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Kawista fruit improves lung histopathology of rats exposed to cigarette smoke

Buah kawista memperbaiki histopatologi paru tikus yang terpapar asap rokok

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Abstract

Background: Free radicals in cigarettes can increase oxidative stress, exacerbating inflammation and activating alveolar macrophage cells. The phenolic components of kawista fruit act as antioxidants that reduce free radicals.
Objective: To determine the effect of kawista fruit on alveolar macrophage activation in Wistar rats exposed to cigarette smoke (CS).
Methods: The experimental research used a post-test only group design with a completely randomized design for 35 days. Wistar 25 male rats were divided into five groups: positive control (exposed to cigarette smoke), negative control (no treatment), O1, O2, and O3 (exposed to cigarette smoke and Kawista fruit sonde at doses O1=0,5, O2=0,6, and O3=0,7 g/kg BW). Data were analyzed using ANOVA one analysis of variance and LSD test.
Results: Kawista fruit given preventively at doses of 0,6 and 0,7 g/kg BW could prevent the activation of lung alveolar macrophages in rats exposed to cigarette smoke (p=0,000). The lowest number of alveolar macrophages was seen at a dose of 0,7 g/kg BW.
Conclusion: Kawista fruit inhibited the increase in the number of alveolar macrophages and improved the lung histopathology of Wistar rats exposed to cigarette smoke.

Keywords
Kawista, antioxidants, cigarette, alveolar macrophages

Abstrak

Latar Belakang: Radikal bebas dalam asap rokok dapat menyababkan stres oksidatif yang memperparah peradangan dan mengaktivasi sel makrofag alveolar. Komponen fenolik dalam buah kawista bertindak sebagai antioksidan yang meredam radikal bebas.
Tujuan: Mengetahui pengaruh pemberian buah kawista untuk mencegah aktivasi makrofag alveolar tikus Wistar yang dipapar asap rokok.
Metode: Penelitian eksperimental menggunakan post-test only group design dengan rancangan acak lengkap, selama 35 hari. Tikus Wistar jantan sebanyak 25 ekor, dibagi dalam 5 kelompok yaitu kontrol positif (dipapar asap rokok), kontrol negatif (tanpa perlakuan), O1, O2, dan O3 (dipapar asap rokok dan sonde buah kawista dosis O1=0.5, O2= 0.6, dan O3=0.7 g/kg BB). Data dianalisa dengan uji *ANOVA one way* dan uji *LSD.*
Hasil: Buah kawista yang diberikan secara preventif pada pada dosis 0,6 dan 0,7 g/kg BB dapat mencegah aktivasi makrofag alveolar paru tikus yang dipapar asap rokok (p=0,000). Jumlah makrofag alveolar terendah terlihat pada dosis 0,7 g/kg BB.
Kesimpulan: Pemberian buah kawista dapat menghambat peningkatan jumlah makrofag alveolar dan memperbaiki histopatologi paru tikus Wistar yang dipapar asap rokok.

Kata Kunci
Kawista, antioksidan, rokok, makrofag alveolar

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Introduction

Smoking is different from other health challenges. Cigarettes are in demand by many consumers and have become a form of public health habit (Chauhan & Setia, 2016). In 2016, 39,5% of Indonesia's population, who were 15 years of age and older, were current smokers, which is 7,4% more than the global average. Furthermore, the proportion of Indonesian men who smoked tobacco has dramatically increased from 56,2% in 2000 to 76,2% in 2015 (Holipah et al., 2020). Smoking is a risky behavior for cardiovascular disease, cancer, tumors, and chronic obstructive pulmonary disease (COPD) (Kotlyarov, 2023). Cigarette smoke (gas and particle phases) contains oxidizing, carcinogenic, and Reactive Oxygen Species (ROS) components that damage genes, macromolecules, and cell membranes (Sharifi-Rad et al., 2020).

The particles in cigarette smoke have a small range of 0,2 to 0,5 µm (Granda-Orive et al., 2022), which enables them to reach the lung's alveoli (Sharifi-Rad et al., 2020). Small particles that enter the alveoli are impacted by gravity and sedimentation when the speed of airflow in the bronchioles slows, causing these particles to settle in the respiratory tract (Thomas, 2013). Cigarette smoke is an irritant, and when it deposits in the alveoli, it triggers an inflammatory reaction (Strzelak et al., 2018). The causes of inflammation are unclear; however, they may include the direct chemoattractant effects of nicotine as well as ROS in cigarette smoke (Caliri et al., 2021; Kumar et al., 2017).

Alveolar macrophage cells are the offspring of monocyte cells in leukocytes and are responsible for the phagocytosis of foreign objects in the lung alveoli. Because leukocyte products that kill microbes also harm healthy host tissue, leukocyte defense mechanisms (macrophages and neutrophils) can result in tissue damage and prolong inflammation (Caliri et al., 2021; Kumar et al., 2017; Leick et al., 2014). After exposure to microbes, chemokines (cytokine chemoattractants), immune complexes, or phagocytic stimulation, which can exacerbate inflammation, macrophages and neutrophils may release free radicals derived from oxygen into the extracellular space (Leick et al., 2014). The main species produced in cells are superoxide (·O2), hydrogen peroxide (H2O2), and hydroxyl radicals (·OH), which can combine with NO to form other reactive nitrogen intermediates (Strzelak et al., 2018). According to the protease-antiprotease theory, damage to the alveolar walls results from an imbalance of proteases and antiproteases, which is exacerbated by an imbalance of oxidants and antioxidants that heightens inflammation (Kumar et al., 2017; Pandey et al., 2017).

·O2, H2O2, and ·OH are thought to be the ROS groups that contribute the most to the process of disease occurrence. The body has a ROS defense mechanism. The endogenous antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) help reduce the harmful effects of ROS. The enzymes SOD, manganese SOD found in mitochondria, and copper-iron SOD found in the cytosol all prevent the stacking of ·O2. The activities of the enzymes catalase and GPx prevent the release of H2O2. In contrast to GPx, which is found in the cytosol and mitochondria, catalase is primarily found in peroxisomes and, to a lesser extent, in the cytosolic and microsome fractions of cells (Kurutas, 2016; Sharifi-Rad et al., 2020). However, endogenous antioxidants are unable to counteract the increased and prolonged exposure to smoking. As a result, O2 and H2O2 build up and can undergo either the Haber–Weiss reaction or the Fenton reaction to produce ·OH. As it causes oxidative stress and exacerbates inflammation, OH is the most reactive ROS group (Biswas, 2016).

The consumption of antioxidants is necessary when the body's defense mechanisms are unable to reduce excess ROS. Kawista fruit has antioxidant activity and is a natural food ingredient. Flavonoids and tannins are among the phenolic compounds found in kawista fruit (Rustiah & Umriani, 2016; Rustiah & Umriani, 2018; Syakri et al., 2021). The phenolic component serves as an effective buffer against OH and O2, thereby defending membrane lipids from harmful oxidation reactions (Raharja et al., 2016). This study was conducted to determine the effect of kawista fruit on the prevention of oxidative stress, which strengthens inflammation due to exposure to cigarette smoke, by observing lung histopathology, namely the activation of alveolar macrophages.

Methods

Research Design

Laboratory experimental research with a completely randomized design, post-test only group. Male Rattus norvegicus furrow Wistar rats that–2-3 months old and 180-200 g in weight) were used as the research subjects. Rats were fed a standard diet containing

17% protein and fluids, and this method was optional. Two cages were used for each treatment group of rats, which were kept in cages. The cage should have adequate ventilation, normal lighting, comfortable temperature, and low humidity. The Public Health Research Ethics Committee at Airlangga University approved the use of experimental animals in this study after conducting an ethical review; their certificate bears number 358-KEPK.

A total of 25 rats were used, which were divided into 5 groups at random: the positive control (exposed to cigarette smoke), the negative control (no treatment), the K1 (cigarette smoke and kawista 0,5 g/kg BW), the K2 (cigarette smoke and kawista 0,6 g/kg BW), and the K3 (cigarette smoke and kawista 0,7 g/kg BW) groups. Kawista fruits were treated orally in a stomach tube. Sonde was administered once daily in the morning for 35 days before exposure to cigarette smoke for the first week of treatment. Mature kawista fruits were purchased from the Sidoarjo fruit market. The fruit of the kawista is taken, the seeds are removed, and the weight is determined by the dose, which is 0,5, 0,6, or 0,7 g/kg BW of rats, dissolved in 1 ml of distilled water/dose. On the eighth day, up to two clove cigarettes per day (afternoon and evening) smoked in a smoking pump were introduced into the system. The rats were killed by anesthetic injection on the 36th day. To remove the lung organs during anesthesia, rats were intramuscularly injected with ketalar in their thighs. For histopathological examination, the lung organs were fixed in 10% formalin solution.

Determination of Cigarette Smoke Exposure Dosage

In a previous study, male Wistar rats exposed to clove cigarette smoke for 21 days at a rate of two cigarettes per day had more alveolar macrophages than the control group (Herdiani et al., 2018). Based on this research, participants were exposed to clove cigarettes for 28 days at a dose of two cigarettes per day.

Determination of Dosage of Kawista Fruit

It has been shown in earlier studies that the antioxidant activity of the kawista fruit can lower the levels of the cigarette-smoke-exposed male Wistar rats serum malondialdehyde (MDA) (Raharja et al., 2016). In this study, the ideal dose of kawista fruit as an antioxidant was determined to doses of 0,5, 0,6, and 0,7 g/kg BW.

Preparation of Kawista Fruit

Use of ripe kawista fruit (Figure 1). The Kawista fruit flesh was filtered to distinguish the fruit from the seeds. 0.5/0.6/0.7 g/kg BW of kawista fruit flesh is measured out per dose, dissolved in 1 ml of distilled water per dose, and then blended with a stirrer. The treatment was administered via a stomach tube (Raharja et al., 2016).

Hematoxylin Eosin Staining in Lung Tissue

The slides that need to be colored are set up on a unique painting rack. Harris hematoxylin was administered for 15 min, followed by 15 min of washing under running water. The preparation was first submerged for up to 2–5 dips in 1% acid alcohol before being submerged in aqueous ammonia. Counterstaining was performed for 15–20 s. The dehydration process in graded alcohol (alcohol 70%, 80%, 96%, and absolute) for 3 min was as follows. The preparation procedure involved administration of Xylol for five minutes. The prepared slide was allowed to dry at room temperature as the last step in the coloring procedure. HE preparations were viewed under a microscope.

Histopathological measurement of the number of alveolar macrophages

The number of alveolar macrophages was the observed indicator in the lung histology slices. In five different lung alveolar fields, beginning from the left, right, top, bottom, and middle areas of the preparation, observations were made using a microscope at 400X. The number of alveolar macrophages was counted after each field of view.

Data analysis

Statistical analysis was performed using the Shapiro-Wilk test for data normality, Levene's test for data homogeneity, and one-way ANOVA, followed by the LSD test to identify differences between treatment groups. Statistical tests were performed at a 95% confidence level.

Result

According to the results of the statistical tests, the data were normally distributed across all groups and the variance of the homogeneous data was equal to p = 0,276. There was a significant difference in the number of alveolar macrophages,

as shown by one-way ANOVA (p=0,000. Table 1 shows the typical distribution of alveolar macrophages.

Table 1. The mean number of alveolar macrophages

Group	Means	P-value
Negative control	5,55 + 1,02[d]	0,000
Positive control	28,54 + 3,53[a]	
O1	24,38 + 3,34[a]	
O2	13,7 + 1,07[b]	
O3	11.46 + 0,82[c]	

The average number of alveolar macrophages increased because of smoking, as shown in Table 1, where the average number was higher in the positive control group (28,54 + 3,53) than in the negative control group (5,55 + 1,02). Groups P.1 (24,38 + 3,34), P.2 (13,7 + 1,07), and P.3 (11,46 + 0,82), where the average number of macrophages was lower than the control group positive, all showed a decrease in the average number of alveolar macrophages.

The results of the LSD test for the number of alveolar macrophages indicated that there was a difference in the number of macrophages between the positive and negative control groups, with a p-value of 0,000 (p<0,05) for this test. There was a difference in the number of alveolar macrophages between groups P2 and P3 and the positive control group (p=0,000 (p<0,05). There was also a significant difference between the P1, P2, and P3 groups and the negative control group (p=0,000 (p<0,05). The P1 and positive control groups, however, did not differ significantly (p=0,057 (p>0,05).

Figure 2 shows that the microscopic appearance of the lung alveoli was normal in the negative control group. The positive control group contained inflammatory cells that were distinguished by an abundance of neutrophils and macrophages as well as what appeared to be cells undergoing fibrosis and destruction of the alveolar septum. In the P1 treatment group, fibrosis-affected cells and cells that destroyed the alveolar septum were also observed. Inflammation was still present in some cells in the P2 and P3 groups, but was less prevalent than in the positive control and P1 groups. There were fewer macrophages in the P2 and P3 groups than in the positive control group. Some cells in the P2 and P3 groups underwent fibrosis and the alveolar septum was destroyed.

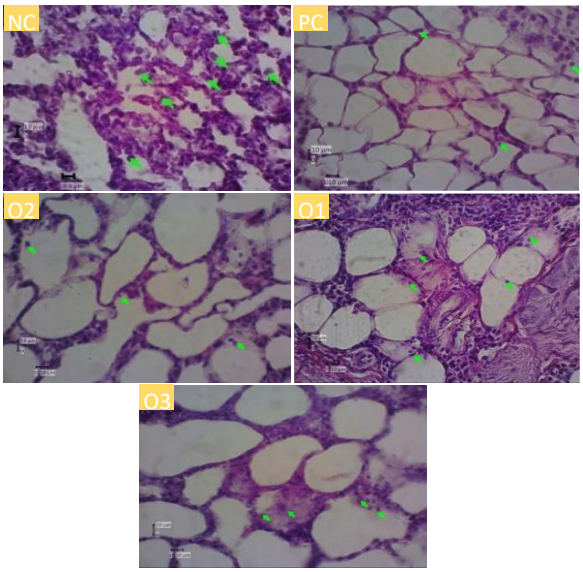

Figure 2. Microscopic observation results of histology of rat lung staining with HE 400X magnification in (NC) negative control, (PC) positive control, (O1) kawista treatment dose of 0,5 g/kg BW, (O2) kawista treatment dose of 0,6 g/kg, (O3) kawista treatment dose of 0,7 g/kg. Alveolar macrophages are visible as green arrows.

Discussion

The results revealed that exposure to cigarette smoke increased the number of macrophages in the alveoli. The mean number of alveolar macrophages in comparison to the negative control group, the positive control group, was significantly higher than that in the negative control group, where there were more alveolar macrophages on average, which has been demonstrated by several earlier studies that cigarette smoke exposure can influence the level of alveolar macrophage activation. Rats exposed to cigarette smoke had noticeably more alveolar macrophages than rats not exposed to cigarette smoke, according to studies by (Okrit et al., 2021), (Virlando Suryadinata et al., 2021), and (Herdiani et al., 2018).

In the group that received Kawista fruit treatment, there were fewer activated macrophages. The mean alveolar macrophage count of the positive control group differed significantly from that of the P2 and P3 groups, whereas there were fewer alveolar macrophages on average in the latter two groups. The average number of activated alveolar macrophages in the P1 group was lower, although

the alveolar macrophage count of the positive control group did not differ significantly from that of the P1 group. This demonstrated that the administration of kawista fruit decreased the activation of alveolar macrophages. The harmful effects of free radicals and inflammation can be lessened by kawista fruit owing to its antioxidant activity (Raharja et al., 2016; Rustiah & Umriani, 2018; Syakri et al., 2021).

Because cigarette smoke particles are very small, measuring between 0,2 and 0,5 µm (Granda-Orive et al., 2022), they can reach the alveoli of the lungs. Small particles that enter the alveoli are affected by gravity and sedimentation as the airflow speed in the bronchioles decreases, causing these particles to settle in the respiratory tract (Thomas, 2013). Alveolar macrophages are activated by discarded particles to perform phagocytosis. Alveolar macrophages are involved in leukocyte defence mechanisms and are derived from blood monocytes. The final and most crucial line of defence against the entry of foreign bodies into the lungs is provided by alveolar macrophages (Hirayama et al., 2018).

Inflammatory reactions are induced by foreign particles in the alveoli. An array of processes that attempt to repair and replace damaged tissue are set off by inflammation, which destroys, saturates, or limits harmful agents (Strzelak et al., 2018). Leukocyte activation and phagocytosis during inflammation also release reactive oxygen species (ROS) into the extracellular space in addition to the phagolysosome. After exposure to microbes, chemokines (cytokine chemoattractants), immune complexes, or phagocytic stimulation, neutrophils and macrophages may release free radicals derived from oxygen into the extra cell. To intensify inflammation, these products have the potential to damage tissues and injure endothelial cells (Kumar et al., 2017; Mittal et al., 2014).

The primary species produced in cells are O2, H2O2, and OH, and these metabolites can combine with NO to form other reactive nitrogen intermediates (Mandal et al., 2022). These extracellular free radicals can strengthen the inflammatory response cascades by increasing the expression of chemokines, cytokines, and endothelial leukocyte adhesive molecules (Mittal et al., 2014).

According to the protease-antiprotease theory, imbalances in oxidants and antioxidants strengthen damage to the alveolar walls, which is caused by proteases and antiproteases. Elastase is a protease released during inflammation by neutrophils and macrophages. Alpha-1 antitrypsin or α1-AT has primary anti-elastase activity in the serum and interstitial tissue. Additionally, neutrophils and activated macrophages secrete ROS, which prevent α1-AT activity. Antiprotease (α1-AT) inactivation causes protease (elastase) activity to become uncontrolled, increasing damage to the alveolar extracellular matrix, and aggravating inflammation (Kumar et al., 2017; Pandey et al., 2017).

Antioxidant defenses in cells shield them from reactive oxygen species (ROS), which can intensify inflammation. SOD, catalase, and GSH are some of the antioxidants. Exogenous antioxidants must be consumed if the endogenous antioxidant enzyme mechanism cannot reduce the impact of free radicals (Biswas, 2016; Kurutas, 2016; Sharifi-Rad et al., 2020). Kawista fruit contains phenolic compounds such as flavonoids and tannins (Raharja et al., 2016; Rustiah & Umriani, 2018; Syakri et al., 2021).

·O2 and ·OH radicals can be effectively stored in phenolic components (Rani et al., 2018). Phenolic compounds contain an aromatic ring and at least one hydroxyl group. For phenolic compounds to function as hydrogen atom donors for free radicals (Platzer et al., 2022; Raharja et al., 2016; Rani et al., 2018), the stability of the oxygen-hydrogen atomic bond is affected by the presence of an aromatic ring. Phenolic substances can reduce inflammation by minimizing the negative effects of free radicals produced by inflamed cells as well as those directly produced by cigarette smoke. The expression of chemokines, cytokines, and endothelial leukocyte adhesive molecules, which cause inflammatory reactions owing to free radicals, can be decreased (Boo, 2019; Hussain et al., 2016). Additionally, elastase activity may be inhibited to lessen harm to the alveolar extracellular matrix, which may otherwise result in the exacerbation of inflammation. Following the findings of this study, rats exposed to cigarette smoke and dragon fruit extract as antioxidants had significantly fewer alveolar macrophages than rats exposed to cigarette smoke alone (Herdiani et al., 2018). The balance between the production and inactivation of these free radicals in cells or tissues determines how ROS affects the inflammatory response (He et al., 2017).

Conclusions

This study demonstrated that kawista fruit administration at doses of 0,6 and 0,7 g/kg BW could prevent alveolar macrophage activation and improve the lung histopathology of rats exposed to cigarette smoke. At a dose of 0,5 g/kg, BW kawista fruit did not have a significant effect on decreasing the number of alveolar macrophage cells, although the number of activated alveolar macrophages was less than that in the positive control group.

This shows that a dose of 0,5 g/kg BW has not been able to provide optimal results for reducing inflammation so the activation of alveolar macrophages was not significantly different from the positive control group. Given that kawista fruit plants are becoming increasingly rare, it is hoped that this fruit can be consumed and re-cultivated because it has been demonstrated to be a functional food with antioxidant bioactivity.

Conflict of Interest

The authors declare that they have no personal interests that may have impacted their work.

Author Contributions

Conceptualization, methodology and original draft preparation, Kristian T. Raharja; Formal analysis, Endang F. Susilawati; Project administration, Ita F. Romadhoni.

References

Biswas, S. K. (2016). Does the Interdependence between Oxidative Stress and Inflammation Explain the Antioxidant Paradox? In *Oxidative Medicine and Cellular Longevity* (Vol. 2016). Hindawi Publishing Corporation. https://doi.org/10.1155/2016/5698931

Boo, Y. C. (2019). Can plant phenolic compounds protect the skin from airborne particulate matter? In *Antioxidants* (Vol. 8, Issue 9). MDPI. https://doi.org/10.3390/antiox8090379

Caliri, A. W., Tommasi, S., & Besaratinia, A. (2021). Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. In *Mutation Research - Reviews in Mutation Research* (Vol. 787). Elsevier B.V. https://doi.org/10.1016/j.mrrev.2021.108365

Chauhan, H., & Setia, P. (2016). Discouraging cigarette smoking through de-marketing strategies. *Future Business Journal*, *2*(1), 31–39. https://doi.org/10.1016/j.fbj.2016.01.002

Granda-Orive, J. I. de, Jiménez-Ruiz, C. A., Unzueta, I. G., Higes-Martínez, E. de, Cabrera-César, E., Sandoval-Contreras, R., & Rábade-Castedo, C. (2022). Effects on health of passive smoking and vape on terraces in the covid-19 pandemic: A Review. In *Open Respiratory Archives* (Vol. 4, Issue 4). Elsevier Espana S.L.U. https://doi.org/10.1016/j.opresp.2022.100204

He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. In *Cellular Physiology and Biochemistry* (Vol. 44, Issue 2, pp. 532–553). S. Karger AG. https://doi.org/10.1159/000485089

Herdiani, N., Budi, E., & Putri, P. (2018). *Prefix-RP seminar nasional hasil riset efek antioksidan ektrak buah naga merah (hylocereus polyrhizus) terhadap makrofag alveolar tikus yang dipapar asap rokok*.

Hirayama, D., Iida, T., & Nakase, H. (2018). The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis. In *International Journal of Molecular Sciences* (Vol. 19, Issue 1). MDPI AG. https://doi.org/10.3390/ijms19010092

Holipah, H., Sulistomo, H. W., & Maharani, A. (2020). Tobacco smoking and risk of all-cause mortality in Indonesia. *PLoS ONE*, *15*(12 December). https://doi.org/10.1371/journal.pone.0242558

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C. B., & Rahu, N. (2016). Oxidative stress and inflammation: what polyphenols can do for us? in *oxidative medicine and cellular longevity* (Vol. 2016). Hindawi Limited. https://doi.org/10.1155/2016/7432797

Kotlyarov, S. (2023). The role of smoking in the mechanisms of development of chronic obstructive pulmonary disease and atherosclerosis. In *International journal of molecular sciences* (Vol. 24, Issue 10). NLM

(Medline). https://doi.org/10.3390/ijms24108725

Kumar, V., Abbas, A., & Aster, J. C. (2017). *Robbins basic pathology* (10th Edition). Elsevier.

Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. In *Nutrition Journal* (Vol. 15, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12937-016-0186-5

Leick, M., Azcutia, V., Newton, G., & Luscinskas, F. W. (2014). Leukocyte recruitment in inflammation: Basic concepts and new mechanistic insights based on new models and microscopic imaging technologies. In *Cell and Tissue Research* (Vol. 355, Issue 3, pp. 647–656). Springer Verlag. https://doi.org/10.1007/s00441-014-1809-9

Mandal, M., Sarkar, M., Khan, A., Biswas, M., Masi, A., Rakwal, R., Agrawal, G. K., Srivastava, A., & Sarkar, A. (2022). Reactive oxygen species (ros) and reactive nitrogen species (rns) in plants– maintenance of structural individuality and functional blend. *Advances in Redox Research*, *5*, 100039. https://doi.org/10.1016/j.arres.2022.100039

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. In *Antioxidants and Redox Signaling* (Vol. 20, Issue 7, pp. 1126–1167). https://doi.org/10.1089/ars.2012.5149

Okrit, F., Chantranuwatana, P., Werawatganon, D., Chayanupatkul, M., & Sanguanrungsirikul, S. (2021). Changes of vitamin D receptors (VDR) and MAPK activation in cytoplasmic and nuclear fractions following exposure to cigarette smoke with or without filter in rats. *Heliyon*, *7*(1). https://doi.org/10.1016/j.heliyon.2021.e05927

Pandey, K. C., De, S., & Mishra, P. K. (2017). Role of proteases in chronic obstructive pulmonary disease. In *Frontiers in Pharmacology* (Vol. 8, Issue AUG). Frontiers Media S.A. https://doi.org/10.3389/fphar.2017.00512

Platzer, M., Kiese, S., Tybussek, T., Herfellner, T., Schneider, F., Schweiggert-Weisz, U., & Eisner, P. (2022). Radical scavenging mechanisms of phenolic compounds: A quantitative structure-property relationship (QSPR) Study. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.882458

Raharja, K. T., Wirjatmadi, B., & Adriani, M. (2016). Pemberian buah kawista menghambat peningkatan kadar malondialdehid serum tikus wistar yang dipapar asap rokok. *Jurnal Kedokteran Brawijaya*, 196–201. https://doi.org/10.21776/ub.jkb.2017.029.03.2

Rani, R., Arora, S., Kaur, J., & Manhas, R. K. (2018). Phenolic compounds as antioxidants and chemopreventive drugs from Streptomyces cellulosae strain TES17 isolated from rhizosphere of Camellia sinensis. *BMC Complementary and Alternative Medicine*, *18*(1). https://doi.org/10.1186/s12906-018-2154-4

Rustiah, W., & Umriani, N. (2018). Uji aktivitas antioksidan dari ekstrak buah kawista (limonia acidissima) menggunakan spektrofotometer uv-vis antioxidant activity test from fruit extract kawista (limonia acidissima) using uv-vis spectrofotometer. In *J. Chem. Res* (Vol. 6, Issue 1).

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., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., … Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. In *Frontiers in Physiology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fphys.2020.00694

Strzelak, A., Ratajczak, A., Adamiec, A., & Feleszko, W. (2018). Tobacco smoke induces and alters immune responses in the lung triggering inflammation, allergy, asthma and other lung diseases: A mechanistic review. In *International Journal of Environmental Research and Public Health* (Vol. 15, Issue 5). MDPI AG. https://doi.org/10.3390/ijerph15051033

Syakri, S., Syahrana, N. A., Ismail, A., Tahir, K. A., & Masri, A. (2021). A review: Testing antioxidant activity on kawista plants (limonia acidissima l.) in indonesia. In *Open Access Macedonian Journal of Medical Sciences* (Vol. 9, Issue F, pp. 281–287). Scientific Foundation SPIROSKI. https://doi.org/10.3889/oamjms.2021.6497

Thomas, R. J. (2013). Particle size and pathogenicity in the respiratory tract. In *Virulence* (Vol. 4, Issue 8). https://doi.org/10.4161/viru.27172

Virlando Suryadinata, R., Prawitasari, D. S., Rochim, I. P., Rungkut, J. R., Rungkut, K., Surabaya, J., & Timur, I. (2021). Efikasi flavonoid pada mulberi merah terhadap penurunan radikal bebas dan alveolar makrofag akibat paparan asap rokok pada tikus wistar. https://doi.org/10.22435/mgmi.v12i2.4021; Copyright