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Cover Page Footnote

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Lipid Peroxidation Inhibition Activity of Sintrong (*Crassocephalum crepidioides*) Leaf Extract in Rats Consuming Arak Jembrana

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Abstract

The present study was conducted to determine the antioxidant activity of sintrong (*Crassocephalum crepidioides*) leaf extract to inhibiting lipid peroxidation by decreased malondialdehyde (MDA) level in the blood plasma of rats consuming arak Jembrana. The process of making sintrong leaf extract used 96% ethanol with maceration techniques. In vivo testing used five groups: (1) a control group, (2) treatment with arak, (3) treatment with arak followed by extract at a dose of 37.5 mg/kgBW, (4) treatment with arak followed by extract at a dose of 50 mg/kgBW, and (5) treatment with arak followed by extract at a dose of 75 mg/kgBW. As much as 1 cc/day of arak was provided for seven days. The extract treatment at a dose of 37.5 mg/kgBW decreased the MDA level from 21.24 nmol/L to 16.33 nmol/L. Based on a Tukey's Honestly Significant Difference (HSDa) test, this treatment was not significantly different from that of the control group (normal condition).

Keywords: sintrong, arak, antioxidant, malondialdehyde, lipid peroxidation

Introduction

Indonesia, which includes the island of Bali, is a country rich in plant biodiversity because its location in a tropical climate region. Some of these plants can be used as food or medicine and many have potential as medicine but are not widely known by the public. Sintrong (*C. crepidioides),* as the Javanese people know it, or *kejompot*, as it is called by the Balinese people, is a wild plant that is considered a weed, and its use is still limited to consumption as a vegetable dish [1].

Sintrong contains a variety of secondary metabolite compounds that are not yet widely known by the public. The content of active compounds found in sintrong plants includes polyphenols, flavonoids, quinones, tannins, monoterpenes, sesquiterpenes, triterpenoids and steroids. This plant is consumed by the people of Benin as a vegetable and traditionally, it is believed to have antibiotic, anti-worm, anti-inflammatory, antidiabetic, and antimalarial properties [2]. Previous studies have suggested that the hot water extract of *C. crepidioides* leaves also has antibacterial activity [3].

In previous studies, the toxicity and antioxidant activity of crude methanol extracts of sintrong leaves (*C. crepidioides*) were determined in vitro using the DPPH method, and IC_{50} values of 369.08% were obtained [4]. The flavonoid content found in the leaves of *C. crepidioides* has potential as an endogenous antioxidant. Antioxidants can inhibit oxidation reactions by free radicals or neutralize and destroy free radicals that can cause damage to cells and biomolecules such as DNA, proteins, and lipoproteins in the body, which can ultimately lead to degenerative diseases [5].

One of the triggers for oxidative stress is ethanol consumption. Ethanol can be sourced from Balinese alcoholic drinks such as arak. Traditionally, arak is produced from the distillation process of coconut juice. Previous research found that the ethanol content in arak Bali is 30–50% (w/v). Chronic consumption of 20% alcohol can cause macroscopic changes in the structure of liver tissue in Wistar rats [6,7]. Ethanol is metabolized in the liver and produces acetaldehyde, which is toxic to the body. The formation of free radicals due to the presence of ethanol can induce cytochrome P450 (CYPs), which can form superoxide radicals [8]. One of the biomarkers for oxidative damage is malondialdehyde (MDA). MDA is the result of lipid peroxidation of carbonyl compounds, which in turn are the result of protein damage due to reactive oxygen species (ROS).

MDA can be used to determine oxidative damage in vivo as a result of lipid peroxidation [9].

Research on the antioxidant activity of *sintrong* leaves is limited to in vivo tests; thus, the present study is aimed at the antioxidant activity of *sintrong* leaf extract to decreasing the MDA levels in Wistar rats under stress conditions due to ethanol exposure sourced from arak Jembrana. This research is important because it provides information to the public about the efficacy of *sintrong* leaves for providing health by examining its antioxidant activity in counteracting free radicals that can trigger various diseases such as cancer, tumors, and other degenerative diseases.

Materials and Methods

General. The secondary metabolite content contained in *sintrong* leaf extract was tested using a phytochemical screening test. The phytochemical test used HCl, Mg powder, Mayer reagents, Dragendrof reagents, ammonia, chloroform, FeCl3 10%, acetic acid, sulfuric acid and NaOH. The determination of antioxidant activity via in vivo testing used 2,2-diphenyl-1-picryl-hydrazyl-hydrat (DPPH). The MDA levels of rats were measured using a spectrophotometer (Merck Amersham Bioscience, type Ultraspec 3100 pro). The analysis of MDA levels used reagents such as EDTA, 15% TCA (*trichloroacetic acid*), 0.37% TBA, and 0.25 N HCl.

Plant material. The leaves of *sintrong* (*C. crepidioides*) were collected from Selat, Karangasem Regency, Bali, Indonesia in May, 2019.

Making ethanol extract of *sintrong* leaves. A total of five kilograms of *sintrong* leaves were cleaned, cut into small pieces, and dried in an oven at 40°C for 9 h. The dried *sintrong* leaves were then blended to a powder and subsequently soaked in 96% ethanol for 24 h. The *sintrong* leaf extract was then evaporated to obtain concentrated ethanol extract. Finally, the *sintrong* leaf extract was tested using a phytochemical screening test and the 2,2-diphenyl-1-picryl-hydrazyl-hydrat (DPPH) the in vitro test was used to determine its radical-scavenging activity (IC_{50}).

Animal experiments and sampling. A total of 25 male Wistar strain rats with an average weight of approximately 200 g were used in this study. The rats were adapted to their environment for a week. They were grouped into five treatment groups of five animals. The rat groupings consisted of a negative control (KN), a positive stress control group (KP), a group treated with stress and ethanol extract of *sintrong* leaves at as much as 37.5 mg/kgBW of rat (P1), a group treated with stress and ethanol extract of *sintrong* leaves at 50 mg/kgBW of rat (P2), and a group treated with stress and ethanol extract of *sintrong* leaves at 75 mg/kgBW of rat (P3). Stress treatment of the treatment groups was conducted by administering as much as 1 cc of arak Jembrana per rat per day for 7 days. From the 8th day to the 21st day, all three treatment groups were given ethanol extract of *sintrong* leaves in varying doses. On the 22nd day, a blood plasma specimen was collected through a *medial canthus sinus orbitalis* (post-test) to determine the MDA levels of the rats.

Analysis of lipid peroxidation (MDA) levels. MDA levels were measured using the *thiobarbituric acid reactive substance* (TBARS) method. A total of 3 cc of Wistar rat blood was added to a centrifuge tube containing two drops of EDTA. From blood samples that had been centrifuged at a speed of 3000 rpm for 30 minutes, as much as 200 µL of supernatant was transferred to an empty centrifuge tube. Then, 2000 µL of a 15% TCA (*trichloroacetic acid*) solution, 2000 µL of 0.37% TBA solution, and 2000 µL of 0.25 N HCl were added to the samples. Next, the sample were heated in a water bath at 95 °C for 60 min, followed by cooling to room temperature above an ice bath for 15 min, followed by centrifugation for 15 minutes at 3000 rpm. Finally, the supernatant was transferred to a cuvette and its absorbance was read using a spectrophotometer at a wavelength of 532 nm [5]. All analyses were performed in triplicate. Data were analyzed via the analysis of variance test (ANOVA) using a pos-hoc test and then continued with a *Tukey HSD*^{a} test.

Results and Discussion

MDA is the final product of fat peroxidation due to the breakdown of fatty acid chains that turn into toxic compounds against cells. MDA can be used as a marker for free radicals in the body to determine if antioxidants are needed to reduce free radicals [10]. MDA level analysis results showed that the positive control group exposed to arak Jembrana had the highest MDA of 21.24 nmol/L. The results of the MDA level analysis of each treatment group are presented in Figure 1.

This result indicates that arak Jembrana exposure can increase MDA levels. Increased levels of MDA are associated with the presence of free radicals, which trigger lipid membrane peroxidation processes. In addition to activity from the enzyme Alcohol Dehydrogenase (ADH), oxidation of alcohol to aldehyde can be mediated by the microsomal CYPs mainly Cytochrom P450 2E1 (CYP2E1), and the order is mediated by peroxisomal catalase [11,12]. The CYP2E1 activity can be significantly induced by chronic alcohol consumption, and during the CYP2E1 catalytic cycle, significant amounts of toxic acetaldehyde and ROS are generated, which may lead to increases in cellular injury, lipid peroxidation, oxidant activity, and mitochondrial damage and contribute to various phatological processes [13,14].

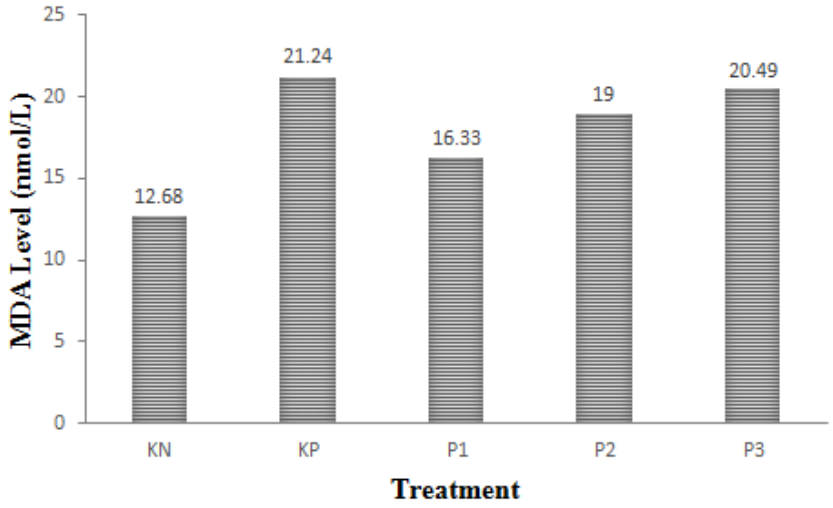

Figure 1. Levels of Malondialdehyde (MDA) (nmol/L) in the Blood Plasma of the Rats Treated with *Sintrong* Leaf Extract

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According to previous studies, chronic alcohol consumption not only activates free radical derivatives but also changes the level of the endogenous antioxidant systems, both enzymatic and nonenzymatic. Catalase, which was first defined as an antioxidant enzyme, can also catalyze the metabolism of alcohols [15]. The capacity of this reaction is limited, however, by the low level of H_2O_2, and catalase consequently plays only a minor role in the overall metabolism of alcohol. This can produce the effects of oxidative stress quickly, thus affecting both the structure and function of the cell membrane and its organelles [16].

The negative control group had the lowest MDA level, 12.62 nmol/L, which indicates that the amount of free radicals formed in the body of a group member not exposed to arak Jembrana is low, so that the MDA levels formed are very low.

Of the three doses given to the extract treatment groups (37.5 mg/kgBW; 50 mg/kgBW and 75 mg/kgBW), the dose of 37.5 mg/kgBW (P1) is the most influential and can reduce MDA levels. The group receiving this dosage shows the lowest MDA levels, 16.33 nmol/L, among the three treatment groups. Further statistical analysis with the Tukey HSDa test, $\alpha = 0.05$, shows that the group given extracts of 37.5 nmol/L (P1) and the negative (normal) controls are not significantly different. This result indicates that the administration of ethanol extract *sintrong* leaves at a dose of 37.5 nmol/L can reduce MDA levels close to the normal MDA level. This result is supposedly caused by the presence of secondary metabolites in the extract, especially the flavonoid class, which has potential as an exogenous antioxidant. The phytochemical test shows that *sintrong* leaf extract contains flavonoids, polyphenols, tannins, terpenoids and steroids. DPPH radical-scavenging activity can be known from the IC_{50} value, which is 187,12 mg/L for *sintrong* leaf extract. Compounds that have an IC_{50} value bellow 200 mg/L are classified as antioxidants with strong activity. That classification indicates the presence of flavonoid compounds in the sintrong leaf extract. According to previous research, flavonoids are exogenous antioxidants that are useful for preventing cell damage due to oxidative stress. Flavonoids act as antioxidants by donating hydrogen ions to neutralize the toxic effects of free radical and by increasing the expression of endogenous antioxidant genes through nuclear activation factor erythroid 2 related factor 2 (Nrf2), resulting in an increase in genes that play a role in the synthesis of endogenous antioxidant enzymes [17].

Lipid peroxidation is a common consequence of oxidative stress. When free radicals target lipids, they can initiate the lipid peroxidation process, a chain reaction that produces multiple breakdown molecules such as MDA. MDA is a secondary product of lipid peroxidation and an end product generated by decomposition of

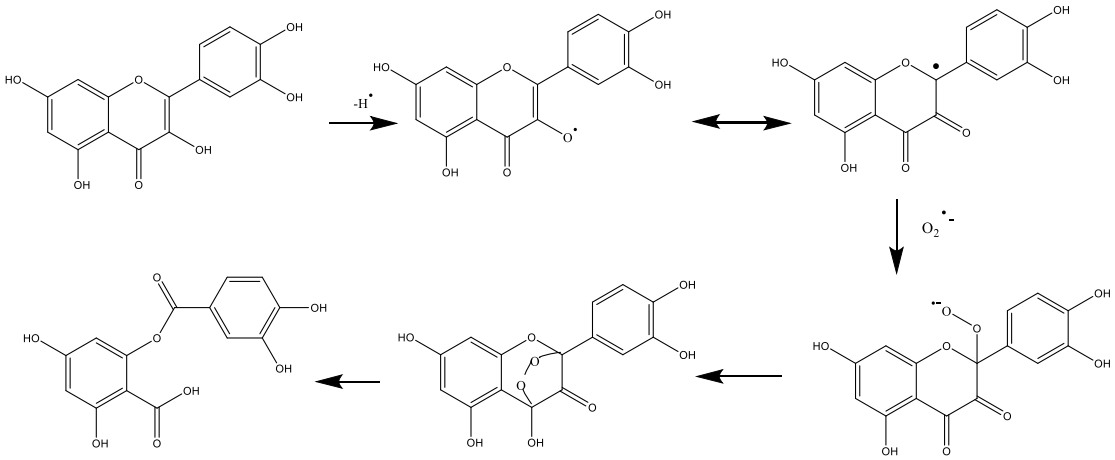

Figure 2. Mechanism of Superoxide Anion Radical-scavenging Activity of Flavonoid

arachidonic acid and larger PUFAs [18]. Flavonoids are a group of natural benzo-γ-pyran derivates that possess strong antioxidant activities. They protect lipids against oxidative damage by various mechanisms [19,20]. Free metal ions enhance ROS formation by the reduction of hydrogen peroxide with generation of the highly reactive hydroxyl radical [21]. Due to their lower redox potentials, flavonoids are thermodynamically able to reduce highly oxidizing free radicals (redox potentials in the range of 2.13-1.0 V) such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation. Because of their capacity to chelate metal ions (iron, copper, etc.), flavonoids also inhibit free radical generation (Figure 2) [22,23].

The strength of the antioxidant activity of a flavonoid depends on the number of hydroxyl groups (-OH) in the compound and their positions. The greater the number of hydroxyl groups is, the higher the antioxidant activity will be. Flavonoids are potential antioxidants and their effectiveness in the inhibition of the lipid peroxidation is related to their metal ion-chelating activity and free radical-scavenging activity, so they can decrease the MDA levels. Three structural groups are important determinants of the radical-scavenging activity of flavonoids. First, the ortho-dihydroxy structure in the B-ring. Second, the 2,3-double bond in conjugation. Third, the 4-oxo function in the C-ring. Flavonoids form complexes with the metal ions using the 3- or 5-hydroxyl and 4-keto substituents or the hydroxyl groups in the ortho position of the B-ring [23].

Increasing the dose of ethanol extract of *sintrong* leaves above 37.5 mg/kgBW actually increases the blood plasma MDA levels. This result is supposedly caused by the presence of secondary metabolite compounds, which have a toxicity effect and potentially inhibit the decrease in blood plasma MDA levels. This hypothesis is supported by previous studies on screening tests and the study of the toxicity of the leaves of the *C. rubens (Juss. Ex Jack.)* S. Moore and *C. crepidioides (Benth.).* The S. Moore species is consumed as a vegetables in Benin. In addition to having antioxidant, antibacterial, and anti-inflammatory potential, the plant has a toxicity effect at higher concentrations [2].

Conclusion

This study demonstrates that *sintrong* leaf extract has antioxidant activity by decreased MDA levels as a result of lipid peroxidation. The effective dose of *sintrong* leaf extract is 37.5 mg/kgBW, which can decrease the MDA levels of rats that consume arak Jembrana to reach 16.33 nmol/L.

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