THE PHYTOCHEMICAL PROFILE OF MANGIFERA RUFOCOSTATA KOSTERM AND ITS ANTIOXIDANT ACTIVITY

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The tandui plant (Mangifera rufocostata Kosterm.) is one of the typical plants of South Kalimantan which belongs to the genus Mangifera. It has the potential as medicine. The local people use boiled water from bark of M. rufocostata to treat diabetes and minor stroke. This study aims to identify compounds and determine the antioxidant activity of leaves and bark ethanol extract M. rufocostata qualitatively and quantitatively. The results of the identification show that ethanol extract of M. rufocostata contains tannins, phenols, flavonoids and saponins. The result of the qualitative antioxidant activity test shows that the ethanol extract of M. rufocostata has an antioxidant activity. The result of quantitative antioxidant activity test reveals that the leave and bark have IC50 value of 7,614 ppm and 8,254 ppm. Based on this research, it can be concluded that ethanol extract of M. rufocostata from Hulu Sungai Tengah District contains various secondary metabolites such as tannins, phenols, flavonoids, and saponins and have a very strong antioxidant activity.

Keywords: Antioxidant; Mangifera rufocostata Kosterm; Phytochemical; Antioxidant

INTRODUCTION

Free radicals produced by metabolism regulate signal transmission between cells and cell growth, and inhibit viruses and bacteria. Excess free radicals will leak intracellularly, which can denature proteins and nucleic acids thereby damaging cells¹. These events are associated with several diseases, both carcinogenic and atherogenesis. A decrease in natural antioxidant levels or an increase in reactive oxygen species (ROS) triggers oxidative stress². Due to the dangers of ROS and free radicals, it is necessary to find natural antioxidants that can prevent oxidative stress³.

Antioxidants are substances that can react and neutralize free radicals. The impact of antioxidants in the body can prevent or reduce the harmful effects. Antioxidants have been known since ancient times to have pharmacological effects and are found in plants or in synthetic products⁴. Antioxidants act as reducing agents and will experience oxidation in their structure. Synthetic and natural antioxidants have hydroxyl groups which take part in redox reactions. Antioxidants can be in the form of vitamins, flavonoids, some minerals and synthetic phenolic compounds⁵.

Secondary metabolites in plants generally consist of several active substances that work synergistically or singly in overcoming various diseases, especially degenerative diseases⁶. One of the plants that has the potential as medicine is Tandui (Mangifera rufocostata Kosterm.). The M. rufocostata plant is one of the endemic plants of Kalimantan which is rarely found and has medicinal properties. The parts of the plant which are often used by the community is the fruit, leaves and bark. The M.
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The *M. rufocostata* plant has empirical efficacy in treating diabetes and stroke. Diabetes Mellitus and stroke are included in degenerative diseases. Compounds that can prevent cell damage caused by free radicals are antioxidants. This study aims to explore the leaves and bark phytochemical profiles and evaluate their antioxidant properties.

**MATERIALS AND METHODS**

**Collection and preparation of extract**

Samples were taken from the village of Tanjung Hanntak, Hanntak District, Hulu Sungai Tengah Regency, South Kalimantan. The determination was carried out at the Banua Botanical Gardens, South Kalimantan. The bark and leave of *M. rufocostata* obtained were immediately wet-sorted, then washed thoroughly with running water. Then, the bark and leave of *M. rufocostata* were chopped to make the drying process more efficient. Furthermore, the drying process was carried out in a drying cabinet at a temperature of 55°C. The dry simplicia was then sorted dry and then crushed using a blender to form coarse powder. The simplicia powder obtained was stored in a tightly closed container.

**Extraction**

Preparation of *M. rufocostata* stem bark extract was done by placing 500 g of simplicia powder into a maceration container, soaking with 96% ethanol until the simplicia powder was submerged. The maceration container was closed and stored for 6 days (remereration) in a place protected from sunlight and stirred every 8 hours. The solvent was replaced every 1x24 hours. Liquid extract and extraction dregs were separated by filtering using Whatman filter paper. The liquid extract obtained was then evaporated using a water bath to obtain a thick extract with a fixed weight.

**Phytochemical screening**

Phytochemical screening was done following standard test tube methods. Extract of samples was used to screen some compounds like alkaloids, flavonoids, saponins, tannins, phenols, steroids, and terpenoids.

**Qualitative Test of Sample Extract**

The ethanol extract of *M. rufocostata* was suspended with methanol:chloroform in a ratio of 1:1 then spotted on a silica Gel254 TLC plate (stationary phase). Then, it was eluted using several ratios of the mobile phase (eluent) n-hexane and ethyl acetate (1:9, 2:8, 3:7 and 5:5) v/v. The TLC plate was sprayed with 0.02% DPPH solution and left to dry. The active compound as an antioxidant formed a yellow stain with a purple TLC plate background.

**Quantitative Test of Sample Extract**

Liquid extract was prepared with a concentration of 500 ppm. Next, a series of concentration solutions with concentrations of 2.5, 5, 7.5, 10, 12.5, and 15 ppm were prepared using a 10 mL volumetric flask. 0.4 mM DPPH solution was added as much as 1 mL to each sample concentration of 4 mL. Furthermore, the solution was allowed to stand for a span of operating time in a dark place. Absorbance readings were carried out using a UV-Vis spectrophotometer in the maximum wavelength range that had been obtained previously.

Quercetin was used as a comparison of antioxidant activity with 100 ppm as an initial concentration. Next, a series of levels were made with concentrations of 0.5, 1, 2, and 4 ppm in a 10 mL volumetric flask. The series of levels that had been made were then taken as much as 4 mL each and then 1 mL of 0.4 mM DPPH solution was added. The grade series solution was left in a dark place for a predetermined operating time. The absorbance was read with a UV-Vis spectrophotometer at the maximum wavelength that has been obtained.

**RESULTS AND DISCUSSION**

**Phytochemical screening**

The extraction results obtained were in the form of filtrate which was then evaporated with a water bath at 50°C to obtain a thick extract with a fixed weight. The amount of yield from the leaves was 2 times more than the bark. This can be caused by the amount of chlorophyll that was still in the yield.
Fig. 1: Percentage of crude extract.

Table 1: Phytochemical compound of bark and leave extract.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Result</th>
<th>Leave</th>
<th>Bark</th>
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<tbody>
<tr>
<td>Tanin</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>+</td>
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</tbody>
</table>

(*) Positive: There is compound, (-) Negative: There is no compound.

Bark and leaves contain tannins, phenols, flavonoids, and saponins. The leaves did not detect any alkaloids. Steroids and terpenoids also produced negative results. Based on their nature, steroids and terpenoids tend to be more non-polar, which means they are difficult to dissolve in polar solvents such as ethanol.

DPPH free radical scavenging activity.

Based on the calculation of the polarity index, it can be seen that with a greater ratio of ethyl acetate, the polarity index value is higher, which means it is more polar. The chromatogram with a semi-polar eluent ratio (5:5) v/v has the most spots with stable separation between spots. This eluent ratio has the lowest polarity compared to the others. Eluent chromatograms with v/v comparisons (3:7, 2:8 and 1:9) have almost the same spot separation. There are several spots that are separated quite well.

Non-polar compounds will move up more easily following the eluent. A compound which has a higher Rf value has low polarity, while a compound with a lower Rf value has higher polarity. The chromatogram that was sprayed with the DPPH spotter changed the color of the stain to yellow with a purple background, indicating the presence of antioxidant activity in the sample."
Fig. 2: Analysis of Rf value with TLC methods, (a) after sprayer with DPPH on UV 254 nm, (b) before sprayer with DPPH on 254 nm, (c) before sprayer with DPPH on 366 nm.

Fig. 3: (a) percent of inhibition bark extract, (b) percent of inhibition leave extract, (c) percent of inhibition Quersetin.
The results of calculating the IC50 value of the sample using linear regression were obtained at 7.614 ppm. These results indicate that the *M. rufocostata* extract sample has very strong antioxidant activity because the IC50 obtained is <50 ppm\(^1\). IC50 value was calculated using probit analysis. The concentration significance value obtained using SPSS is 0.000, which means it is lower than 0.05. This shows that the concentration of *M. rufocostata* extract samples has a significant effect on the inhibition percentage. An independent t-test was used to compare whether there was a significant difference between the IC50 value of the positive control quercetin comparator and the ethanol extract samples of *M. rufocostata*. The normality test and homogeneity test were carried out first as a condition before carrying out the independent t-test\(^1\). The normality test obtained data that were normally distributed while the homogeneity test obtained data that were not homogenously distributed\(^1\). The independent t-test was carried out with the significance value used in the equal variances not assumed section because the homogeneity test of the data is not homogeneous. The results of the independent t-test obtained a significance value of <0.05. So, it can be concluded that there is a significant difference between the average IC50 value of the positive control quercetin comparator and the ethanol extract samples of *M. rufocostata*\(^1\).

Conclusions

It can be concluded that the ethanol extract of the leaves and bark of *M. rufocostata* from Hulu Sungai Tengah District contains tannins, phenols, flavonoids, and saponins and it has a very strong antioxidant activity.

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REFERENCE


A study of the phytochemical analysis of the plant ingredients of Rofokostana Koostirum and its antimicrobial activity

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(Labu tawar Mangkurat Rofokostana Koostirum) is a type of traditional plants from South Kalimantan which belongs to the Mangkurat species. It is used in the local treatment of many diseases, such as diabetes, and the study conducted on this species showed that a high level of quality is achieved in the treatment of the disease. The study determined the antimicrobial activity of the extract, which contains active ingredients such as vitamins, fatty acids, glycolipids, and sterols. The study showed that the extract has a strong inhibitory effect on the growth of bacteria and fungi, and it can be used as a natural antimicrobial agent. The results of the study confirm the high quality of the extract, and it can be used as a natural agent in the treatment of diseases.