Fisheries Journal, 14(4), 2230-2239 (2024)
http://doi.org/10.29303/jp.v14i4.1297

EVALUATION OF ANTIOXIDANT PROPERTIES AND SENSORY CHARACTERISTICS OF *Rhizophora mucronata* LEAF TEA INFUSION

Evaluasi Potensi Antioksidan Dan Kualitas Sensori Seduhan Daun Mangrove *(Rhizophora mucronata)*

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(Received November 25th 2024; Accepted December 13th 2024)

ABSTRACT

Rhizophora mucronata leaves are known to have antioxidant properties, including the presence of flavonoids and phenolic compounds. The infusion of these mangrove leaves has the potential for development into herbal drinks. This study aims to evaluate the antioxidant potential of *R. mucronata* leaves tea infusion that has been dried using sunlight and an oven. The process involved cleaning the *R. mucronata* mangrove leaves, drying them (either in the sun or in an oven), and grinding them into a powder. This mangrove leaf powder was then brewed with hot water and subsequently dried. The antioxidant capacity of the leaves tea infusion extract was assessed using the DPPH method. Additionally, the sensory acceptance of the mangrove leaves tea infusion was evaluated based on its appearance (color) and aroma. The results demonstrated that the percentage inhibition of the mangrove leaves tea infusion extract dried in the sun ranged from 2.80% to 68.83%, with an IC_{50} of 79.99 ± 0.21 µg/mL. Conversely, the percentage inhibition of the oven-dried mangrove leaves tea infusion extract ranged from 19.25% to 95.41%, with an IC_{50} of 51.55 ± 0.12 µg/mL. Panelists expressed a moderate appreciation for the appearance (color) and aroma of the *R. mucronata* leaves tea infusion. No significant differences were observed in the assessments of appearance and aroma between the oven-dried and sun-dried samples. Overall, the findings suggest that *R. mucronata* leaves possess the potential to be developed into antioxidant-rich herbal drinks that can be appealing to consumers.

Keywords: Antioxidant, DPPH, Infusion, *Rhizophora*, Tea

ABSTRAK

Daun *Rhizophora mucronata* diketahui memiliki sifat antioksidan, karena terdapat kandungan senyawa flavonoid dan fenolik. Seduhan daun mangrove ini berpotensi untuk dikembangkan menjadi minuman herbal. Penelitian ini bertujuan untuk mengevaluasi potensi antioksidan dari

seduhan daun *R. mucronata* yang telah dikeringkan dengan menggunakan sinar matahari dan oven. Proses penelitian meliputi membersihkan daun mangrove *R. mucronata*, mengeringkannya (di bawah sinar matahari maupun menggunakan oven), dan menggilingnya hingga menjadi serbuk. Serbuk daun mangrove kemudian diseduh dengan air panas dan selanjutnya dikeringkan. Kapasitas antioksidan dari ekstrak seduhan daun mangrove dievaluasi dengan menggunakan metode DPPH. Selain itu, penerimaan sensori dari seduhan daun mangrove dievaluasi berdasarkan kenampakan (warna) dan aromanya. Hasil penelitian menunjukkan bahwa persentase penghambatan ekstrak seduhan daun mangrove yang dikeringkan di bawah sinar matahari berkisar antara 2,80% hingga 68,83%, dengan IC_{50} sebesar 79,99 ± 0,21 µg/mL. Sebaliknya, persentase penghambatan ekstrak seduhan daun mangrove yang dikeringkan dengan oven berkisar antara 19,25% hingga 95,41%, dengan IC_{50} sebesar 51,55 ± 0,12 µg/mL. Panelis menilai agak suka terhadap penampilan (warna) dan aroma seduhan daun *R. mucronata*. Tidak ada perbedaan signifikan yang diamati dalam penilaian penampilan dan aroma antara sampel yang dikeringkan dengan oven dan sampel yang dikeringkan dengan sinar matahari. Secara keseluruhan, hasil penelitian ini menunjukkan bahwa daun *R. mucronata* memiliki potensi untuk dikembangkan menjadi minuman herbal dengan antioksidan yang dapat diterima konsumen.

Kata kunci: Antioksidan, DPPH, Seduhan, *Rhizophora*, Teh

INTRODUCTION

Mangrove *Rhizophora mucronata*, known as black mangrove, is a species with significant potential due to its various bioactive compounds (Cahyono *et al*., 2024). Phytochemical screening has identified diverse secondary metabolites from *R. mucronata* extracts, such as flavonoids, terpenoids, and saponins, contributing to its diverse biological properties (Akasia *et al*., 2021). *Rhizophora Mucronata* extracts have been reported to have antibacterial (Mangrio *et al*., 2016) anticancer (Mahmiah *et al*., 2020) and antidiabetic (Sain *et al*., 2020) activities.

In addition, *Rhizophora mucronata* is also known for its antioxidant properties, especially the leaves. Previous studies have reported the antioxidant ability of *R. mucronata* leaves, such as ethyl acetate and n-hexane extracts (Kasitowati *et al*., 2017; Ridlo *et al*., 2017). Sasmito *et al*., (2016) also reported the potential of *R. mucronata* leaf tannin extract with antidiabetic and antioxidant properties. *Rhizophora mucronata* leaf extract from North Sulawesi is also reported to contain pigments with antioxidant properties (Rumengan *et al*., 2021). Previous research also reported that *R. mucronata* leaf extract contains bioactive compounds such as flavonoids and phenolics (Rahmawati *et al*., 2023). Flavonoid and phenolic derivative compound groups are reported to scavenge free radicals, thus potentially preventing oxidative stress-related diseases (Treml & Šmejkal, 2016). Suganthy & Pandima Devi (2016) also reported (+)-catechin compounds with antioxidant properties. The results of these studies have proven the ability of the leaves of *R. Mucronata* to be a source of natural antioxidants in food and health products (Setyawan *et al*., 2022).

Antioxidant compounds play an important role in the health of the human body, especially their function against oxidative stress due to free radicals (Dewanto *et al*., 2018). Antioxidant compounds are the first line of defense against free radical damage, thus preventing oxidative damage that can lead to aging and disease progression (Nimse & Pal, 2015). In addition, free radicals can damage cells and contribute to various chronic diseases such as atherosclerosis, cardiovascular disease, cancer, and neurodegenerative disorders (Roy *et al*., 2022). Antioxidant compounds can help reduce the risk of chronic diseases by minimizing oxidative damage to lipids, proteins, and DNA (Franco *et al*., 2019).

Previous studies have reported the utilization of mangrove leaves in herbal drinks infusion with hot water, namely *Sonneratia ovata* mangrove leaves (Dandi *et al*., 2022), *Sonneratia alba* leaves (Mandang *et al*., 2021), *Sonneratia caseolaris* leaves (Rohman, 2024), *Achantus illicifolius* leaves which have anticancer properties (Safitri *et al*., 2018; Wijayanti *et al*., 2023). The previous literature reported the potential of tea infusion beverages from *R. mucronata* mangrove leaves, namely Analuddin *et al*., (2018) reported the anticholesterol potential of mangrove leaf infusion.

From the description above, it is known that *R. mucronata* leaves contain compounds with antioxidant properties and have the potential to be developed as an infusion beverage. This study evaluated the antioxidant potential of *R. mucronata* leaves tea infusion, which was dried under sunlight and in the oven. This study will also assess the panelists' acceptability of the appearance and aroma of *R. mucronata* mangrove leaf tea infusion.

RESEARCH METHODS

Time and place

This research was conducted in June – November 2023 and took place in the Laboratory of Fishery Product Technology, Sekolah Tinggi Perikanan dan Kelautan (STPL) Palu. The chemistry and analysis in Chemistry Laboratory, Tadulako University.

Materials

The materials used are *Rizhophora mucronata* mangrove leaves, distilled water, and 1,1-diphenyl-2-picrylhydrazyl crystal (DPPH-Sigma). The equipment used, namely glass cups for organoleptic tests, plastic funnels, pans, aluminum cups, wooden stirrers, stoves, filter cloths, ovens (Finco), 100 ml goblets, *rotary vacuum evaporators* (EYELA), *freeze dryers* (Labfreez), desiccators, analytical scales, thermometers, test tubes, erlenmeyers, vortexes, UV-Vis spectrometers ($T90^+$ PG Instruments Ltd).

Preparation of *Rizhophora mucronata* Mangrove Leaf Tea Infusion

Rizhophora mucronata mangrove leaves were obtained from the coast of Palu Bay (Figure 1). The mangrove leaves obtained were at the top and had a soft texture. After that, mangrove leaves are washed using clean water and separated from the leaf bones. The leaves separated from the leaf bones are soaked using clean water for 48-60 hours, and every 4-6 hours, water is changed. After that, boiling is done with 5% whiting water solution for 15 minutes at 100 ^oC. Then, rinsing is done with running water until the rinse water becomes clear again.

Figure 1. Mangrove leaves of *Rizhophora mucronata*

Furthermore, mangrove leaves underwent two types of drying processes: 1) sun drying for approximately one week, with a drying time of 7 hours per day, and 2) oven drying for one

week at approximately 50 ºC, also for 7 hours per day. Once the mangrove leaves were dried, they were ground into a powder.

To prepare the infusion, 2 grams of the mangrove leaf powder were brewed with 100 mL of boiling distilled water (100 ºC). The mixture was then filtered to separate the infusion extract from any debris. Following filtration, the infusion extract was concentrated using a rotary vacuum evaporator and subsequently dried using a freeze-dryer (Figure 2).

Figure 2. *Rhizophora mucronata* Mangrove Leaves Infusion Extract

Antioxidant Activity Assay

The antioxidant activity of *R. mucronata* mangrove leaves infusion extract was evaluated using the DPPH method (Dewanto *et al*., 2021; Molyneux, 2004). The infusion extract of *R. mucronata* leaves was added to ethanol so that a concentration of 100 µg/mL was obtained, and a dilution series of 20, 40, 60, 80, and 100 µg/mL was made. Then, 2 mL of each concentration was added to 2 mL of 50 µM DPPH solution. After that, the mixture was homogenized and kept for 30 minutes in a dark room. Then, measurements were obtained using a UV-Vis spectrophotometer (λ = 517 nm).

The absorbance value of the DPPH solution was measured to determine the IC_{50} value of the infusion extract and vitamin C (comparative control). Then, the percentage inhibition value was plotted on the y-axis, and the extract concentration on the x-axis. The IC_{50} value was evaluated with a linear regression equation (y=a+bx). The assay was performed for three repetitions, and the measurement results were expressed with standard deviation. The percentage inhibition of the infusion extract was calculated using the equation:

$$\text{Inhibition Capacity } (\%) = \frac{Absorbance\ DPPH - Absorbance\ Extract}{Absorbance\ \ DPPH} \times 100\%$$

Sensory Assessment

The sensory assessment was conducted using a hedonic scale to determine panelist acceptance of *R. mucronata* mangrove leaves tea infusion dried in the sun and oven. Assessment parameters include appearance (color) and aroma. The number of panellists was 25 semi-trained panellists. The scores used were 7 (Very like), 6 (Like), 5 (somewhat like), 4 (Neutral), 3 (somewhat dislike), 2 (dislike), and 1 (very dislike).

Data Analysis

Data analysis was carried out by analysis of variance in a randomized group design pattern to determine the effect of drying type on the percentage of inhibition. Sensory assessment data were analyzed with Kruskal Wallis. Analysis using SPSS 20.0.

RESULTS

The capacity inhibition values of *R. mucronata* mangrove leaves infusion extract dried in the sun and oven are presented in Figure 3.

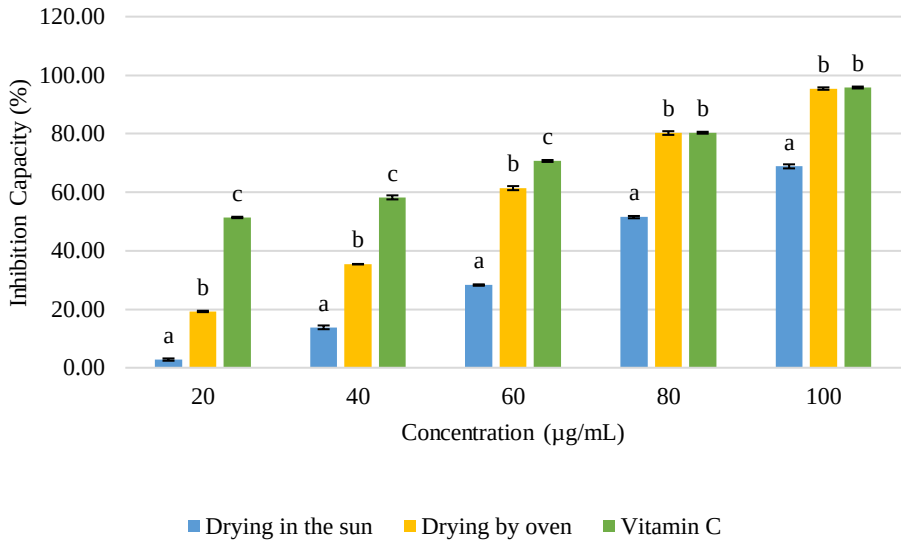

Figure 3. Inhibition of *Rhizophora Mucronata* Mangrove Leaf Infusion Extract

Figure 3 shows that the inhibition ability of mangrove leaves infusion extract dried using the oven is comparable to vitamin C. Statistical analysis showed that different drying methods and concentrations significantly affected the percentage of extract inhibition ($F<0.05$). Furthermore, the IC_{50} value was also evaluated, as seen in Table 1, and was determined to determine the antioxidant power of *R. mucronata* mangrove leaf infusion extract. Table 1 shows the antioxidant power of mangrove leaf infusion extract, both oven-dried and sun-dried, including a strong antioxidant category based on the Blois Category.

Table 1. IC_{50} of *Rhizophora mucronata* Mangrove Leaves Infusion Extract

Sample	IC_{50} (µg/mL)	Regression Equation
Drying by oven	51,55 ± 0,12	y = 0.9858x - 0.8122 R^2 = 0.9923
Drying in the sun	79,99 ± 0.21	y = 0.8486x - 17.88 R^2 = 0.9839
Vitamin C	21,63 ± 0,68	y = 0.5546x + 38.001 R^2 = 0.9848

This study also evaluated the sensory assessment of the appearance and aroma of *R. mucronata* mangrove leaf tea infusion. The sensory assessment of the appearance (color) and aroma of *R. mucronata* mangrove leaf tea infusion can be seen in Figure 4.

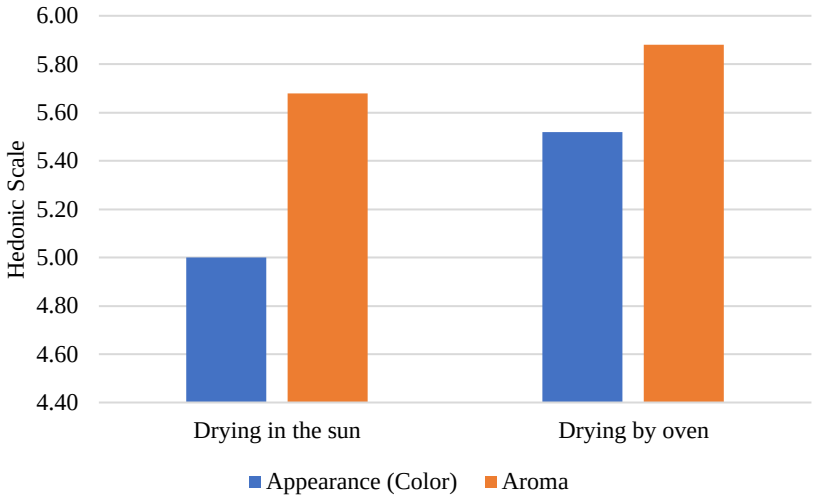

Figure 4. Sensory Assessment of *Rhizophora mucronata* Mangrove Leaf Tea Infusion

Figure 4 shows how panelists rated somewhat, such as the appearance (color) and aroma of *R. mucronata* mangrove leaf tea infusion. Statistical analysis showed no difference (F>0.05) in assessing the appearance and aroma of *R. mucronata* mangrove leaf tea infusion between oven-dried and sun-dried. The appearance (color) of *R. mucronata* mangrove leaf tea infusion can be seen in Figure 5.

Figure 5. *Rhizophora mucronata* Mangrove Leaf Tea Infusion

DISCUSSION

The percentage of DPPH radical inhibition is a parameter used to assess a compound's antioxidant ability (Rohmah, 2022). DPPH is a free radical commonly used in antioxidant activity testing because of its stability and ease of absorbance measurement (Hasanah *et al*., 2023). The results demonstrated differences in the DPPH radical scavenging capacity of infusion extracts using mangrove leaves dried in the sun and using an oven.

Drying natural materials using different methods can affect the antioxidant activity. Based on previous research, it was reported that there is a significant difference between oven drying and sun drying in terms of antioxidant activity (Wahdaningsih *et al*., 2023). Oven drying of natural ingredients tends to produce higher antioxidant activity, while sun drying of natural ingredients often produces lower antioxidant activity (Apsari *et al*., 2021). Oven drying can maintain the content of bioactive compounds such as flavonoids and phenols due to better temperature control and avoiding oxidation due to ultraviolet light (Rababah *et al*., 2015; Sihombing *et al*., 2024). Suganthy & Pandima, (2016) reported that the percentage of inhibition of *R. mucronata* leaves extract with a 100-500 µg/mL concentration ranged from 20.21 to 91.05%, depending on the solvent used. The percentage of inhibition of *R. mucronata* leaves

extract with a concentration of 50-400 µg/mL ranged from 22 to 95%, depending on the solvent used (Ridlo *et al*., 2017). Meanwhile, vitamin C was reported at a concentration of 10 µg/mL C showed an inhibition percentage of 52.74%, and at 60 µg/mL, the inhibition percentage increased to almost 99.86% (Kholifah *et al*., 2023; Nariya *et al*., 2013).

Based on the IC_{50} evaluation, the tea infusion of *R. mucronata* leaves is a strong antioxidant. Blois (1958) categorizes antioxidant power, namely <50 µg/mL (very strong), 50-100 µg/mL (strong), 101-150 µg/mL (moderate), 151-200 µg/mL (weak), and >200 µg/mL (very weak). Rahmawati *et al*. (2023) reported IC_{50} value of *R. mucronata* leaves extract ranged from 59.89-184.78 µg/mL, depending on the solvent used. Another study reported the IC_{50} value of *R. mucronata* leaf extract which is 0.04 µg/mL using methanol solvent and 2.34 µg/mL with ethyl acetate solvent (Bulan *et al*., 2022). Meanwhile, the IC_{50} vitamin C value was reported to be 2,260-12,360 µg/mL (Kholifah *et al*., 2023; Maryam *et al*., 2023).

The results of the sensory assessment demonstrated that panelists liked the aroma of *R. mucronata* leaf infusion because it contains various chemical compounds, including flavonoids and phenolic compounds, which can provide a distinctive and fragrant aroma (Parikesit, 2019). Literature studies report that aroma-giving compounds are found in tea leaves, and also found in *R. mucronata* leaf infusion extract. Suganthy & Pandima, (2016) reported that catechin compounds in *R. mucronata* leaf extract can provide a distinctive aroma. In addition, quercetin derivatives have also been reported (Adhikari *et al*., 2016). Another study also reported the presence of benzophenone compounds in *R. mucronata* leaf extract (Rahmawati *et al*., 2023). Benzophenone has a soft sweet aroma, similar to rose (Wang & Liang, 2014). The results indicate that *R. mucronata* mangrove leaves have the potential to be developed into herbal drinks with antioxidant properties and can be accepted by consumers.

CONCLUSIONS

This study demonstrates that the tea infusion of *Rhizophora mucronata* mangrove leaves, both oven-dried and sun-dried, has strong antioxidant activity and has received favorable sensory evaluation because it has an appearance and aroma similar to steeping tea leaves. In developing an antioxidant herbal drink, mangrove leaf infusion extract must be tested for toxicity.

ACKNOWLEDGMENTS

All authors would acknowledge the students of STPL Palu for being panelists in the sensory testing. The authors would also accept the Director of STPL Palu so that this research can be carried out.

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