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# Optimizing Ultrasonic Extraction of *Enhalus acoroides* to Enhance Physicochemical Traits and Antioxidant Activity

## Optimalisasi Ekstraksi Ultrasonik *Enhalus acoroides* untuk Meningkatkan Sifat Fisikokimia dan Aktivitas Antioksidan

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**ABSTRACT**

*Enhalus acoroides* is a seagrass species known for its antioxidant potential due to its flavonoid content, including quercetin. This study aimed to optimize the extraction time using the ultrasound-assisted extraction (UAE) method based on physicochemical parameters and antioxidant activity. The simplicia was extracted for 20, 30, and 40 minutes, each in triplicate, using 96% ethanol acidified with 1% HCl. The results demonstrated that extraction time significantly influenced the physicochemical properties, including yield (highest at 30 minutes: 40.2%), total phenolic content (1.153 mg GAE/g), total flavonoid content (0.318 mg QE/g), quercetin content (0.316 mg/g), and antioxidant activity ($IC_{50}$ = 58.44 ppm), whereas no significant effect was observed on total ash content. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and triterpenoids. Based on these findings, a 30-minute extraction time is recommended as the optimal condition for obtaining *E. acoroides* extract with the highest antioxidant activity.

**ABSTRAK**

Lamun (*Enhalus acoroides*, seagrass) yang berpotensi sebagai sumber antioksidan alami karena kandungan flavonoid, termasuk kuersetin. Penelitian ini bertujuan mengoptimalkan waktu ekstraksi menggunakan metode *ultrasound-assisted extraction* berdasarkan karakteristik fisikokimia dan aktivitas antioksidan. Simplisia diekstraksi selama 20, 30, dan 40 menit menggunakan pelarut etanol 96%-HCl 1%. Hasil menunjukkan bahwa waktu ekstraksi berpengaruh terhadap rendemen, kadar fenolik (1,153 mg GAE/g), flavonoid (0,318 mg QE/g), kuersetin (0,316 mg/g), dan aktivitas antioksidan ($IC_{50}$ = 58,44 ppm), tetapi tidak berpengaruh signifikan terhadap kadar abu total. Uji fitokimia menunjukkan keberadaan alkaloid, flavonoid, tanin, saponin, dan triterpenoid. Waktu ekstraksi 30 menit merupakan kondisi optimal.

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## 1. INTRODUCTION

*Enhalus acoroides* is a species of seagrass commonly found in the shallow marine and coastal waters of Indonesia. Among the various species of seagrass, *E. acoroides* is considered the most abundant and often dominates seagrass meadows (Sarinawaty et al., 2020). Previous studies have revealed that *E. acoroides* extract contains various secondary metabolites, such as flavonoids, alkaloids, tannins, and saponins, which exhibit notable biological activities, including antioxidant properties (Senthilkumar et al., 2016; Dewi et al., 2018).

The antioxidant activity of *E. acoroides* is strongly associated with its flavonoid content. Tangon et al. (2020) reported that methanol extracts of *E. acoroides* demonstrated an $IC_{50}$ value of 0.301 mg trolox/g, indicating moderate to strong antioxidant capacity. Additionally, *E. acoroides* has shown superior inhibition of the enzyme α-glucosidase compared to other seagrass species, such as *Thalassia hemprichii* and *Cymodocea rotundata* (Widyanto, 2018). Flavonoids exert their antioxidant effects by neutralizing free radicals, reducing reactive oxygen species (ROS), and enhancing endogenous antioxidant defense mechanisms (Menajang, 2020; Thakur et al., 2018).

The efficacy of bioactive compounds is largely influenced by the extraction method employed. Conventional methods like maceration present several limitations, including lengthy extraction times, high solvent volumes, and potential thermal degradation of compounds. As a sustainable alternative, green extraction technologies such as Ultrasound-Assisted Extraction (UAE) offer improved efficiency. UAE operates through acoustic cavitation, producing microbubbles in the liquid phase below boiling point, which disrupt plant cell walls and facilitate the rapid release of active compounds (Kumar et al., 2021).

One of the critical parameters in UAE is extraction time. An extraction period that is too brief may not sufficiently extract bioactive compounds, while overly extended durations can lead to compound instability due to oxidative or thermal degradation, thereby reducing extract potency (Xu et al., 2017; Miluska et al., 2023). Other variables influencing UAE include frequency, power, duty cycle, temperature, solvent type, and the solvent-to-material ratio (Kumar et al., 2021).

This study aims to determine the optimal extraction time using the UAE method for *E. acoroides* by evaluating the physicochemical characteristics and antioxidant activity of the resulting extracts.

## 2. METHODS

### 2.1. Preparation of *E. acoroides* Simplicia

Fresh *E. acoroides* seagrass was collected from the coastal waters of Kalih Island, Serang, Banten. The samples were manually cleaned to remove debris and epiphytes. Following sorting, the material was dried in a drying chamber for 14 days, ground using a hand blender, and sieved through an 80-mesh screen. The resulting powder was stored in a dry, dark container until further use.

### 2.2. Extraction of *E. acoroides*

The extraction was conducted using the Ultrasound-Assisted Extraction (UAE) method with a UP400St sonicator (Hielscher Ultrasonics, Teltow, Germany). Fifty grams of dried simplicia were immersed in 500 mL of 96% ethanol containing 1% HCl (v/v) (Merck, Darmstadt, Germany). The mixture underwent sonication at 40 kHz and 30°C for 20, 30, and 40 minutes, respectively, in triplicate. The filtrate was separated from the residue using Whatman No. 40 filter paper and concentrated using a rotary evaporator (Heidolph, Schwabach, Germany) at 40°C to obtain a viscous extract.

### 2.3. Physicochemical Analysis

The physicochemical parameters assessed included yield, moisture content, total ash content, total phenolic content (using the Folin–Ciocalteu method with gallic acid standard; Merck, Darmstadt, Germany), total flavonoid content (via $AlCl_3$ complexation with quercetin standard; Sigma-Aldrich, St. Louis, USA), and quercetin concentration determined by spectrophotometry. Phytochemical screening was performed to identify secondary metabolite groups: alkaloids using Mayer's and Dragendorff's reagents, flavonoids using magnesium and HCl, saponins via foam test, tannins with $FeCl_3$, and steroids/triterpenoids using Liebermann–Burchard reagent.

### 2.4. Antioxidant Activity Assay

The antioxidant activity of the extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Sigma-Aldrich, St. Louis, USA). A stock solution was prepared by dissolving 10 mg of extract in ethanol to a final volume of 10 mL, yielding a concentration of 1000 ppm. This stock solution was then diluted to 50, 100, 150, 200, and 250 ppm. For each concentration, 2 mL of sample was mixed with 2 mL of 100 ppm DPPH solution. The mixtures were vortexed and incubated at 37°C for 30 minutes in the dark. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The inhibition percentage was calculated using the formula:

$$\%\ Inhibition = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

The $IC_{50}$ value, representing the concentration at which 50% of DPPH radicals were inhibited, was calculated using a regression equation derived from the plot of inhibition percentage against sample concentration (Bo-Wei Zhang et al., 2017).

### 2.5. Experimental Design and Sampling Technique

A completely randomized design (CRD) was employed with a single factor: extraction time (20, 30, and 40 minutes), each performed in triplicate. Samples were selected using simple random sampling.

### 2.6. Data Analysis

Statistical analysis of physicochemical and antioxidant activity data involved the Kolmogorov-Smirnov test for normality and Levene's test for homogeneity. When assumptions were met ($p > 0.05$), one-way ANOVA was applied. Significant differences ($p < 0.05$) were further analyzed using Tukey's HSD post hoc test. All analyses were conducted using IBM SPSS Statistics version 28 (IBM Corp., New York, USA).

## 3. RESULTS AND DISCUSSION

### 3.1. Yield of Extract

Yield is a fundamental parameter to evaluate the extraction efficiency and indicates the amount of bioactive compounds extracted. As shown in **Table 1**, the yield increased from 23.8% ± 2.3% at 20 minutes to a peak of 40.2% ± 3.1% at 30 minutes, then declined slightly to 35.0% ± 3.3% at 40 minutes.

Analysis of variance (ANOVA) revealed a significant effect of extraction time on yield ($p = .004$; $\eta^2 = 0.42$). Further analysis using Tukey HSD showed that the 30-minute yield was significantly higher than at 20 minutes ($p = .003$), while no significant difference was found between 30 and 40 minutes ($p = .085$). These findings confirm 30 minutes as the optimal duration for maximum extraction efficiency, as prolonged ultrasonication may lead to the degradation of sensitive compounds.

Ultrasonic cavitation contributes to yield enhancement by disrupting the plant cell wall, thereby increasing solvent penetration and compound diffusion (Zhu et al., 2016). However, overexposure can result in functional group breakdown and oxidation of phenolics and flavonoids (Kumar et al., 2021).

**Table 1.** Physicochemical Characteristics of *E. acoroides* Extracts at Different Extraction Times

| Extraction Time (min) | Moisture Content (%) | Ash Content (%) | Yield (%) | Total Phenolics (mg GAE/g) | Total Flavonoids (mg QE/g) | Quercetin (mg/g) |
|---|---|---|---|---|---|---|
| 20 | 2.94 ± 0.19 | 4.11 ± 0.54 | 23.8 ± 2.3 | 0.905 ± 0.103 | 0.256 ± 0.031 | 0.246 ± 0.024 |
| 30 | 3.19 ± 0.34 | 4.36 ± 0.44 | 40.2 ± 3.1 | 1.153 ± 0.129 | 0.318 ± 0.024 | 0.316 ± 0.023 |
| 40 | 4.14 ± 0.48 | 4.88 ± 0.36 | 35.0 ± 3.3 | 0.997 ± 0.091 | 0.258 ± 0.028 | 0.249 ± 0.021 |

### 3.2. Moisture Content

Moisture content is a critical parameter in evaluating extract quality, as excessive moisture can accelerate microbial growth and reduce chemical stability during storage. According to the Indonesian Herbal Pharmacopeia (2017), the moisture content of concentrated extracts should not exceed 10%.

As shown in **Table 1**, moisture content increased progressively with extraction time: 2.94 ± 0.19% at 20 minutes, 3.19 ± 0.34% at 30 minutes, and 4.14 ± 0.48% at 40 minutes. All values remained well within the acceptable range, indicating effective drying and minimal solvent residue.

The observed increase in moisture content with extended sonication can be attributed to the greater solvent penetration into plant tissues, facilitated by ultrasonic disruption. This enhances the water-binding capacity of the matrix, which is then retained in the extract (Buanasari et al., 2019).

ANOVA results revealed that extraction time had a statistically significant effect on moisture content ($p = .021$; $\eta^2 = 0.31$). Tukey HSD post hoc analysis showed a significant difference between the 40-minute and 20-minute groups ($p = .016$), while no significant differences were observed between 20 and 30 minutes ($p = .093$) or between 30 and 40 minutes ($p = .087$).

These findings are consistent with the results of Kruszewski and Boselli (2024), who reported that longer extraction durations led to increased moisture content in blackcurrant pomace due to enhanced solvent absorption. Thus, while longer extraction may increase moisture, all levels remained within the recommended limits for stable storage.

### 3.3. Ash Content

Ash content is a nonspecific quality indicator for herbal extracts, reflecting residual mineral content following high-temperature incineration. This parameter encompasses both natural inorganic constituents and external contaminants such as soil particles or insoluble residues.

**Table 1** indicates that ash content across the three extraction durations was relatively stable: 4.11% at 20 minutes, 4.36% at 30 minutes, and 4.88% at 40 minutes. These values are well below the maximum threshold of 16.6% set by the Indonesian Herbal Pharmacopeia (2017).

Statistical analysis via ANOVA showed no significant differences in ash content among the extraction times ($p = .147$; $\eta^2 = 0.11$). The stability of ash content may be attributed to the inert nature of mineral substances, which are generally unaffected by the sonication process. This outcome aligns with findings from Rathnakumar (2017), who reported similar trends in pineapple peel extract, where extraction duration had no substantial impact on ash content.

### 3.4. Phytochemical Screening

Qualitative phytochemical screening **(Table 2)** revealed that *E. acoroides* extract contains various secondary metabolites,

including alkaloids, flavonoids, tannins, triterpenoids, and saponins. These findings are consistent with previous reports by Dewi et al. (2018) and Widyanto et al. (2018), which identified similar bioactive compounds in *E. acoroides*. Supporting this, Amudha et al. (2017) also reported the presence of phenolics, flavonoids, tannins, alkaloids, saponins, and steroids in extracts of *E. acoroides*, underscoring its phytochemical richness.

The antioxidant potential of *E. acoroides* is largely attributed to its phenolic and flavonoid content. These compounds are known for their free radical scavenging properties and ability to modulate oxidative stress (Shi et al., 2022). Tannins, particularly proanthocyanidins, contribute to antioxidant activity through hydrogen donation and metal ion chelation, thereby preventing the formation of reactive oxygen species (ROS) (Cosme et al., 2025). Saponins, with their amphipathic structure, help stabilize cell membranes and are known to enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in vitro (Juang & Liang, 2020).

Alkaloids also play a role in antioxidant defense by inhibiting ROS-producing enzymes and directly interacting with reactive species like superoxide and hydroxyl radicals. Heinrich et al. (2021) highlighted the potential of certain alkaloids as lipid oxidation inhibitors, providing a protective mechanism for cellular membranes under oxidative stress.

**Table 2.** Phytochemical Constituents Identified in *E. acoroides* Extract

| Compound Group | Test Reagent | Observation | Result |
|---|---|---|---|
| Alkaloids | Mayer & Dragendorff | White and orange precipitates | + |
| Flavonoids | Mg + HCl | Yellow-orange coloration | + |
| Saponins | Foam Test | Persistent froth for ~10 min | + |
| Tannins | $FeCl_3$ | Greenish-black coloration | + |
| Steroids | Liebermann–Burchard | No reddish-brown solution | - |
| Triterpenoids | Liebermann–Burchard | Reddish-purple coloration | + |

(+): Present; (-): Not detected.

### 3.5. Total Phenolic Content

Phenolic compounds are key secondary metabolites with strong antioxidant properties. In this study, the total phenolic content (TPC) of *E. acoroides* extracts was measured at three different extraction times. As presented in **Table 1**, the TPC values were 0.905 ± 0.103 mg GAE/g at 20 minutes, 1.153 ± 0.129 mg GAE/g at 30 minutes, and 0.997 ± 0.091 mg GAE/g at 40 minutes.

Statistical analysis confirmed a significant influence of extraction time on TPC ($p = .009$; $\eta^2 = 0.39$). Tukey HSD post hoc analysis revealed a significant increase in phenolic content at 30 minutes compared to 20 minutes ($p = .007$), while no significant difference was observed between 30 and 40 minutes ($p = .091$).

These results highlight 30 minutes as the optimal extraction time for phenolic recovery. When compared with previous findings by Widyanto et al. (2018), who reported only 0.2876 mg GAE/g using maceration, the UAE method demonstrates superior extraction efficiency. This enhancement can be attributed to the ability of ultrasonic waves to disrupt plant cell walls and release bound phenolic compounds more effectively (Kumar et al., 2021).

A similar pattern was observed in the study by Biswas et al. (2023), where the TPC of *Corchorus olitorius* leaves increased up to an optimal time of 37.2 minutes before plateauing. This suggests that excessive extraction time may reduce phenolic content due to degradation or oxidative breakdown.

Phenolic compounds, including simple phenols, phenolic acids, tannins, lignins, and flavonoids, exhibit antioxidant activity by donating electrons or hydrogen atoms to neutralize free radicals. Their chemical structure—particularly the hydroxyl groups on aromatic rings—enables interactions with ROS and metal ions that catalyze oxidative stress (Tangon et al., 2020; Shi et al., 2022).

### 3.6. Total Flavonoid Content

As presented in **Table 1**, the total flavonoid content of *E. acoroides* extracts showed a marked increase at 30 minutes (0.318 ± 0.024 mg QE/g), compared to 20 minutes (0.256 ± 0.031 mg QE/g) and 40 minutes (0.258 ± 0.028 mg QE/g).

ANOVA results confirmed that extraction time significantly affected total flavonoid content ($p = .012$; $\eta^2 = 0.38$). Tukey HSD post hoc test revealed significant differences between 30 and 20 minutes ($p = .011$), as well as between 30 and 40 minutes ($p = .015$), but not between 20 and 40 minutes ($p = .998$).

The increase observed at 30 minutes likely reflects optimal disruption of plant cell structures, facilitated by acoustic cavitation from ultrasonic waves. These microbubbles collapse violently, creating localized high energy that enhances solvent penetration, membrane permeability, and solute diffusion (Chemat et al., 2022).

However, prolonged extraction beyond 30 minutes may lead to thermal degradation of flavonoids. These compounds are known to be thermolabile and susceptible to oxidative damage, resulting in structural breakdown and reduced concentration. This degradation process aligns with kinetic principles governing bioactive compound stability (Nguyen, 2022).

These findings are consistent with Biswas et al. (2023), who noted that flavonoid content in *C. olitorius* peaked at 37.2 minutes and declined thereafter. Additionally, Tangon et al. (2020) reported a

significantly lower flavonoid content (0.096 mg QE/g) from *E. acoroides* extracted via maceration, further demonstrating the advantages of UAE.

Flavonoids, as a subclass of polyphenols, are present in smaller quantities than total phenolics. According to Thakur (2018), polyphenolic groups include phenolic acids, coumarins, flavonoids, tannins, and lignins, each contributing to antioxidant activity through distinct mechanisms.

### 3.7. Quercetin Content

Quercetin, a flavonol subclass of flavonoids, is recognized for its potent antioxidant activity. As indicated in **Table 1**, the quercetin concentration in *E. acoroides* extracts varied with extraction time: 0.246 ± 0.024 mg/g at 20 minutes, 0.316 ± 0.023 mg/g at 30 minutes, and 0.249 ± 0.021 mg/g at 40 minutes.

Statistical analysis (ANOVA) showed a significant effect of extraction time on quercetin levels ($p = .004$; $\eta^2 = 0.51$). Tukey HSD analysis confirmed that the 30-minute extract had significantly higher quercetin content compared to 20 minutes ($p = .004$) and 40 minutes ($p = .006$), with no significant difference between 20 and 40 minutes ($p = .998$).

The increased quercetin yield at 30 minutes likely results from optimal ultrasonic energy facilitating cell lysis and release of intracellular compounds. At 20 minutes, cavitation may be insufficient to fully disrupt cellular structures, while at 40 minutes, degradation processes such as thermal breakdown, isomerization, or cross-interactions may reduce quercetin levels (Chemat et al., 2022; Miluska et al., 2023).

Kumar and Smita (2014) reported similar trends in cabbage, where quercetin concentration peaked at 40 minutes of UAE. Likewise, Kamal et al. (2023) found that UAE of *Chromolaena odorata* yielded higher quercetin content than maceration, with maximum concentration at 60 minutes.

Given quercetin's capacity to neutralize free radicals and chelate transition metals (Xu et al., 2019), its concentration is a critical determinant of antioxidant potency. Thus, the increased quercetin content at 30 minutes may contribute significantly to the antioxidant activity observed in this study.

### 3.8. Antioxidant Activity

The antioxidant activity of *E. acoroides* extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. As shown in **Figure 1**, $IC_{50}$ values were recorded as 68.53 ± 1.49 ppm at 20 minutes, 58.44 ± 2.06 ppm at 30 minutes, and 64.83 ± 1.54 ppm at 40 minutes. According to the classification by Thakur, Ashwini, and Awanish (2018), all extracts exhibited strong antioxidant activity ($IC_{50}$ between 50–100 ppm), with the 30-minute extract demonstrating the highest potency.

$IC_{50}$ represents the concentration required to inhibit 50% of DPPH radicals. A lower $IC_{50}$ value indicates greater antioxidant capacity. Compared to the ethanol extract obtained via maceration, which had an $IC_{50}$ of 168.15 ppm (Widyanto, 2018), the UAE method clearly improved antioxidant efficacy. This enhancement is attributed to the acoustic cavitation mechanism of UAE, which facilitates rapid and efficient release of bioactive compounds (Chemat et al., 2022).

ANOVA revealed significant differences in $IC_{50}$ across extraction times ($p = .002$; $\eta^2 = 0.55$). Tukey HSD post hoc analysis showed that the 30-minute extract was significantly different from both 20 minutes ($p = .002$) and 40 minutes ($p = .009$), while the 20-minute and 40-minute extracts also differed significantly ($p = .041$).

The lowest $IC_{50}$ value obtained at 30 minutes coincides with the highest levels of total phenolics, flavonoids, and quercetin (see **Table 1**), suggesting a synergistic effect of these compounds in enhancing antioxidant activity. This observation is consistent with kinetic principles of extraction, wherein insufficient extraction duration limits compound release, while overexposure can cause thermal and oxidative degradation (Xu et al., 2017; Chemat et al., 2022).

Notably, Tjandrawinata and Nurkolis (2024) reported that UAE extracts of *E. acoroides* exhibited greater antioxidant potential than Trolox, a water-soluble vitamin E analog commonly used as a standard. Trolox is known for its high stability in in vitro assays, underscoring the robust antioxidant nature of *E. acoroides*.

Chaudhary et al. (2023) highlighted that phenolics and flavonoids function as electron donors, terminate oxidative chain reactions, and protect biomolecules such as DNA, proteins, and lipids from oxidative stress.

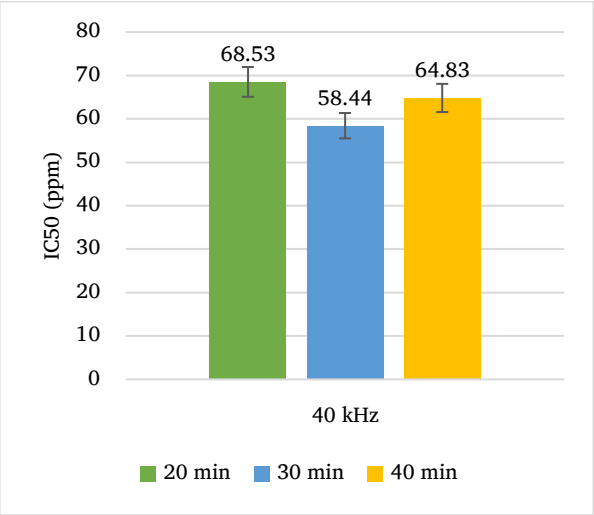


**Figure 1.** $IC_{50}$ Values of *E. acoroides* Extracts at Various Extraction Durations

## 4. CONCLUSION

This study demonstrates that extraction time significantly influences the physicochemical characteristics and antioxidant activity of *Enhalus acoroides* extracts obtained through ultrasound-assisted extraction (UAE). Among the evaluated parameters, 30 minutes was identified as the optimal extraction duration, yielding the highest values for extract recovery (40.2%), total phenolic

content (1.153 mg GAE/g), total flavonoid content (0.318 mg QE/g), quercetin (0.316 mg/g), and antioxidant activity ($IC_{50}$ = 58.44 ppm). In contrast, extraction time had no significant effect on total ash content, which remained within pharmacopeial limits.

Qualitative phytochemical screening confirmed the presence of major antioxidant-related compounds such as alkaloids, flavonoids, tannins, saponins, and triterpenoids. These constituents collectively contribute to the antioxidant capacity of *E. acoroides*, validating its potential as a promising natural antioxidant source.

Overall, the application of UAE with a 30-minute extraction time offers an efficient, reproducible, and green approach for obtaining high-quality antioxidant-rich extracts from *E. acoroides*, with broad implications for nutraceutical and pharmaceutical product development.

## AUTHOR CONTRIBUTIONS

Siti Mardiyanti: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization.

Effionora Anwar*: Supervision, Resources, Writing – review & editing.

Yesi Desmiaty: Methodology, Validation, Data curation, Visualization.

Siti Sadiah: Validation, Formal analysis, Writing – review & editing.

All authors have read and agreed to the published version of the manuscript.

## INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

## INFORMED CONSENT STATEMENT

Not applicable.

## DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## REFERENCES

Biswas, A., Dey, S., Xiao, A., Deng, Y., Birhanie, Z. M., Roy, R., Akhter, D., Liu, L., & Li, D. (2023). Ultrasound-assisted extraction of antioxidant phenolics from *Corchorus olitorius* leaves: A response surface optimization. *Chemical and Biological Technologies in Agriculture, 10*, Article 64. https://chembioagro.springeropen.com/articles/10.1186/s40538-023-00443-2

Bo-Wei, Z., Xia, L., Wen-Long, S., Yan, X., Zhi-Long, X., Chun-Lin, Z., & Yue-Sheng, D. (2017). Dietary flavonoids and akarbose synergistically inhibit α-glucosidase and lower postprandial blood glucose. *Journal of Agricultural and Food Chemistry, 65*(38), 8319–8330. Here's the DOI for that article, https://doi.org/10.1021/acs.jafc.7b02531

Buanasari, F., Febrianto, Y., Cholifah, & Chakim, A. (2019). Potensi metode ultrasound-assisted extraction (UAE) dalam mengekstrak senyawa aktif dari bahan alam. *Jurnal Farmasi & Sains Indonesia, 2*(1), 106–112. https://doi.org/10.35891/jfsi.v2i1.15

Chaudhary, A., Yadav, R., & Singh, P. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Antioxidants, 12*(5), 789. https://doi.org/10.3390/antiox12050789

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2022). Ultrasound-assisted extraction of food and natural products: Mechanisms, techniques, combinations, protocols and applications. *Ultrasonics Sonochemistry, 88*, 106040. https://doi.org/10.1016/j.ultsonch.2022.106040

Cosme, F., Pinto, T., & Vilela, A. (2025). Phenolic compounds and antioxidant activity: Contribution of tannins. *Molecules, 30*(2), 312. https://doi.org/10.3390/molecules30020312

Dewi, C. S. U., Kasitowati, R. D., & Siagian, J. A. (2018). Phytochemical compounds of *Enhalus acoroides* from Wanci Island (Wakatobi) and Talango Island (Madura) Indonesia. *IOP Conference Series: Earth and Environmental Science, 137*, 012030. https://doi.org/10.1088/1755-1315/137/1/012045

Heinrich, M., Bertin, J., Frei, B., & Georgiev, V. (2021). Alkaloids used as medicinal and antioxidant agents: A comprehensive review. *Molecules, 26*(20), 6335. https://doi.org/10.3390/molecules26206335

Juang, T., & Liang, H. (2020). Saponins as natural antioxidants and anticancer agents: A review. *Molecules, 25*(20), 4716. https://doi.org/10.3390/molecules25204716

Kamal, A. A., Mohamad, M., Masri, M. N., Ter, T. P., Shoparwe, N. F., & Lim, J. W. (2023). Comparison of quercetin yield obtained using conventional and ultrasound-assisted extraction from *Chromolaena odorata*. *AIP Conference Proceedings, 2669*(1), 030012. https://doi.org/10.1063/5.0122022

Kementerian Kesehatan Republik Indonesia. (2017). *Farmakope Herbal Indonesia* (Edisi ke-2). Jakarta: Kementerian Kesehatan Republik Indonesia.

Kruszewski, B., & Boselli, E. (2024). Blackcurrant pomace as a rich source of anthocyanins: Ultrasound-assisted extraction under different parameters. *Applied Sciences, 14*(2), 821. https://doi.org/10.3390/app14020821

Kumar, B., Smita, K., & Kumar, B. (2014). Ultrasound-assisted extraction of quercetin from cabbage. *International Journal of Pharmaceutical Sciences and Research, 5*(9), 3779–3783. https://ijpsr.com/bft-article/ultrasound-assisted-extraction-of-quercetin-from-cabbage

Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound-assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrasonics Sonochemistry, 70*, 105325. https://doi.org/10.1016/j.ultsonch.2020.105325

Menajang, F. S., Mahmudi, M., Yanuhar, U., & Herawati, E. Y. (2020). Evaluation of phytochemical and superoxide dismutase activities of *Enhalus acoroides* (Lf) Royle from coastal waters of North Sulawesi, Indonesia. *Veterinary World, 13*(4), 676–681. https://doi.org/10.14202/vetworld.2020.676-680

Miluska, C. Y., Vesela, I. C., Hristo, R. K., Dasha, M., Albert, I. K., & Anna, L. (2023). Ultrasound-assisted extraction of antioxidant bioactive compounds from wastes of rapeseed industry and their application in delaying rapeseed oil oxidation. *Environmental Technology & Innovation, 30*, 102242. https://doi.org/10.1016/j.eti.2023.103081

Nguyen, A. T. (2022). Investigation of the effects of extraction temperature and time on bioactive compounds content from garlic (*Allium sativum* L.) husk. *Frontiers in Sustainable Food Systems, 6*, 1004281. https://doi.org/10.3389/fsufs.2022.1004281

Rathnakumar, K., Anal, A. K., & Lakshmi, K. (2017). Optimization of ultrasonic-assisted extraction of bioactive components from pineapple waste. *International Journal of Agriculture, Environment and Biotechnology, 10*(5), 553–563. https://ndpublisher.in/admin/issues/IJAEBv10n5e.pdf

Sarinawaty, P., Idris, F., & Nugraha, A. H. (2020). Karakteristik morfometrik lamun *Enhalus acoroides* dan *Thalassia hemprichii* di Pesisir Pulau Bintan. *Journal of Marine Research, 9*(4), 474–484. https://doi.org/10.1016/j.eti.2023.103081

Senthilkumar, P., Santhosh Kumar, D. S. R., Sudhagar, B., Vanthana, M., Parveen, M. H., & Sarathkumar, S. (2016). Seagrass-mediated silver nanoparticles synthesis by *Enhalus acoroides* and its α-glucosidase inhibitory activity from the Gulf of Mannar. *Journal of Nanostructure in Chemistry, 6*, 275–280. https://doi.org/10.1007/s40097-016-0200-7

Shi, L., Zhao, W., Yang, Z., Subbiah, V., & Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environmental Science and Pollution Research, 29*, 81112–81129. https://doi.org/10.1007/s11356-022-23337-6

Tangon, E., Canencia, O. P., Del Rosario, R. M., & Alivio, E. R. (2020). Antioxidant activity of methanol extracts of *Enhalus acoroides* and *Thalassia hemprichii* from the coastal water of Carmen, Agusan Del Norte, Philippines. *International Journal of Biosciences, 16*(6), 178–184. https://doi.org/10.12692/ijb/16.6.178-184

Thakur, P., Kumar, A., & Kumar, A. (2018). Targeting oxidative stress through antioxidants in diabetes mellitus. *Journal of Drug Targeting, 26*(9), 766–776. https://doi.org/10.1080/1061186X.2017.1419478

Tjandrawinata, R. R., & Nurkolis, F. (2024). Ultrasound-assisted extraction of *Enhalus acoroides* and its antioxidant activity against DPPH radical. *Journal of Marine-Based Natural Products, 12*(2), 101–110. https://doi.org/10.3390/md22080365

Widyanto, A., Anwar, E., & Nurhayati, T. (2018). In vitro assay of alpha-glucosidase inhibitor activities of three seagrasses from Banten Bay, Indonesia. *Pharmacognosy Journal, 10*(5), 907–910. https://doi.org/10.5530/pj.2018.5.152

Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. *Molecules*, *24*(6), 1123. https://doi.org/10.3390/molecules24061123

Xu, D. P., Zheng, J., Zhou, Y., Li, Y., Li, S., & Li, H. B. (2017). Ultrasound-assisted extraction of natural antioxidants from the flower of *Limonium sinuatum*: Optimization and comparison with conventional methods. *Food Chemistry, 217*, 552–559. https://doi.org/10.1016/j.foodchem.2016.09.013

Zhu, Z., Guan, Q., Guo, Y., He, J., Liu, G., Li, S., Barba, F. J., & Jaffrin, M. Y. (2016). Green ultrasound-assisted extraction of anthocyanin and phenolic compounds from purple sweet potato using response surface methodology. *International Agrophysics, 30*, 113–122. https://doi.org/10.1515/intag-2015-0066