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EFFECT OF ETHANOL EXTRACT OF KATUK LEAVES (*Sauropus androgynus*) ON MALONDIALDEHYDE LEVELS AS AN ANTIOXIDANT

(PENGARUH EKSTRAK ETANOL DAUN KATUK SEBAGAI ANTIOKSIDAN DALAM MENGURANGI MALONDIALDEHIDA MENGGUNAKAN METODE TBARS)

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ABSTRACT

The tooth movement that occurs when mechanical forces are applied to an orthodontic appliance can cause irritation and inflammation. This inflammation can contribute to increased oxidative stress in the oral cavity. The formation of oxidative stress can turn into a chain reaction that can continue to form new free radicals. Katuk leaves (*Sauropus androgynus L. Merr*) have antioxidant content that can slow down the process of free radicals**.** The purpose of this study was to determine the effect of the ethanol extract of katuk leaves as an antioxidant on reducing blood plasma MDA levels. The research method is a laboratory experiment using four treatment groups: blood plasma as a negative control, plasma oxidized with $CuCl_2$ as a positive control, blood plasma given $CuCl_{2,\text{ and}}$ ethanol extract of katuk leaves and plasma given $CuCl_2$, and vitamin C as an antioxidant control. MDA levels were examined

using the thiobarbituric acid reactive substance (TBARs) method. The data were analyzed by Anova and Tukey ($p<0.05$). The results showed that there was an effect of the ethanol extract of katuk leaves on malondialdehyde levels ($p = 0.035$) with MDA values of $CuCl_2$ plasma (0.005 mg/dL), plasma with katuk (0.003 mg/dL), and plasma with vitamin C (0.021 mg/dL). It can be concluded that the use of ethanol extract from katuk leaves has antioxidant potential to decrease MDA levels in blood plasma.

Keywords: Antioxidant, Katuk Leaf, Malondialdehyde, TBARs Method

ABSTRAK

Pergerakan gigi yang terjadi saat ada penerapan gaya mekanik dalam alat ortodonti dapat menimbulkan iritasi dan peradangan. Peradangan ini dapat berkontribusi pada peningkatan stres oksidatif di dalam rongga mulut. Terbentuknya stress oksidatif dapat berubah menjadi sebuah reaksi berantai yang dapat terus menerus berlanjut membentuk radikal bebas yang baru. Daun katuk (Sauropus Androgynus L. Merr) memiliki kandungan antioksidan yang mampu memperlambat proses terjadinya radikal bebas. Tujuan penelitian ini adalah mengetahui pengaruh ekstrak etanol daun katuk sebagai antioksidan dalam menurunkan kadar malondialdehid (MDA) plasma darah. Metode penelitian adalah eksperimen laboratorik, menggunakan empat kelompok perlakuan yaitu plasma darah sebagai kontrol negatif, plasma yang dioksidasi dengan $CuCl_2$ sebagai kontrol positif, plasma darah yang diberi $CuCl_2$ ekstrak etanol daun katuk dan plasma yang diberikan $CuCl_2$, dan vitamin C sebagai kontrol antioksidan. Kadar MDA diperiksa menggunakan metode thiobarbituric acid reactive substance (TBARs). Data dianalisis dengan Anova dan Tukey ($p<0,05$). Hasil penelitian menunjukkan terdapat pengaruh ekstrak etanol daun katuk terhadap kadar malondialdehid ($p=0,035$) dengan nilai MDA plasma $CuCl_2$ (0,005 mg/dL), plasma dengan katuk (0,003 mg/dL), plasma

dengan vitamin C (0,021 mg/dL). Dapat disimpulkan bahwa penggunaan ekstrak etanol daun katuk berpotensi antioksidan dapat menurunkan kadar MDA plasma darah.

***Kata kunci**: Antioksidan, Daun Katuk, Malondialdehid, Metode TBARs*

INTRODUCTION

Malocclusion is a condition of discrepancy from the normal relationship of teeth in position due to developmental changes in teeth, such as size, shape, and position.[1] One of the treatments used to overcome malocclusion is a fixed orthodontic appliance that has the function of restoring the position of the teeth to their original position according to the jaw arch. The tooth movement that occurs due to the application of orthodontic forces causes an inflammatory process. This inflammatory factor affects the process of tissue remodeling for tooth movement because the bone will constantly undergo bone remodeling, which is influenced by osteoblast cells, which, in addition to forming bone, are also responsible for the activation and recruitment of osteoclast precursors.[2]

The use of katuk leaves (*Sauropus androgynus (L.) Merr*) as an herbal plant in Indonesia is easily available and often consumed by the community because it can be used as an antioxidant, anti-inflammatory, and antimicrobial.[3,4] The flavonoids and vitamin C in katuk leaves can capture reactive oxygen compounds inside and outside cells and prevent oxidized compounds.[5] Oxidative stress can cause changes in DNA structure due to interference with the cell division process. Another component that can be damaged is the destruction of lipids in the cell membrane, so that it will further change the structure and function, because lipids are the most sensitive molecules to free radicals and will then produce secondary results, namely malondialdehyde (MDA).[6-8]

The mechanism of action of antioxidants themselves can neutralize free radicals, reduce peroxide concentration, improve oxidation, encourage the production of reactive oxygen species (ROS), and neutralize ROS with lipid metabolism, short-chain free fatty acids, and cholesterol esters.[9] Based on the explanation above, the researcher intends to examine the effect of giving an ethanol extract of katuk leaves as an antioxidant on reducing malondialdehyde with the TBARs method. Therefore, this study aimed to

evaluate the effect of the ethanol extract of katuk leaves (*Sauropus androgynus*) on reducing malondialdehyde levels using the TBARS method.

METHOD

This study is analytical laboratory experimental-type research that aims to determine the effect of the ethanol extract of katuk leaves used as an antioxidant to reduce free radicals using the TBARs method in vitro. The research proposal has been submitted to the Medical Research Ethics Commission of the Faculty of Medicine, Padjadjaran University, with an ethics letter number 1232/UN6.KEP/EC/2023.

The tools for making ethanol extract from katuk leaves consist of a rotary evaporator, test tubes, centrifugation tubes, micropipettes, marbles, a water bath, a cuvette, a centrifuge, and a spectrophotometer. The materials used in this study were blood plasma, ethanol extract of katuk leaves, vitamin C, $CuCl_2$ solution, distilled water, sodium dodecyl sulfate solution 8.1g in 100 ml H_2O, acetic acid solution (200 ml 96% acetic acid in 76 ml H_2O), thiobarbituric acid solution (dissolve 0.8 thiobarbituric acidwith7mlNaOH1Nandadd H2O to 100 ml), BHT solution, and EDTA.[10]

The method of making ethanol extract from katuk leaves is that 1 kg of katuk leaves has been washed, dried, and mashed for kinetic maceration for 1 hour using ethanol solvent. The solution was allowed to stand overnight, then filtered and separated into the pulp and filtrate. Re-macerate the pulp three times. The filtrate obtained from filtering was collected, and the extract mixture was concentrated with a rotary evaporator and evaporated with a water bath at ± 60 °C.

The TBARS (Thiobarbituric Acid Reactive Substance) method was used to measure MDA levels based on the reaction of malondialdehyde with thiobarbituric acid, which aims to detect oxidative stress. The number of samples in this study was as many as 24.

This study used four treatment groups: blood plasma as a negative control, plasma oxidized with $CuCl_2$ as a positive control, blood plasma given $CuCl_2$, ethanol extract of katuk leaves, plasma given $CuCl_2$, and vitamin C. The tubes were closed using marbles and then heated using

a marble. The tubes were closed using marbles and then heated in boiling water at 100 °C for 30 minutes. After heating, the plasma will change to produce a pink compound, and then the test tube is centrifuged to separate the liquid and pulp.

Read the absorbance at a wavelength of 532 nm through a spectrophotometer.

The results of the research data will be tested for normality first using Shapiro Wilk (<50) to determine whether the data is normally distributed or not. The sample used in this study amounted to 24 samples.

RESULT

In this study, researchers examined the MDA levels of the four groups using a spectrophotometer with a wavelength of 532 nm. There were four treatment groups: blood plasma mixed with distilled water as a negative control, blood plasma oxidized by $CuCl_2$ as a positive control, blood plasma oxidized by $CuCl_2$ and then given an ethanol extract of katuk leaves as a treatment group, and blood plasma oxidized by $CuCl_2$ and then given vitamin C as a treatment group. The analysis was performed with a one-way ANOVA parametric test to determine the significant effect on each treatment group. The results of the study of the effect of the ethanol extract of katuk leaves as an antioxidant in reducing malondialdehyde using the TBARs method can be seen in Table 1.

Table 1. Effect of ethanol extract of katuk leaves as an antioxidant in reducing malondialdehyde using the TBARs method for each treatment group

No	Group	Mean	Std Deviation	P-Value
1	Blood plasma	0.004	0.003	
2	$CuCl_2$	0.005	0.003	
3	Ethanol extract of katuk leaves	0.003	0.001	0.023*
4	Vitamin C	0.021	0.020	

*) One-way ANOVA test, $p \leq 0.05$ (There is a significant effect)

The results of the statistical analysis, as output in Table 1, using the one-way ANOVA test, obtained a p-value of 0.023. The probability value obtained from the results of this analysis is smaller than 0.05, so it can be concluded that there is a significant effect on each treatment group.

This test was carried out as a continuation of the ANOVA test, namely the post hoc test, to find out about the differences between each group. The comparison can be seen in Table 2.

Table 2. Post hoc test of groups of ethanol extracts of katuk leaves and vitamin C on blood plasma malondialdehyde levels

Group	Blood plasma	$CuCl_2$	Ethanol extract of katuk leaves	Vitamin C
Blood plasma	-	-	-	-
$CuCl_2$	0.996	-	-	-
Ethanol extract of katuk leaves	0.999	0.985	-	-
Vitamin C	0.047*	0.072	0.035*	-

* Tukey post hoc test p<0.05 (there is a significant difference)

Based on Table 2 post hoc test between groups, the probability value is smaller than 0.05, so it is concluded that there is a significant influence between groups in this study, with the group of ethanol extract of katuk leaves being more influential than the vitamin C group (p = 0.035 <0.05).

DISCUSSION

Orthodontic tooth movement can cause oxidative stress phenomena. When orthodontic mechanical forces are applied, mechanical stress causes various biological reactions such as root resorption, gingival inflammation, attachment loss, and gingival retraction, as well as local inflammatory responses and the repair of surrounding bone tissue. At high concentrations, ROS can function as mediators that can damage cell structures, nucleic acids, lipids, and proteins. Free radicals are responsible for lipid peroxidation and can also decrease the activity of other antioxidant defense system enzymes, such as catalase (CAT) and glutathione peroxidase (GPx), thereby damaging the ribonucleotides required for DNA synthesis.[11-14]

Katuk (*Sauropus androgynus (L.) Merr.*) is an herbal plant that is widely used by the community as a traditional medicine, and one of the uses of katuk leaves is as an antioxidant. According to previous research by Herawati et al. (2021) regarding the acute toxicity test of the ethanol extract of katuk leaves, it did not cause toxic symptoms. Based on the level of effect, katuk can be categorized as a medicinal or functional food that has the potential to treat several diseases.[15,16]

The phytochemicals of katuk leaves include flavonoids as antioxidants that can reduce and protect the body from ROS. Flavonoids can oxidize free radicals by donating flavonoid hydrogen atoms to free radicals so that the free radicals become inactive and stable.[17,18]

The mechanism of antioxidant action can be suppression of ROS formation by inhibition of enzymes involved in free radical formation, capture of ROS

compounds, and enhancement of antioxidant defense protection. Flavonoids can inhibit enzymes such as microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase. The effects mediated by them can come from a combination of radical scavenging activity and interaction with enzyme function. Some of the above mechanisms of flavonoid antioxidant activity can be influenced by the configuration, substitution, and number of hydroxyl groups in the core structure. The arrangement of functional groups in the core structure determines the antioxidant activity of flavonoids.[19,20]

The results of this study can be applied to patients who are undergoing orthodontic treatment, in line with previous research by Herawati et al. in 2021 on the use of ethanol extract of katuk leaves effective in orthodontic treatment, for increasing the production of guinea pig alveolar bone osteoblasts. in orthodontic treatment and reducing alveolar bone osteoclasts in the bone remodeling process, which can maximize the process of wearing orthodontic devices. Research by Cikita in 2016 using the ethanol extract of katuk leaves as an antioxidant was able to reduce peroxide levels significantly, affecting free radicals and making them more stable.[18,21]

There are confounding factors for the limitations of the study "The Effect of Katuk Leaf Ethanol Extract as an Antioxidant in Reducing Malondialdehyde Using the TBARs Method," carried out, namely, the limitations of the respondents. This is because researchers do not take random samples from other respondents, which can affect the effectiveness of research materials, so that they cannot be pursued optimally.

This study has several limitations. The sample was not randomly selected from a broader population, which may limit the generalizability of the findings. Therefore, the effectiveness of the intervention may not be fully optimized, and further studies with larger and randomized samples are recommended.

CONCLUSION

Based on the results of the research and data analysis that have been done, the hypothesis is that there is an effect of using the ethanol extract of katuk leaves as an antioxidant on blood malondialdehyde levels. This suggests its potential use as a natural antioxidant during orthodontic treatment.

CONFLICT OF INTEREST

We declare that there is no conflict of interest in the scientific articles.

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