiatica* was then ground and immersed in 70% ethanol. Further, the formulation was prepared by dissolving 15 grams of dried *Centella asiatica* in 150 mL of 70% ethanol, and left to macerate at room temperature for 24 hours. Following that, the extraction solution was filtered using a filter paper and a glass funnel. The resulting filtrate biomass was transferred to a sealed glass beaker, and the filtrate was then evaporated using a rotary evaporator set at 40 °C and 100 Mbar pressure to yield 23.39 grams of the extracts (Rahayu *et al.*, 2020). Stratified doses of *Centella asiatica* extract were administered at 120, 180, and 240 mg/kg body weight.

2.2. Phytochemical Test

Wagner and Bouchardart tests revealed a positive reaction for the alkaloid, as evidenced by the formation of a brownish-to-yellowish precipitate. The saponin test further confirmed the presence of glycosides, demonstrated by the formation of foam in aqueous hydrolysed containing glucose and related compounds. The results of the 5% FeCl₃ test confirmed these positive findings through the formation of a blackish-green precipitate. Later, the addition of 5% FeCl₃ to extract samples containing flavonoid compounds resulted in the formation of black flavium salt. The Salkowski test was employed to detect triterpenoids and steroids. A positive result for triterpenoids was indicated by a reddish color change, meanwhile a positive result for steroids was denoted by a greenish-blue color change. In addition, the Liebermann-Bouchard

confirmed the presence of triterpenoids by the formation of a brown ring, and the presence of steroids was marked by a greenish-blue color change (Harborne, 2012; Shabur, 2019).

2.3. Experimental Design

This study was a true experiment with a *post-test control-only* design. Wistar rats were divided into six groups ($n = 4$): normal, negative control (three cigarettes daily without extract), positive control (three cigarettes daily with vitamin E), and three treatment groups that received three cigarettes daily with *Centella asiatica* extract at doses of 120, 180, and 240 mg/kg body weight for 28 days.

2.4. Animal Treatment

Male Wistar white rats aged 10 to 16 weeks and weighing between 150 and 200 g were obtained from the Experimental Animal Development Unit of the Universitas Sumatera Utara, Indonesia. Wistar rats were allowed to adapt for seven days. Twenty-four adult male rats were assigned to six groups of four animals each. Group I served as the normal group, and Group II acted as the negative control group, exposed only to CS. Then, Group III was assigned as the positive control group; exposed to CS in addition to 100 IU of vitamin E. Groups IV, V, and VI were exposed to CS and *Centella asiatica* extract at doses of 120, 180, and 240 mg/kg body weight, respectively. On the eighth day after acclimatisation, the rats were exposed to cigarette smoke daily for 28 consecutive days. The exposure was carried out in a smoking chamber by burning three DJ cigarettes per day. DJ cigarettes containing 2.9 mg nicotine and 44.3 mg tar were purchased from the market and used in this study. The dosage of cigarettes per exposure was based on the nicotine LD50 value in the rats (50 mg/kg body weight) (Henstra et al., 2022).

An hour following exposure to cigarette smoke, rats received *Centella asiatica* extract at various doses as designed. On the twenty-ninth day, all rats were euthanized by cervical dislocation under anesthesia, and lung tissues were collected and prepared for histological examination.

2.5. Histopathological Examination of the Lung

Lung samples were collected and fixed in 10% phosphate-buffered formalin solution for 24 hours before routine processing and staining with hematoxylin and eosin (H&E). Subsequently, the samples were examined using an Olympus CX33 microscope, and photomicrographs were captured using the CellSen imaging software (Olympus Corporation, Japan). All samples were evaluated in five fields of view at a magnification of 400X, and the mean scores were used to evaluate any changes in the lung histopathology. The Manja-Roegnik scoring system was used to assess lung histopathology, including damage to the alveolar membrane, alveolar lumen, and interalveolar connections. The alveolar membrane scores were as follows: (1) intact alveolar membrane, containing complete cell nuclei with >75% normal endothelial cells; (2) intact alveolar membrane, containing complete cell nuclei with 25–75% endothelial cells; and (3) intact alveolar membrane, containing complete cell nuclei with <25% normal endothelial cells. The alveolar lumen scores were assigned as follows: (1) round alveolar lumen with a proportional size greater than 75% normal; (2) round shape with a proportional size between 25 and 75% normal; and (3) round shape with a proportional size less than 25% of normal. Inter-alveolar connecting score: (1) density greater than 75% normal; (2) density between 25 and 75% normal; and (3) density less than 25% approaching normal (Herdiani & Putri, 2018).

2.5. Data Analysis

The Kruskal-Wallis test was performed to investigate any significant differences in histological changes in the lungs between the different groups assigned. Subsequently, a post-hoc Bonferroni correction test was performed to determine the optimal dose of *Centella asiatica* extract to ameliorate histological changes in the lungs of rats exposed to CS. Statistical significance was set at p -value < 0.05.

3. FINDINGS

The phytochemical composition of the ethanol extract of *Centella asiatica* is presented in Table 1. The results revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids, and terpenoids in ethanol extracts.

Table 1. Qualitative determination of the phytochemical composition of the extract.

Secondary metabolites	Reagent	Result
Alkaloid	Bouchardart	+
	Maeyer	+
Saponin	Aquadest + Alkohol 96% +HCl 2 N	+
Tannin	FeCl ₃ 5%	+
Flavonoid	FeCl ₃ 5%	+
Steroid	Salkowsky	+
	Liebermann-Bouchard	+
Terpenoid	Salkowsky	+
	Liebermann-Bouchard	+

Note: + = Phytochemically detected (present)

The Kruskal–Wallis test revealed that the degree of lung damage was significantly different between the groups (Table 2). The lung damage scores at doses of 120 mg/kg body weight and 240 mg/kg body weight were 2.16 and 2.65, respectively, while the dosage of 180 mg/kg body weight was 1.83. The highest lung damage score was observed in Group II (2.67), which was higher than the normal group (1.83). The lung damage scores were 2.16 %, 2.65 %, and 1.83% at 120, 240, and 180 mg/kg body weight, respectively.

Table 2. Differences in lung damage score between groups.

Group	Median (IQR)	<i>p</i> -value
I (Normal)	1 (0)	0.001*
II (negative control)	2.67 (0.25)	
III (Positive control)	1.67 (0.25)	
IV (120 mg/kg body weight)	2.16 (0.59)	
V (180 mg/kg body weight)	1.83 (0.33)	
VI (240 mg/kg body weight)	2.65 (0.66)	

Notes: The Kruskal–Wallis test was used for comparisons between groups ($p = 0.001$); IQR = interquartile range.

*Statistical significance was set at $p < 0.05$.

Post-hoc Bonferroni correction analysis revealed statistically significant differences in the degree of lung damage between the normal group and all treatment groups, and a significant difference was observed between groups V (180 mg/kg body weight), II (negative control group), and III (positive control group) ($p < 0.05$). Furthermore, significant differences were observed between groups V (180 mg/kg body weight) and VI (240 mg/kg body weight) ($p = 0.002$). However, there were no significant differences between groups III (positive control group) and V (180 mg/kg body weight) ($p = 1.000$) (Table 3). Therefore, the extract at 180 mg/kg body weight was as effective as vitamin E in alleviating lung damage caused by cigarette smoke. Figure 1 shows the histological changes in the lungs according to the structures observed in the alveolar membrane, alveolar lumen, and interalveolar connections of the lung tissues.

Table 3. Comparison of lung damage scores between the different treatment groups.

Group	Comparison	S.E.	p-value	95% CI	
				Lower bound	Upper bound
I	II	0.169	0.000*	-2.324	-1.181
	III	0.169	0.005*	-1.3240	0.181
	IV	0.169	0.000*	-1.821	0.678
	V	0.169	0.002*	-1.406	0.263
	VI	0.169	0.000*	-2.236	-1.093
II	III	0.169	0.000*	0.428	1.571
	IV	0.169	0.122	0.069	1.074
	V	0.169	0.001*	0.346	1.489
III	VI	0.169	1.000	0.484	0.659
	IV	0.169	1.131	-1.069	0.074
	V	0.169	1.000	-0.654	0.489
IV	VI	0.169	0.001*	-1.484	0.341
	V	0.169	0.368	-0.156	0.986
V	VI	0.169	0.368	-0.986	0.156
	VI	0.169	0.002*	-1.401	-0.258

Notes: A Bonferroni correction post hoc test was used to assess significant differences within each group; I = normal group; II = negative control; III = positive control; IV = 120 mg/kg body weight; V = 180 mg/kg body weight; VI = 240 mg/kg body weight; CI = 95% confidence interval; * indicates a statistically significant difference (p -value < 0.05); S.E: Standard Error.

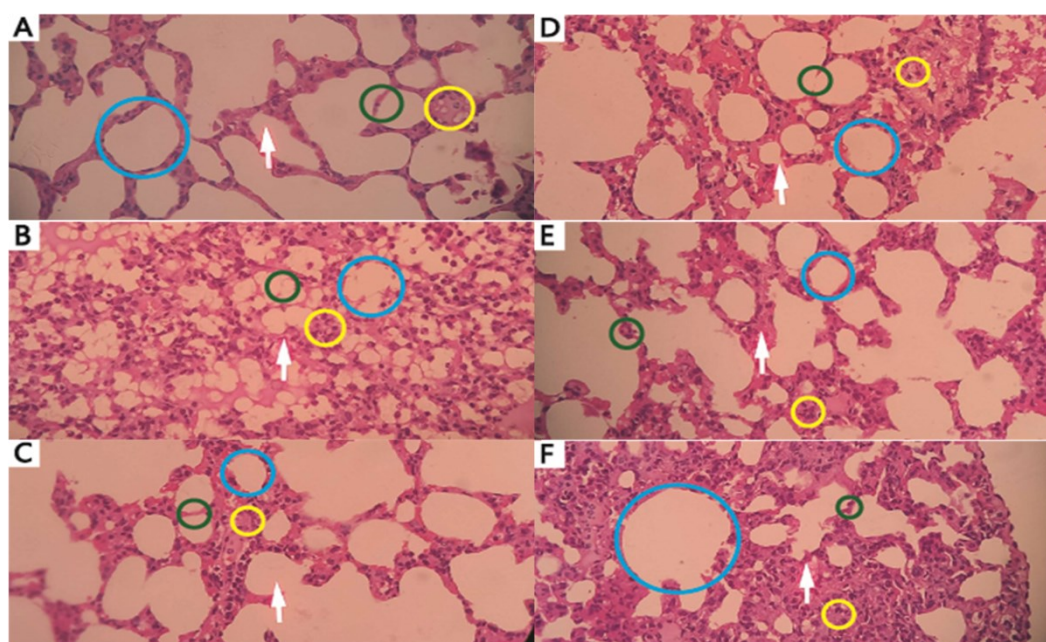


Figure 1. Light micrographs of lung sections from control and extract-treated rats stained with H&E (400X). Normal control (A), negative control (B), positive control (C), *Centella asiatica* extract group at doses of 120 mg/kg BW (D), group at doses of 180 mg/kg BW (E), group at doses of 240 mg/kg (F). Note: The round shape of the alveolar lumen (blue), intact alveolar membrane with complete endothelial cells of the nuclei (yellow), and discontinuous interalveolar relationship (green) were compared between groups.

4. DISCUSSION and CONCLUSION

This study aimed to investigate differences in lung damage caused by various doses of *Centella asiatica* extract in Wistar rats exposed to cigarette smoke. Our findings indicated that the control group exhibited minor pulmonary damage. This pulmonary damage could be attributed to extraneous factors beyond our control, such as the psychological effects of a high-decibel environment and repeated treatment. Endogenous free radicals, which are typically present under normal physiological conditions, could also contribute to pulmonary damage. These

antioxidants are endogenously produced in the body to prevent damage caused by reactive oxygen species (Sharifi-Rad *et al.*, 2020).

Numerous pathological changes have been observed in the lungs of rats exposed to cigarette smoke. Histopathology was used to analyze damage to the alveolar membrane, alveolar lumen, and interalveolar connection. The highest lung damage score was observed in the negative control group. Histopathological examination of the negative control group revealed widening of the alveolar lumen, indicating emphysema. Emphysema is a condition in which the lungs enlarge beyond the normal size of the air cavities in the distal part of the terminal bronchioles, destroying the alveolar wall. Cigarette smoke induces the production of cytokines that stimulate the infiltration of inflammatory cells and the decrease of endothelial cells in the lung alveoli. The accumulation of inflammatory cells in the alveolar wall causes thickening of the alveolar wall structure, which can destroy the alveolar junction and result in rounded alveoli. Thickening of the alveolar septa may depend on increased blood capillary volume, inflammatory infiltration, and edema. Moreover, cigarette smoking activates neutrophil and macrophage elastase, which damage the elastic fibers and cause emphysema (Sharifi-Rad *et al.*, 2020; Suryadinata *et al.*, 2019).

The formation of free radicals from exposure to cigarette smoke causes an imbalance between proteases and antiproteases, disrupting cell membrane integrity and damaging the interalveolar connection. It is presumed that the observed disruptive changes in lung tissues are probably associated with oxidative stress and dysfunction of the blood-air barrier caused by the toxic substances present in smoke (Sharifi-Rad *et al.*, 2020; Suryadinata *et al.*, 2019).

The present study identified secondary metabolites, including alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids, in *Centella asiatica* extract, which demonstrated antioxidant activity. The treatment groups of graded doses of *Centella asiatica* extract were analyzed by comparison with the negative control group to determine the ability of *Centella asiatica* extract in reducing the level of lung damage. Analysis of the treatment group administered stratified doses of *Centella asiatica* extract, with the negative control group showing a significant difference between the treatment group administered a dose of 180 mg/kg and the negative control group ($p = 0.001$). These findings indicate a significant difference in lung damage at this dose compared with that in the negative group. Administration of *Centella asiatica* extract improved lung morphology, although this effect was not maximized. The decrease in alveolar damage is hypothesized to be attributable to the binding of free radicals to antioxidants present in *Centella asiatica* extract, resulting in diminished alveolar damage compared to the group treated with a dose of 120 mg/kg and the negative control group.

The present study demonstrated that *Centella asiatica* extract, a potent antioxidant, significantly mitigated tissue damage induced by cigarette smoke in rat lungs at a dose of 180 mg/kg. These findings are particularly noteworthy given the established correlation between smoking and elevated oxidative stress and inflammation, which are fundamental to the pathogenesis of numerous chronic diseases, including chronic obstructive pulmonary disease. Alkaloids and flavonoids have exhibited potential as antioxidants capable of attenuating lung damage through the inhibition of inflammatory cell release, specifically alveolar macrophages and neutrophils (Agatonovic-Kustrin *et al.*, 2018; Mitra *et al.*, 2022). Flavonoids have been shown to function as protease inhibitors, mitigate inflammatory responses, inhibit lung tissue degradation, and attenuate alveolar dilation. Consequently, these compounds enhance the elasticity of lung tissue (Al-Khayri *et al.*, 2022; Santana *et al.*, 2021).

Saponins exhibit anti-inflammatory and antioxidant properties. Steroids and terpenoid compounds that function as secondary metabolites have been demonstrated to attenuate inflammatory lung damage through the suppression of pro-inflammatory mediator synthesis, including LPS and carrageenan, in murine models of pulmonary injury (Merdana *et al.*, 2022; Mieres-Castro & Mora-Poblete, 2023). Terpenoids possess the capacity to diminish the catalytic activity of enzymes responsible for producing ROS while simultaneously offering

protection against oxidative damage through diverse antioxidant mechanisms. Tannins demonstrate robust anti-inflammatory and antioxidant properties and efficiently neutralize stable radicals, including hydroxyl, hydrogen peroxide, and superoxide radicals. These characteristics may play a role in alleviating pathological alterations (Del Prado-Audelo *et al.*, 2021; Merdana *et al.*, 2022).

Our findings suggest that the administration of *Centella asiatica* extract at 180 mg/kg body weight is as effective as vitamin E in mitigating lung damage caused by exposure to cigarette smoke. Vitamin E acts as an antioxidant by inhibiting ROS activity, neutralizing free radicals, and preventing oxidative chain reactions (Niki, 2021; Rajasekaran *et al.*, 2021). Microscopic examination revealed the highest lung damage score in the group administered *Centella asiatica* extract at 240 mg/kg body weight, which exceeded that of the other groups. These results indicate that lung damage did not decrease with increasing doses of the extract. *Centella asiatica* is known for its immunomodulatory properties, which can influence immune functions. In the present study, immunomodulatory effects were observed at a dose of 240 mg/kg body weight. However, doses greater than 180 mg/kg reduced the anti-inflammatory effects of the extract, leading to increased lung inflammation due to increased immune cell production (Griana, 2019). The proposed effect of the *Centella asiatica* extract at a dose of 240 mg/kg body weight stimulates an inflammatory response that can exacerbate lung damage. Further research is required to confirm this hypothesis.

The present study concluded that *Centella asiatica* extract has the potential to reduce lung tissue damage in rats exposed to cigarette smoke. This potential, attributed to the presence of secondary metabolites that function as antioxidants. Further, the optimal dose of *Centella asiatica* extract for reducing lung tissue damage was determined to be 180 mg/kg, highlighting the crucial role of regular consumption of plant-based foods with medicinal properties for effective prevention of this chronic disease.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number:** Ethics Research Commission of the Faculty of Medicine, Universitas Malikussaleh, Aceh Province, Indonesia, 23/KEPK/FKUNIMAL-RSUCM/2023.

Authorship Contribution Statement

Mulyati Sri Rahayu: Investigation, Methodology, Validation, Resources, Formal Analysis, Writing -original draft, and Writing - Review. **Juwita Sahputri:** Investigation, Methodology, Validation, Resources, Formal Analysis. **Daffa Faruq Arrazzaq Albin:** Investigation, Visualization, Software, and Writing.

Orcid

Mulyati Sri Rahayu  <https://orcid.org/0000-0001-7480-2485>

Juwita Sahputri  <https://orcid.org/0000-0001-7072-1354>

Daffa Faruq Arrazzaq Albin  <https://orcid.org/0009-0005-4565-3659>

REFERENCES

Agatonovic-Kustrin, S., Morton, D.W., Mizaton, H.H., & Zakaria, H. (2018). The relationship between major polyphenolic acids and stigmasterol to antioxidant activity in different extracts of *Myrmecodia platytyrea*. *South African Journal of Botany*, 115, 94–99.

- Al-Khayri, J.M., Sahana, G.R., Nagella, P., Joseph, B.V., Alessa, F.M., & Al-Mssallem, M.Q. (2022). Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, 27(9), 2901.
- American Cancer Society. (2020). *Harmful Chemicals in Tobacco Products*. <https://www.cancer.org/content/dam/CRC/PDF/Public/8344.00.pdf>
- Badan Penelitian and Pengembangan Kesehatan. (2018). Hasil Riset Kesehatan Dasar (Riskesdas) Tahun 2018 [Basic Health Research 2018]. In *Kementerian Kesehatan RI*.
- Cheng, T., Reilly, S. M., Feng, C., Walters, M.J., & Holman, M.R. (2022). Harmful and potentially harmful constituents in the filler and smoke of tobacco-containing tobacco products. *ACS Omega*, 7(29), 25537–25554.
- Del Prado-Audelo, M.L., Cortés, H., Caballero-Florán, I.H., González-Torres, M., Escutia-Guadarrama, L., Bernal-Chávez, S.A., Giraldo-Gomez, D.M., Magaña, J.J., & Leyva-Gómez, G. (2021). Therapeutic applications of terpenes on inflammatory diseases. *Frontiers in Pharmacology*, 12, 704197.
- Griana, T.P. (2019). Potential effect of Pegagan (*Centella asiatica* (L.) Urban) and Widuri (*Calotropis gigantea* (L.)) as immunomodulator [The English name of the source should be given in square brackets]. *Journal of Food and Pharmaceutical Sciences*, 7(2), 55–72.
- Harborne, J.B. (2012). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Springer Netherlands. <https://books.google.co.id/books?id=BIMpBgAAQBAJ>
- Henstra, C., Dekkers, B.G.J., Olgers, T.J., ter Maaten, J.C., & Touw, D.J. (2022). Managing intoxications with nicotine-containing e-liquids. *Expert Opinion on Drug Metabolism & Toxicology*, 18(2), 115–121. <https://doi.org/10.1080/17425255.2022.2058930>
- Herdiani, N., & Putri, E.B.P. (2018). Gambaran histopatologi paru tikus wistar setelah diberi paparan asap rokok [Histopathology of Wistar Rat Lungs After Exposure to Cigarette Smoke]. *Medical and Health Science Journal*, 2(2).
- Idris, F.N., & Mohd Nadzir, M. (2021). Comparative studies on different extraction methods of *Centella asiatica* and extracts bioactive compounds effects on antimicrobial activities. *Antibiotics*, 10(4), 457.
- Maisetta, G., Batoni, G., Caboni, P., Esin, S., Rinaldi, A.C., & Zucca, P. (2019). Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant *Cytinus*. *BMC Complementary and Alternative Medicine*, 19, 1–11.
- Merdana, I.M., Mahottama, I.G.P.T., Berata, I.K., & Sudimartini, L.M. (2022). Struktur Histopatologi Paru-Paru Tikus Putih yang di Berikan Ekstrak Sarang Semut dan Diinduksi Parasetamol dengan Dosis Toksik [Histopathological Structure of Lung White Rat Given Myermecodia Pendans Extract and Induced Toxic Dose of Paracetamol]. *Buletin Veteriner Udayana Volume*, 14(2), 103–109.
- Mieres-Castro, D., & Mora-Poblete, F. (2023). Saponins: research progress and their potential role in the post-COVID-19 pandemic era. *Pharmaceutics*, 15(2), 348.
- Mitra, S., Anand, U., Ghorai, M., Vellingiri, B., Jha, N.K., Behl, T., Kumar, M., Radha, Shekhawat, M.S., & Proćków, J. (2022). Unravelling the therapeutic potential of botanicals against chronic obstructive pulmonary disease (COPD): molecular insights and future perspectives. *Frontiers in Pharmacology*, 13, 824132.
- Mustika, A., Rahaju, A.S., Sudjarwo, S.A., Agil, M., & Mertaniasih, N.M. (2021). Mechanism of *Centella asiatica* extract in increasing alveolar macrophages apoptosis in rat tuberculosis models. *Journal of International Dental and Medical Research*, 14(2), 812–819.
- Niki, E. (2021). Lipid oxidation that is, and is not, inhibited by vitamin E: Consideration about physiological functions of vitamin E. *Free Radical Biology and Medicine*, 176, 1–15.
- Rahayu, N.K.T., Permana, I.D.G.M., & Diah, G.A.K. (2020). Pengaruh Waktu Maserasi Terhadap Aktivitas Antioksidan Ekstrak Daun Pegagan (*Centella asiatica* (L.) Urban) [Effect of maceration time on antioxidant activity of pegagan (*Centella asiatica* (L.) Urban leaf extract)]. *J Ilmu Dan Teknol Pangan*, 9(4), 482. <https://doi.org/10.24843/itepa.2020.v09.i04.p12>

- Rajasekaran, S., Rajasekar, N., & Sivanantham, A. (2021). Therapeutic potential of plant-derived tannins in non-malignant respiratory diseases. *The Journal of Nutritional Biochemistry*, 94, 108632.
- Rohmani, A., Yazid, N., & Rahmawati, A.A. (2018). Rokok elektrik dan rokok konvensional merusak alveolus paru [Both Electric Cigarette and Conventional Cigarette Destruct the Pulmonary Alveolus Structure]. *Prosiding Seminar Nasional Unimus*, 1.
- Santana, F.P.R., Thevenard, F., Gomes, K.S., Taguchi, L., Câmara, N.O.S., Stilhano, R.S., Ureshino, R.P., Prado, C.M., & Lago, J.H.G. (2021). New perspectives on natural flavonoids on COVID-19-induced lung injuries. *Phytotherapy Research*, 35(9), 4988–5006.
- Shabur, J.T. (2019). *Fitokimia Tinjauan Metabolit Sekunder dan Skrining Fitokimia* (1st ed.). Universitas Islam Indonesia.
- Sharifi-Rad, M., Anil Kumar, N.V, Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P.V., Azzini, E., & Peluso, I. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694.
- Suryadinata, R.V., Wirjatmadi, B., Adriani, M., & Sumarmi, S. (2019). The effects of exposure duration to electronic cigarette smoke on differences in superoxide dismutase and malondialdehyde in blood of Wistar rats. *International Journal of Current Pharmaceutical Research*, 11(3), 13–16.
- Susetyarini, E., Wahyono, P., Latifa, R., & Nurrohman. (2020). Struktur morfologi dan anatomi pegagan (*Centella asiatica* L. (L.) Urban.) di Kota Malang [Morphological and anatomical structure of *Centella asiatica* L. (L.) Urban. in Malang City]. *Seminar Nasional Biologi*, 91–98.
- WHO. (2023). *Chronic obstructive pulmonary disease (COPD)*. [https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-\(copd\)](https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd))
- Yuyun, X., Xi, C., Qing, Y., Lin, X., Ke, R., & Bingwei, S. (2018). Asiatic acid attenuates lipopolysaccharide-induced injury by suppressing activation of the Notch signaling pathway. *Oncotarget*, 9(19), 15036–15046. <https://doi.org/10.18632/oncotarget.24542>