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A combination of eucalyptus, peppermint, and lavender essential oil ameliorates serum lipid peroxidation and endogenous antioxidant enzymes in allergic asthma rat model

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Abstract

Asthma is a chronic inflammatory disease and is caused by several allergens. In asthma, Reactive Oxygen Species (ROS) are produced endogenously by metabolic reactions and exogenously by environmental factors, which can lead to a decrease in endogenous antioxidant activity. The purpose of this study was to determine the effect of treatment herbal essential oils with various compositions of eucalyptus, lavender, and peppermint on serum lipid peroxidation and endogenous antioxidant enzymes in ovalbumin-induced rats. The sample in this study was 24 white rats (Rattus novergicus L.) which were divided into six groups, namely the negative control group, the positive control group, three groups of varying doses of herbal essential oil, and the standard budesonide drug group. Serum lipid peroxidation markers that was determined in this research were malondialdehyde (MDA) and nitrite oxide (NO), while the endogenous antioxidant enzyme that was observed was glutathione (GSH) and superoxide dismutase (SOD). The results of the statistical analysis of the One Way Anova test showed that there was a significant difference in the levels of SOD ($p = 0.000$ (<0.05)) and NO ($p = 0.043$ (<0.05)), but there was no significant difference in the levels of GSH ($p = 0.377$ (>0.05)) and MDA ($p = 0.074$ (>0.05)) in ovalbumin-induced rats. The conclusion of this study was that there was a significant effect of herbal essential oils on SOD and NO levels, but there was no significant effect on GSH and MDA levels in ovalbumin-induced rats.

Keywords: *Herbal essential oil, SOD, NO, GSH, MDA.*

1. Introduction

Asthma is a non-communicable disease characterized by shortness of breath, wheezing, tightness in the chest area, and coughing. Symptoms usually occur at night or when a person is exposed to allergens such as cigarette smoke, dust, and so on. Exposure to allergens can also be caused by exposure to ovalbumin. Ovalbumin is a protein in egg whites and has a high level of allergenicity. Ovalbumin is often used to stimulate allergic reactions (Sharif, 2022). According to Liu K (2022), there were as many as 30% of people experience stress when they contract asthma, and this results in an imbalance of antioxidants and free radicals. According to Almohawes & Alruhaimi, (2020) several studies that shortness of breath or asthma is one of the determining factors of oxidative stress. Oxidative stress can occur due to interference between oxidants and antioxidants or conditions where the production of free radicals exceeds the amount of antioxidants in the body, which in turn has the potential to cause cell damage (Liu K, 2022). This situation can be overcome with antioxidants. Antioxidants are substances that, in low concentrations, can delay or prevent the occurrence of oxidative stress (Tan, 2018).

Herbal Essential oil includes antioxidants that can prevent the occurrence of oxidative stress and can prevent the negative effects of free radicals on the body (Mar, 2020). Herbal essential oil, known as essential oil, is a product of natural extracts from plant species. Eucalyptus essential oil has the potential to protect the human body against damage and disease caused by free radicals that trigger oxidative stress. The 1,8-cineol compound in eucalyptus leaf essential oil has empirical properties: overcoming respiratory disorders such as asthma and being an antioxidant agent (Nakamura, 2020). Peppermint essential oil contains menthol, which can relieve the respiratory system or bronchitis, sinusitis, and asthma (Kim, 2021). While lavender essential oil has ability as an antioxidant and anti-asthma in ovalbumin-induced guinea pigs (Almohawes & Alruhaimi, 2020). Based on previous research, lavender essential oil supplementation has the potential to improve growth performance, intestinal microbiota balance, intestinal morphology, and antioxidant activity in broiler chickens (Barbarestani, 2020). Peppermint, parsley, and their mixture oils also have strong radical scavenging activity and high antioxidant activity; at 0.5 ml of peppermint oil, has a significant increase in the activity of SOD and GSH and decreasing in MDA. Therefore, the results of this study show that essential oils led to a protective effect against hepatotoxicity induced by cyclophosphamide (Sheweita, 2016).

Referring to the several studies on the effectiveness of giving lavender, peppermint, and eucalyptus above, no research has yet combined the three essential oils to determine the lipid peroxidation profiles and endogenous enzyme activity. Therefore this study was conducted to analyze the effectiveness of inhalation of a combination of lavender, peppermint, and eucalyptus essential oils on lipid peroxidation profiles (MDA, NO) and endogenous enzyme activity (GSH, SOD) in ovalbumin-induced rats.

2. Materials and methods

2.1 Materials

The materials used in this study were 1% ovalbumin, 0.9% NaCl, PBS rat serum, 20% TCA, 0.067% TBA, Bioassay EnzyChromTM Super Oxide Dismutase Assay Kit (ESOD-100), sodium nitrite powder, 1% sulfanilic acid, glacial acetic acid, 0.1% NED solution, 0.1M phosphate buffer pH 7.0, DNTB (Dithiobis 2-Nitrobenzoic acid), $Al(OH)_3$, ether, standard drug budesonide, essential oil of Eucalyptus, Lavender and Peppermint, husks (as a substitute for

soil), mouse feed and cotton. Ovalbumin 1% was prepared by dissolving 0.1 mg ovalbumin added 0.1 mg aluminum hydroxide, dissolved in 0.5 ml saline. The combination of herbal essential oils is prepared with product formulations from plant species derived from eucalyptus, lavender, and peppermint with a ratio of 1:2:2 (E1), 2:2:1 (M1), and 2:1:2 (L1), respectively. Budesonide drug from the stock is taken as much as 1 ml induced in standard group rats by inhalation using nebulizers (Han, 2022).

2.2 Data collection procedures

This research has received ethical approval from Nahdlatul Ulama University Surabaya with number 052/EC/KEPK/UNUSA/2022. Experimental animals are prepared by preparing a place for keeping experimental animals, such as cages, husks, places to eat, drinking places, and feeding and drinking places for experimental animals. A total of 24 white rats weighing 200 grams were adapted for seven days in the UNUSA animal laboratory. The adaptation process aims to prevent stress in the experimental animals and to equate their diet and lifestyle with the new environment so that the experimental animals will have the same conditions and are expected to provide valid results on the assay. Then, all mice were randomly divided into six groups. Each group contains four white rats with different treatments in each group: The positive control group is the ovalbumin-induced group (asthma model). Injections are carried out every 2 days for 1 week, the negative control group is without treatment, treatment group 1 is the group that was induced by ovalbumin and treated with E1, treatment group 2 is the group that was induced by ovalbumin and was treated with L1, treatment group 3 is the group that was induced by ovalbumin and treated with M1. Induction of E1, L1, and M1 is carried out on the 14^{th} to the 28^{th} day with herbal essential oil induction intervals every 2 days for 3 hours by inhalation. The standard group was the ovalbumin-induced group and was treated with budesonide for 30 minutes/2 days on days 14 to 28.

The rats that had been treated were euthanized with ether and were dissected; the blood was taken from the heart using a 2-3cc syringe and then accommodated in an EDTA vacutainer tube. Malondialdehyde levels were measured using the thiobarbituric acid (TBA) method, which will react with the MDA carbonyl group to form a red complex compound. In the nitric oxide (NO) measurement method using the stress reaction, nitric is added with sulfanilamide reagent in an acidic medium to form a temporary diazonium salt, which is then reacted with N-naphthyl ethylenediamine (NED) to form azo compound In. The measurement of nitric oxide levels uses a maximum wavelength of 540 nm, and nitrile analysis with a high level of sensitivity produces a purple color (Goshi, 2019). Measurement of SOD levels was based on the EnzyChromTMSuperOxide Dismutase Assay Kit (ESOD-100) procedure at a wavelength of 450 nm. GSH was then measured in the clear supernatant by colorimetry using DTNB, i.e., 5,5'-dithiol-bis (2-nitrobenzoic acid), in an aqueous solution at pH 7.8.

2.3 Data analysis

The analysis of the data obtained was processed with the help of the Statistical Product and Service Solution (SPSS) computer program.The Parametric T Dependent Test consists of 2 paired groups with interval and ratio data scales, with a P value = 0.05.

3. Results

3.1 Respiration Rate (Rr)

The results of respiratory rate measurements are based on breaths in rats (Rattus novergicus), which have been induced by Herbal Essential Oil (HBO) with a count per minute.

Then, the value is obtained for the average respiratory rate, which will be presented in Table 1 below.

Table 1. Result of *Respiration Rate* after treatment of HBO

Groups	Mean ± SD (/minute)*	P value
Negative Control	109,5 ± 4,20	0.029 (P<0.05) There is significant difference
Positive Control	120,75 ± 6,84	
E1	112 ± 1,70	
M1	110 ± 4,54	
L1	109 ± 8,28	
Budesonide	113 ± 2,06	

*Normal range Rr = 75-115/minute

Treatment with ovalbumin induction for seven consecutive days resulted in an asthmatic condition with an average RR value in the positive control group of 120.75 ± 6.84/minute while in the negative control group it was 109.5 ± 4.20. The HBO E1 formulation can reduce the RR value after being treated every two days for 3 hours with an RR value of 112 ± 1.70/minute. The treatment of the HBO M1 formulation was given to rats with asthma, resulting in an average RR of 110 ± 4.54/minute. In the treatment group with the HBO L1 formulation treatment, the RR value was 109 ± 8.28/minute. As a comparison, seen from the standard budesonide drug group, the average Rr was 113 ± 2.06/minute. From each treatment group after HBO induction, resulted that the P value of respiration rate was 0.029 (P<0.05), so it can be stated that treatment of HBO E1, M1, and L1 can reduce asthma conditions significantly to normal values in the range of 70-115/min with an average of almost the same as standard drugs budesonide.

3.2 Malondialdehyde (MDA)

Following are the results of making a standard curve using 1,1,3,3-tetramethoxypropane (TMP) for measuring malondialdehyde levels. The average MDA value in each treatment group MDA levels are calculated using the TMP standard curve equation y = 0.0111x + 0.0019 with the following results in Table 2 are obtained.

Table 2. Result of MDA levels

Groups	Mean ± Sd of MDA levels (µM)	P value
Negative Control	1,99 ± 0.422	0.074 (P>0.05) There is no significant difference
Positive Control	3,161 ± 0.714	
E1	2,328 ± 0.996	
M1	2,643 ± 0.168	
L1	2,486 ± 0.364	
Budesonide	2,778 ± 1.889	

3.3 Nitrite Oxide (NO)

Following are the results of nitrite oxide levels between treatment groups measured using a UV-Vis spectrophotometer with the principle of nitrite diazotization with sulfanilic

acid in an acidic medium to form temporary azo compounds and with the addition of N-naphthyl-ethylenediamine (NED) which can form stable azo compounds, as shown in Table 3.

Table 3. Result of NO levels

Groups	Mean ± SD	P value
Negative control	1,765 ± 7,33	
Positive control	1,835 ± 1,29	
E1	1,6625 ± 0,50	0.043 (P<0.05)
M1	1,665 ± 0,57	There is significant difference
L1	1,6675 ± 0,50	
Standard drug	1,73 ± 9,12	

3.4 Glutathione (GSH)

Measurement of glutathione levels in rat plasma was carried out by using the DTNB (Dithiobis 2-Nitrobenzoic acid) with the principle based on the reaction between dithiobis 2-nitrobenzoic acid and glutathione, which produces a stable yellow dianioin thionitro benzoate compound.

Table 4. Result of GSH Levels

Groups	Mean ± SD	P value
Negative control	35,151 ± 9,231	
Positive control	50,695 ± 17,236	
E1	43,738 ± 2,196	0.377 (P>0.05)
M1	42,651 ± 19,898	There is no significant difference
L1	41,390 ± 9,953	
Standard drug	46,431 ± 2,926	

3.5 Super Oxide Dismuthase (SOD)

Measurement of SOD levels was carried out based on the procedure EnzyChromTM Superoxide Dismutase Assay Kit (ESOD-100) at a wavelength of 450nm. The following are the results of measuring SOD levels in the control and treatment groups.

Table 5. Result of SOD Levels

Groups	Mean ± SD	P value
Negative control	2,2850 ± 0,1927	
Positive control	1,2975 ±0,1941	
E1	2,9100 ± 0,2575	0.000 (P<0.05)
M1	2,3325 ± 0,1470	There is significant difference

L1	2,4025 ± 0,1376
Standard drug	2,5250 ± 0,1377

4. Discussion

Based on the results in Table 1 are in line with Sharif's research (2022), where ovalbumin is an ingredient used as an immune response sensitizer to activate B cells to become plasma B cells, thereby producing IgE. The IgE produced will bind to mast cells and cause degranulation of mast cells, resulting in the production of inflammatory mediators such as histamine, cytokines, prostaglandins, and leukotrienes. The products of these inflammatory mediators will cause inflammation, resulting in damage to the respiratory tract. Sensitization with ovalbumin, either by inhalation, orally, or intraperitoneally, has been shown to alter the immune response, causing allergic reactions in experimental animals. Respiratory rate is the number of breaths taken in a respiratory cycle which is calculated within 1 minute or 60 seconds (Ristanto & Zakaria, 2018). The respiratory rate in rats is normal, at 70-115 breaths per minute (Flintoff, 2011). The decrease in respiration rate in the HBO treatment group could be due to the content in eucalyptus, which has a compound composition of 1,8 cineol, which has long been used to treat infections and respiratory disorders, where this compound has a mucotic effect that can thin phlegm and a bronchodilating effect that can relieve breathing. This is in line with the research by Agustina and Suharmianti (2017), which stated that eucalyptus can be used as an alternative to prevent acute respiratory infections.

From Table 2 the positive control group showed an average MDA level higher than the negative control and treatment groups. The increase in MDA levels was due to high levels of free radicals, which were caused by exposure to ovalbumin induction. Ovalbumin exposure can trigger the production of inflammatory mediators in the form of histamine, leukotrienes, prostaglandins, and proinflammatory cytokines, which cause an increase in ROS beyond normal limits and trigger lipid peroxidation. Lipid peroxidation produces free radicals which interact with PUFAs with the end result of MDA. The significance value for the mean MDA level in each treatment group was 0.074 ($p>0.05$), which means that there was no significant effect between the administration of herbal essential oil on the MDA levels of ovalbumin-induced white rats.

Based on the Table 3, it is known that NO levels in the positive control group were higher than in the other groups. This is in line with Wardhani's research (2018), which stated that ovalbumin can bind NO levels, causing shortness of breath in white rats, and administering ovalbumin can increase IgE and inflammation. The significantly lower NO levels in the treatment and standard drug groups could be due to the antioxidant activity which acts as a substance that can counteract the harmful effects of free radicals from oxidative metabolism (Hafiz, 2016). This happens because the content in HBO is a compound of antioxidant compounds such as flavonoids, phenolic compounds, and essential oils (Silvany, 2016). 1,8-cineola hydroxycinnamic acid, rossmarinic acid, and beta-pinama act as antioxidants (Sitepu, 2021). In addition, the menthol aromatherapy contained in mint leaves has anti-inflammatory properties and opens the respiratory tract, which can be affected by the menthol content (Aprilliawati, 2017). Antioxidants have an activity to stop or break free radical chain reactions by providing electrons, making free radicals non-radical, or creating new oxidants, which tend

to be more stable (Hafiz, 2016). One of the compounds contained is 1,8-cineole, which can suppress the production of intracellular reactive oxygen species (ROS) and increase the expression of antioxidant enzymes (Sudradjat, 2020).

Based on the average value of GSH levels in mice, although no significant difference statistically however, it can be seen from Table 4 that there were still differences in levels in the groups of healthy, sick, and mice given medicine or herbal essential oils. This shows that the administration of herbal essential oils affected GSH levels, but the increase was not significant. In the negative control group, GSH levels tend to increase; this indicates that the body is in good health and does not need antioxidants to ward off free radicals, so GSH levels in the body are in a balanced state compared to the positive control group where GSH levels tend to decrease because the body is being exposed to free radicals so that endogenous antioxidants such as GSH work in counteracting free radicals in the body. Whereas, in the E1, M1, and L1 treatment groups, the average GSH level was increased compared to the positive control group. This indicates that herbal essential oils, with the compounds they contain, are beneficial as antioxidants in counteracting free radicals. In line with Seif's study (2014), the normal group of mice had increased GSH levels compared to the group of mice that were given hepatoxic-induced alcohol exposure or the diseased group. Research by Sultan, et al (2015) also stated that GSH levels in the negative control tended to increase compared to the positive control group which tended to decrease. The mechanism for increasing glutathione levels in the E1, M1, and L1 dose variation groups was due to the presence of compounds contained in eucalyptus, peppermint, and lavender, which function as exogenous antioxidants.

Eucalyptus has several compounds contained in it; the largest content is 1.8 cineol, of which 1.8 cineol is found in eucalyptus, peppermint, and lavender. 1,8-Cineol is a monoterpene compound that has anti-inflammatory and antioxidant properties. This is in line with research conducted by Zao (2021), which stated that 1,8-Cineol is used as a non-steroidal anti-inflammatory agent that can potentially suppress inflammation in the airways. In addition to the content of 1.8 cineol, eucalyptus, peppermint, and lavender also contain flavonoids. The mechanism of flavonoids as antioxidants, according to research by Wijaya & Toyib (2018), is divided into two, namely, directly and indirectly. The mechanism of flavonoids as antioxidants is directly donating one of the hydrogen ions from the hydroxyl group so that they are able to capture free radicals and stop the formation of free radicals. Meanwhile, the indirect mechanism of flavonoids is through a mechanism in increasing gene expression by activating the nuclear factor erythroid two related factor 2 (Nrf2), and then there is an increase in genes that function as the synthesis of endogenous antioxidant enzymes (Wijaya & Toyib, 2018). This is in line with Anggia's research (2015), which stated that compounds that act as antioxidants are flavonoids, where the mechanism of action of flavonoids as antioxidants indirectly is by increasing the expression of endogenous antioxidant genes in the manufacture of soursop leaf extract using the maceration method. Herbal essential oils with varying doses can relieve asthma using the compounds contained in them, which are respiratory relievers and antioxidants, while Budesonide is a corticosteroid class of drugs; corticosteroids function as anti-inflammatories in the respiratory tract, thereby preventing the recurrence of asthma, thereby reducing symptoms of asthma attacks (Machado et al., 2020).

Ovalbumin is the main protein in egg white, which can cause a type 1 allergic reaction. It can immediately affect an inflammatory reaction or inflammation, one of which is shortness

of breath/asthma. These conditions can cause an increase in Reactive Oxygen Species (ROS). Superoxide radicals will increase and cause a decrease in antioxidant defense systems such as SOD (Almohawes & Alruhaimi, 2020). Superoxide Dismutase (SOD) acts as the first defense against ROS by catalyzing superoxide radicals (O2-) into H2O2 and O2, then H2O2 will be converted into water molecules (H2O) by the enzymes catalase and peroxidase (Wang et al., 2018). Influence of giving herbal essential oil on SOD levels in ovalbumin-induced white rats (Rattus novergicus) showed that the average SOD level in the negative control group (K-) increased. Namely, an average SOD level of 2.28 U/ml was obtained due to the negative control group without any special treatment. The positive control group (K+), which was induced by ovalbumin, experienced a decrease in SOD levels of 1.28 U/ml. Meanwhile, the E1 treatment group experienced an increase in SOD levels of 2.91 U/ml. SOD levels also increased in the M1 and L1 groups. The M1 group showed an average SOD level of 2.33 U/ml, while the L1 group showed an average SOD level of 2.40 U/ml. In the Standard group, the average SOD level was 2.52 U/ml. Based on these results, it can be seen that the formulation of herbal essential oil E1 showed the highest average SOD level; this indicated that the E1 group was the most effective dose for increasing SOD levels.

Based on the statistical test in Table 5.6, a p-value of 0.000 <0.05 is obtained, which means that H1 is accepted and H0 is rejected. From these results it can be interpreted that there is a significant effect between herbal administration essential oil on Superoxide Dismutase (SOD) levels in the blood of ovalbumin-induced white rats (Rattus novergicus). This is supported by research conducted by Almohawes & Alruhaimi (2020) that there was a significant effect of giving lavender extract on SOD levels in ovalbumin-induced Guinea pigs. The content of flavonoid compounds such as luteolin and phenolic compounds in lavender extract provides antioxidant activity that can reduce IgE levels in ovalbumin-induced guinea pigs. Based on research conducted by Akinrinde & Adebiyi (2019), eucalyptus has an effect on SOD levels in aflatoxin B1-induced white rats with a p-value <0.05. The content of the compound eucalytol (1,8-cineole) in eucalyptus has anti-inflammatory ability in rats conditioned by gastrointestinal injury. Meanwhile, in a study conducted by Khalil et al. (2015), peppermint extract also had an effect on SOD levels in rats induced by carbon tetrachloride (CCl4). The content of menthol compounds (35.9%) and potent antioxidant activity in peppermint extract gave a significant increase in SOD levels in mice induced by CCl4. Herbal Essential Oil, made from eucalyptus, lavender, and peppermint, contains antioxidants that can increase SOD levels. Based on research by Alvarez, (2021) eucalyptus leaves contains flavonoid compounds which act as a source of antioxidants that can counteract oxidative stress and damage caused by free radicals. The content of flavonoid compounds in eucalyptus is also owned by lavender and peppermint, which function as antioxidants to inhibit free radicals (Zheng, 2022).

5. Conclusion

This study found that in ovalbumin-induced rats, the treatment of a combination of herbal essential oils, eucalyptus, lavender, and peppermint, had a substantial influence on SOD and NO levels because of its bioactive compound but no significant effect on GSH and MDA levels on allergic asthma models.

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Conflict of interest

All authors certify in this publication no competing personal or financial interests that could influence the studies reported in this paper

References

Almohawes, Z. N. and Alruhaimi, H. S. (2020) 'Effect of lavandula dentata extract on ovalbumin-induced asthma in male Guinea pigs', Brazilian Journal of Biology, 80(1), pp. 87–96. doi: 10.1590/1519-6984.191485.

Akinrinde AS, Adebiyi OE. Neuroprotection by luteolin and gallic acid against cobalt chloride-induced behavioural, morphological and neurochemical alterations in Wistar rats. Neurotoxicology. 2019 Sep;74:252-263. doi: 10.1016/j.neuro.2019.07.005. Epub 2019 Jul 27. PMID: 31362009.

Álvarez, X.; Cancela, Á.; Merchán, Y.; Sánchez, Á. Anthocyanins, Phenolic Compounds, and Antioxidants from Extractions of Six *Eucalyptus* Species. *Appl. Sci.* **2021**, *11*, 9818. https://doi.org/10.3390/app11219818

Barbarestani, S.Y., Jazi, V., Mohebodini, H., Ashayerizadeh A., Shabani A., Toghyani M., Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens, Livestock Science, Volume 233, 2020, ISSN 1871-1413, https://doi.org/10.1016/j.livsci.2020.103958.

Goshi E, Zhou G, He Q. Nitric oxide detection methods *in vitro* and *in vivo*. Med Gas Res. 2019 Oct-Dec;9(4):192-207. doi: 10.4103/2045-9912.273957. PMID: 31898604; PMCID: PMC7802420.

Han, J.M, Kim M.H., Choi, Y., Kim, G., Yang, W.M. Exploring the Potential Effects and Mechanisms of *Asarum sieboldii* Radix Essential Oil for Treatment of Asthma. Pharmaceutics. 2022 Mar 3;14(3):558. doi: 10.3390/pharmaceutics14030558.

Khalil, A. F., Elkatry, H. O., & El, H. F. (2015). Protective effect of peppermint and parsley leaves oils against hepatotoxicity on experimental rats. Annals of Agricultural Sciences, 60 (2), 353–359. https://doi.org/10.1016/j.aoas.2015.11.004

Kim MH, Park SJ, Yang WM. Inhalation of Essential Oil from Mentha piperita Ameliorates PM10-Exposed Asthma by Targeting IL-6/JAK2/STAT3 Pathway Based on a Network Pharmacological Analysis. Pharmaceuticals (Basel). 2020 Dec 22;14(1):2. doi: 10.3390/ph14010002. PMID: 33374928; PMCID: PMC7821947.

Machado L. A, Oliveira WN, Moreira-Oliveira SS, Pereira DT, Alencar ÉN, Tsapis N, Egito EST. Use of Natural Products in Asthma Treatment. Evid Based Complement Alternat Med. 2020 Feb 13;2020:1021258. doi: 10.1155/2020/1021258. PMID: 32104188; PMCID: PMC7040422.

Mar, P.D, El Khalfi, B., Soukri, A. Protective effect of oregano and sage essentials oils against the effect of extracellular H2O2 and SNP in Tetrahymena thermophila and Tetrahymena pyriformis, Journal of King Saud University - Science, Volume 32, Issue 1, 2020, Pages 279-287, ISSN 1018-3647, https://doi.org/10.1016/j.jksus.2018.05.005.

Nakamura T, Yoshida N, Yamanoi Y, Honryo A, Tomita H, Kuwabara H, Kojima Y. Eucalyptus oil reduces allergic reactions and suppresses mast cell degranulation by

downregulating IgE-FcεRI signalling. Sci Rep. 2020 Dec 1;10(1):20940. doi: 10.1038/s41598-020-77039-5. PMID: 33262354; PMCID: PMC7708995.

Sheweita SA, El-Hosseiny LS, Nashashibi MA. Protective Effects of Essential Oils as Natural Antioxidants against Hepatotoxicity Induced by Cyclophosphamide in Mice. PLoS One. 2016 Nov 1;11(11):e0165667. doi: 10.1371/journal.pone.0165667. PMID: 27802299; PMCID: PMC5089748.

Sudradjat, S. E. (2020). Minyak Kayu Putih, Obat Alami dengan Banyak Khasiat: Tinjauan Sistematik. jurnal kedokteran mediatek, 26(2), 51-59. doi:10.36452/jkdoktmeditek.v26i2.1843

Liu K, Hua S, Song L. PM2.5 Exposure and Asthma Development: The Key Role of Oxidative Stress. Oxid Med Cell Longev. 2022 Apr 4;2022:3618806. doi: 10.1155/2022/3618806. PMID: 35419163; PMCID: PMC9001082.

Sharif M, Anjum I, Shabbir A, Khurram Syed S, Mobeen I, Hassaan Shahid M, Sarwar K. Amelioration of Ovalbumin-Induced Allergic Asthma by *Juglans regia* via Downregulation of Inflammatory Cytokines and Upregulation of Aquaporin-1 and Aquaporin-5 in Mice. J Trop Med. 2022 Mar 30;2022:6530095. doi: 10.1155/2022/6530095. PMID: 35401757; PMCID: PMC8986429.

Sultan, M.T., Butt, M.S., Karim, R. *et al.* Nigella sativa fixed and essential oil modulates glutathione redox enzymes in potassium bromate induced oxidative stress. *BMC Complement Altern Med* **15**, 330 (2015). https://doi.org/10.1186/s12906-015-0853-7

Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol. 2018 Oct 16;9:1162. doi: 10.3389/fphar.2018.01162. PMID: 30405405; PMCID: PMC6204759.

Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol. 2018 Jun 4;217(6):1915-1928. doi: 10.1083/jcb.201708007. Epub 2018 Apr 18. PMID: 29669742; PMCID: PMC5987716.

Zheng, Y.Z., Geng Deng, Yu-Cang Zhang. Multiple free radical scavenging reactions of flavonoids. Dyes and Pigments, Volume 198, 2022, 109877, ISSN 0143-7208, https://doi.org/10.1016/j.dyepig.2021.109877.

Zhao C, Cao Y, Zhang Z, Nie D, Li Y. Cinnamon and Eucalyptus Oils Suppress the Inflammation Induced by Lipopolysaccharide In Vivo. Molecules. 2021 Dec 6;26(23):7410. doi: 10.3390/molecules26237410. PMID: 34885991; PMCID: PMC8659246.