# Bay leaves and tomato extract: The formulation of anti-aging drink

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# Bay Leaves and Tomato Extract: The Formulation of Anti-Aging Drink

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**Abstract.** UV light exposure affecting in reducing collagen and skin elastin collagen (photoaging). This research aimed at obtaining a formula of bay (*Syzygium polyanthum* [Wight.] Walp.) leaf and tomato (*Lycopersicum pyriform*) fruit extract for soft drink package as an anti-aging potential in term of avoiding aging process caused by UV-B light. The measured indicators were superoxide dismutase (SOD) activity, Copper (Cu) content, Zinc-SOD (Zn-SOD), and malondialdehyde (MDA) level. This experimental research employed a post-test only control group design by giving various extracts of bay leaves and tomato fruits to mice. The treatments given were: P0, P1, P2, P3, and P4. The data obtained were analyzed using ANOVA, which then continued to LSD test. The results showed that the treatment of 8 g/kg BW of tomato fruit extract and 3 g/kg bay leaves extract has been proven to be effective in stabilizing MDA level, SOD activity and Cu level, as well as preventing the decreasing Zn-SOD as the effect of UV-B radiation in mice skin.

## INTRODUCTION

Photoaging is an aging process that occurs due to various factors from outside the body, such as sunlight[1]. Skin changes that occur are not comprehensive and not following the truth. Photoaging is a process that involves the reduction of collagen and skin elastin fibers due to solar UV radiation[2], which has adverse effects such as wrinkles, pigmented spots, decreased skin elasticity, and rough texture. The process of early aging can be inhibited or prevented by avoiding factors that accelerate the process.

The UV radiation in living cells can cause various photochemical risks such as photo-oxidation and photoisomerization. Photo-oxidation reactions occur due to the release of reactive oxygen species (ROS) [3] in the form of superoxide anions ($O_2$●), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (OH●) by chromophores which absorb ultraviolet light. Skin reactions to UV radiation, including the formation of free radicals ($O_2$● and OH●) and direct cell death [4]. The pathobiological effect of ultraviolet light (UV-B) produces free radicals and causes damage to DNA, and this free radical is allegedly the main factor that accelerates the process of premature aging. Thus, there have been various efforts, including researches, done to solve this problem [5].

Bay leaves contain essential oils, especially citral and eugenol, as well as tannins, flavonoids, and polyphenol [6]. Besides that, it is also known to be efficacious as an agent of antibacterial [7], neuroprotective [6], and improve glucose and lipid profile [8]. Bay leaves are one of the natural ingredients that have antimicrobial effects. By having these anti-bacterial properties, bay leaves can be a food preservative naturally. While tomatoes have lycopene[9,10], β-carotene, and vitamin C, which can reduce free radicals ($O_2$● and HO●) [11] by binding to these free radicals. However, to date, the combination of bay leaf extract and tomatoes, which possessed both potentials, has no explanation in terms of the formula, which is useful as anti-aging properties in preventing aging as the effect of UV-B radiation.

This study aimed to obtain a liquid extract formula of bay leaves and tomatoes in the form of soft drink packaging as an anti-aging potential. The aspects of testing carried out were SOD activity, Cu content, Zn-SOD, and MDA tests level. Specifically, this research was conducted to obtain the effective anti-aging formula the drink made from bay leaves and tomato extracts to prevent aging caused by UV-B rays.

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## EXPERIMENTAL DETAILS

This true experimental research used the Pre-Post-Test Control Group Design. The population in this study was Wistar strain rats aged four months fromi nbreeds. The tomato (*Lycopersicum pyriforme*) materials were planted in the Sengkaling area, Mulyoagung Village, Dau District, Malang.

The ripe fruits were used after they were washed and extracted. The aqueous part of the extract was used to treat the samples. Meanwhile, the bay leaves were obtained from the trees planted in Sengkaling, Mulyoagung, Dau, Malang. The leaves were boiled in 1.5 L water until it remained a liter.

The treatments given were: (1) P0 = the negative control group (without UV-B radiation and stewed bay leaves and tomato extract); (2) P1 = the positive control group (exposed with 150 mJ/cm$^2$ of UV-B radiation without stewed bay leaves and tomato extract for); (3) P2 = the group treated with 11 g/kg bw of tomato fruit extract once every two days given four hours before were exposed with UV-B; (4) P3 = the group treated with 8 g/kg bw of tomato fruit extract and 3 g/kg bw of stewed bay leaves given once every two days before were exposed with 150 mJ/cm$^2$ UV-B; (4) P4 = the group treated with 3 g/kg bw of tomato fruit extract and 8 g/kg bw of stewed bay leaves given once every two days before were exposed with 150 mJ/cm$^2$ UV-B; (5) P5 = the group treated with 11 g/kg bw of stewed bay leaves given once every two days before were exposed with 150 mJ/cm$^2$ UV-B. All treatments were given for six weeks.

The production of tomato fruit extract and stewed bay leaves, as well as the mice raising, were conducted in the Laboratory of Chemistry of Universitas Muhammadiyah Malang. Meanwhile, the standard plant and tomato fruit extract assessments, as well as plant determination, were done in Laboratory of Botany-Pharmacognition, Faculty of Pharmation, Universitas Muhammadiyah Malang, Indonesia. The MDA level, SOD activity, and CU, Zn-SOD tests were conducted in the Laboratory of Chemistry, Medical Faculty, Universitas Muhammadiyah Malang, Indonesia.

### Analysis of MDA level

The MDA OXISResearch Reagen was employed to examine the MDA level in the back skin tissue of the Wistar mice in all treatments. The MDA levels were measured using a fluoro-meter at the excitation wavelength of 515 nm and 559 nm emission. After the endogenous peroxidase was inactivated, the tissue pieces were incubated in anti-Cu monoclonal antibodies, Zn-SOD (sigma S2147), and continued with the incubation in secondary incubation (Dako K1391). Antigen-antibody reaction products were visualized by the addition of diaminobenzidine (DAB). The antioxidant profile of Cu-Zn-SOD in skin tissues was observed based on the distribution and frequency of the antioxidant in the skin tissues.

### Analysis of Rat Skin SOD Activity

The observation of SOD activity in which the kit used was SOD Sigma Aldrich. As much as 400 µL of 37.5/62.5 (v/v) cold chloroform/ethanol solution was added into 150 µL of skin lysate. This mixture then was mixed using vortex for 3 seconds and centrifuged at 400 rpm at 4ºC for ten minutes. A total of 2.9 mL of A solution (a mixture of xanthine and cytochrome c solution) plus 50 µL of standard (control) or sample solution was slowly homogenized using a vortex. The reaction was started by adding 50 µL of solution B (xanthine oxidase) and slowly divorteks. The observation of the changes that occurred in the absorbent was done with a so-called micro-photometer.

### Analysis of Cu,Zn-SOD Levels

The examination of Cu,Zn-SOD level using spectrophotometry method.

### Data Analysis

Through the random allocation into six experimental groups (two control groups and four experimental groups), the screening before treatment with the inclusion criteria involved age, sex, and bodyweight of mice. Meanwhile, the exclusion, as the health statement of veterinarians which were evaluated after 14 × 24 hours, behaving aggressive (e.g., tend to fight with the other mice in the same cage). Normality test (Kolmogorov-Smirnov Z), homogeneity test (Levene's test), and ANOVA and LSD were conducted.

## RESULTS AND DISCUSSION

Based on the normality test, it can be concluded that the data was normally distributed in which the sig. for MDA: SOD; and Cu,Zn-SOD were 0.119, 0.200, and 0.110, respectively. Meanwhile, the homogeneity test showed that the variances were homogeny in which the *p* values were 0.227 for MDA, 0.174 for SOD, and 0.672 for Cu, Zn-SOD. The ANOVA test results are served in Table 1.

**TABLE 1.** ANOVA test results of MDA level, SOD activity, Cu,Zn-SOD level as the effect of the bay leaves, and tomato fruit extract under 150 mJ/cm$^2$ UVB radiation.

| | Sum of Square | Df | Mean Square | F | Sig |
|---|---|---|---|---|---|
| MDA Level | 21.114 | 5 | 4.223 | 3.198 | 0.000 |
| SOD Activity | 3659.957 | 5 | 731.991 | 98.403 | 0.000 |
| Cu,Zn-SOD Level | 3.092 | 5 | 0.618 | 441.643 | 0.000 |

### The MDA level as the indicator of ROS activity

The MDA OXISResearch Reagen was employed to examine the MDA level in the back skin tissue of the Wistar mice in all treatments. The ANOVA test results (Table 1) showed that there was a significant difference in MDA level of mice ($p$<0.01). Moreover, the LSD test showed the significant differences between P1 and P0, P4, P2, and P3; even though there was no significant difference from P5. This implied that the skin cells experienced the elevation of its MDA level as the effect of stewed bay leaves and tomato fruit extract (Fig 1).

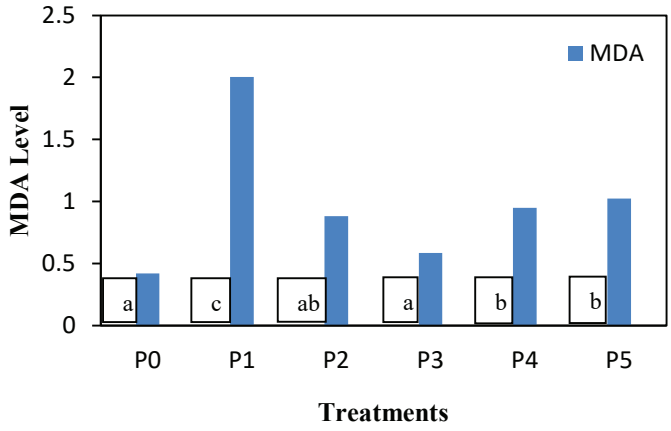


**FIGURE 1.** Graphic of MDA level of mice skin tissue treated using bay leaves and tomato fruit extract. (Description: The bars with the same alphabet imply the insignificant difference)

It can be seen from Fig. 1 that the highest MDA value was possessed by P1 treatment (0.2003 ± 0.063 nmol/g). Contrarily, the lowest MDA occurred in P0 (0.420 ± 0.027 nmol/mL). Meanwhile, albeit that there was a significant difference between P4 and P5, but there was no significant difference between P2 and P3. This showed that there was no increase of ROS activity in mice skin tissue which were treated with 11 g/kg bw of tomato fruit extract or those which were treated with the combination of 8 g/kg bw of tomato fruit extract and 3 g/kg bw of stewed bay leaves and exposed with 150 mJ/cm$^2$ of UV-B. These results are in line with some previous researches which reported that used several kinds of foods such as skin gelatin of Pacific cod [12] and tomato paste [9] to reduce the MDA level. Malondialdehyde (MDA) is the final product of lipid oxidation. High levels of MDA are primarily influenced by lipid peroxidation levels [1], which also indirectly show high free radicals.

After doing statistical analysis with LSD test, the results (Fig. 1) showed that there were differences between the treatments given. This means that UV-B treatment had a significant effect on the increase of free radicals in the body. Furthermore, the administration of stewed bay leaves and tomato extract could reduce those free radicals with

indicators of MDA reduction. The same results were reported by [9], who stated that the administration of tomato paste had decreased the MDA level in the hamster's blood.

The combination of stewed bay leaves and tomato fruit extract is a fairly effective treatment in reducing MDA levels in rats that are irradiated with 150 mJ/cm$^2$ UV-B rays. The presence of lycopene or β-carotene from tomatoes in the rat's body will be able to bind free radicals[13] such as hydroxyl ions and superoxide anions. This due to the radicals (OH●) and (O●-), are resulted from the formation of ROS which are previously been bounded by lycopene, β-carotene, and vitamin C before damaging cell components such as DNA[14] which results in the decreased MDA levels. Thus, the administration of lycopene or β-carotene can prevent the increase in MDA due to the 150 mJ/cm2 UV-B radiation. This in line with the previous researches, which reported that the provision of vitamin C can reduce MDA of elderly group [11]. Whereas Lycopene is the most potent carotenoid in changing singlet oxygen and ROS (Di Mascio, et al. 1989; Conn, et al., 1991). Based on the study results, it can be said that carotenoids can significantly reduce MDA and also prevent lipid peroxidation in cells. Thus, giving lycopene or β-carotene, will reduce ROS, which is characterized by decreased levels of MDA.

## SOD activity

SOD Sigma Aldrich kit was used to examine the SOD activity in mice skin tissue in all treatments. The ANOVA (Table 1) analysis results showed that there was a significant difference ($p$<0.01) of SOD activity. Furthermore, the LSD test results showed that there were significant differences among P1, P0, P4, and P2, even though there was no significant difference between P1 and P5. This proved that the SOD activity in the group treated with stewed bay leaves and tomato fruit extract was not decreased (Fig. 2).

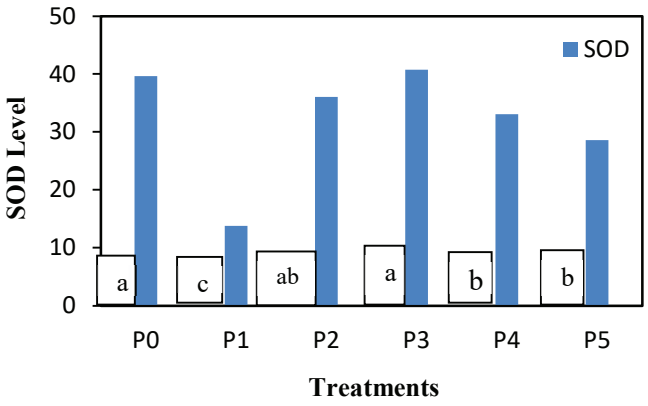


**FIGURE 2.** Graphic of SOD activity in mice skin tissue

Fig. 2 shows that the lowest SOD activity was witnessed by P1 treatment group (13,763 ± 3,114 U/g), while the highest one was found in P3 group (40.720±1.69 U/g). The significant difference was found between P4 and P5, while the insignificant difference was found in P0 and P2. This proved that the SOD activity in skin tissue have not decreased even though the mice were treated with 11 g/kg bw of tomato fruit extract or the combination of 8 g/kg bw tomato fruit extract and 3 g/kg bw of stewed bay leaves which gave before the 150 mJ/cm$^2$ UV-B exposure.

Superoxide dismutase (SOD) is an enzyme in the intracellular fluid that participates in the degradation process of intracellular free radical compounds. SOD activity has been proven to increase against oxidative damage [15]. This enzyme has an oligo atomic logo element on its active side. Superoxide Dismutase catalyzes the dismutation of $O_2$● to $H_2O_2$. This enzyme inhibits the simultaneous presence of $O_2$● and $H_2O_2$ originating from the formation of free radicals (OH).

The results of the analysis of SOD activity showed that the treatment of UV-B exposure had a significant effect on decreasing SOD activity in the body, but the application of stewed bay leaves and tomato fruit extract could increase SOD activity in rat skin. This in line with the findings of [16][16] who reported that lycopene had no significant effect on blood SOD activity of Cyprinus carpio with pyrethroid deltamethrin exposure.

## The Cu, Zn-SOD level of mice skin tissue

The SOD Sigma Aldrich kit was used to examine the Cu, Zn-SOD level in mice skin tissue of all treatment groups. The ANOVA test results (Table 1) showed that there was a significant difference in the Cu, Zn-SOD level in mice skin tissue ($p$<0.01). Furthermore, the LSD test showed a significant difference between P1 and P0, P3, and P4, even though there was an insignificant different in P5. This means that there was no decreasing level of the Cu, Zn-SOD content in the administration of the stewed bay leaves and tomato fruit extract (Fig. 2).

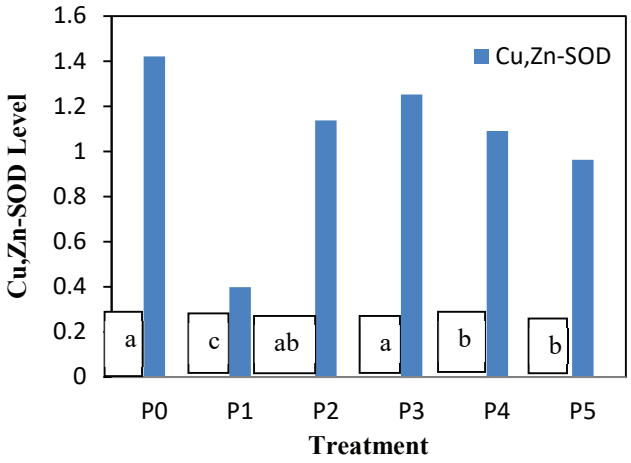


**FIGURE 3.** Graphic of Cu, Zn-SOD level in mice skin tissue of all treatments

Figure 3 shows that P1 was the treatment with the lowest Cu, Zn-SOD value (0.399±0.037 U/mg), while the most significant value occurred in P0 treatment (1.421 ± 0.45 U/mg). Nevertheless, there was no significant difference between P2 and P3, while the significant difference was found between P4 and P5. This proved that there was no decreasing level of SOD activity in mice skin tissue which was treated with 11 g/kg bw of tomato fruit extract or the combination 8 g/kg bw of tomato fruit extract and 3 g/kg bw of stewed bay leaves which were administered before the 150 mJ/cm$^2$ UV-B exposure.

Immunohistochemical staining results showed that Cu,Zn-SOD was present in the nucleus and cytoplasm of skin cells. Qualitatively, the Cu,Zn-SOD content profile was seen in the stress group compared to the control group. This decrease was shown by the number of browns mixed with blue in the cytoplasm and rat liver cells in the stress group compared to the control group.

The Cu,Zn-SOD observations showed that the UV-B exposure treatment had a significant effect in decreasing SOD activity in the rat's body, but the application of stewed bay leaves and tomato fruit extract could increase SOD activity in rats skin. These results are in line with the findings of Sasaki, Akamatsu, and Horio [15] who reported that UV-Bligh exposure has significantly decreased the amount of Mn-SOD as significant as increased Cu,Zn-SOD level in human keratinocytes. On the other hand, Punnonen *et al.* [17] found that chronic UV-B irradiation treatment-induced SOD activity to provide the epidermis a way of defending itself against the effects of UV-B irradiation.

## SUMMARY

The results of the study showed that the administration of formula 8 g/kg bw of liquid tomato extract with a decoction of 3 g/kg bw of leaves proved to be effective in maintaining MDA levels to be in a low level, maintaining SOD activity and prevent the Cu,Zn-SOD levels to elevate as the result of UV-B radiation to the skin rat.

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