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# Extraction of bioactive components on Indonesian seagrass (*Syringodium isoetifolium*) using green emerging technology

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**Abstract**

*Syringodium isoetifolium* was seagrass widely grown on the coast of Indonesia, and was the phytochemical source. This research was conducted to maximalize the extraction of their phytochemicals and bioactive compounds. Firstly, seagrass powder was extracted with different solvent polarities i.e. water, 50% ethanol, and 100% ethanol. Secondly, extraction was continued using different extraction techniques i.e. microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE-Bath system and UAE-Probe system), and conventional with the best solvent. Phytochemicals, bioactive compounds, antioxidant activity, and scanning electron microscope (SEM) were analyzed on the seagrass extracts. As result, the total phytochemical kind (i.e. terpene, polyphenols, alkaloid, and amino acid derivative groups) in the most dominant was had by 50% ethanol extract. Accordingly, extraction using the UAE-Probe with 50% ethanol as solvent able to obtain the richest bioactive compound, the most damaged cell microstructure, and the strongest antioxidant activity. Interestingly, quercetin was only detected dominant in UAE-Probe extract. Therefore, UAE-Probe with 50% ethanol solvent was the best method for recovering the valuable components in seagrass.

**Keywords:** seagrass; ultrasound; microwave; bioactive compound; quercetin.

**Practical Application:** Extraction of rich bioactive compounds in *Syringodium isoetifolium* using an ultrasonic-probe system is the yieldest.

## 1 Introduction

*Syringodium isoetifolium* is a seagrass that can be found in several seacoasts in Indonesia. *Syringodium isoetifolium* grows widely to form a seagrass meadow which plays an important role in the coastal ecosystem. Recently, seagrass being interested to be explored due to its rich phytochemicals. Seagrass such as *Enhalus acoroides, Halophila decipiens, Cymodocea rotundata, Thalassia hemprichii,* and *Cymodocea cerulata* contain bioactive compounds such as flavonoids, tannins, and phenolics. These species are commonly used by local residents an external medicine (itching-skin diseases and external wounds) and internal medicine, such as heart, cancer, and kidney disease (Zulkifli et al., 2021).

The extraction technique with appropriated solvent polarities plays an important role in the extraction of phytochemical compounds. (Susilo et al., 2021) resulted in the extraction of rich antibacterial compounds in seaweed (*Sargassum cristaefolium*) using different solvent polarities. Solvent polarities for extraction in polar up to non-polar (such as water, ethanol, acetone, and their mixtures) significantly affected phenolic, flavonoids, resveratrol, and antioxidant content on extracted oil of *Vitis labrusca* seeds (Dalposso et al., 2021).

Recently, extraction was assisted by certain energy such as microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) able to disintegrate the material cell severely. Therefore, they ease phytochemical components released which leads to enhanced extraction yield, and extracted component quality is maintained in the optimum process (Lefebvre et al., 2021; Yin et al., 2021). In this research, extraction of bioactive components on *Syringodium isoetifolium* was done in two steps. Firstly, extraction used different solvent polarities (polar up to non-polar) to get the best solvent in total phytochemical extraction. Secondly, the extraction was maximalized by different extraction techniques i.e. MAE, UAE-bath, UAE-probe, and conventional with the best solvent.

## 2 Materials and methods

### *2.1 Seagrass pulverized*

Fresh seagrass (*Syringodium isoetifolium*) from Indonesia sea was washed with fresh water to remove attached dirt and salt particles, afterward dried using an oven at 40 °C to achieve constant weight (moisture 13.87 ± 0.20%). Dried seagrass was powdered using a blender, and the seagrass powder (50 mesh) was used for the extraction of bioactive components.

### *2.2 Extraction of bioactive components*

*First step;* Seagrass powder was extracted with different solvents namely water, 50% ethanol, and 100% ethanol.

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The chosen solvents were based on the solvent kind able to extract completely phytochemical polarity (polar up to non-polar organic compounds). In addition, they have been recognized that leading to being non-toxic or safe in the obtained extracts (Ahmad et al., 2021; Molino et al., 2018).

*Second step;* The best solvent from the 1st step was based on the total types of extracted phytochemicals (water = 13 compounds, 50% ethanol = 15 compounds, 100% ethanol = 7 compounds) (see Table 1). The 50% ethanol was chosen for extraction of bioactive components on the seagrass using different techniques i.e. conventional, MAE (Anton Paar-Multiwave Pro), UAE-probe system (Branson-Digital Sonifier), and UAE-bath system (SONICA). These different extraction techniques were used in order to discover the best extraction technique with the extracted bioactive compounds maximum. Concisely, the process condition of seagrass extraction with different techniques was presented in Table 2. The obtained liquid extracts in both the 1st and 2nd steps were filtered, centrifuge (3000 rpm, 5 min), and vacuum-evaporated (40 °C). Thus, the dry extracts were obtained for the proceeded analysis.

**Table 1.** Extracted seagrass with different solvent polarities to obtain the richest total phytochemical content.

| Produced extracts from the different solvent polarity | Phytochemical group | | | |
|---|---|---|---|---|
| | Terpenes | Polyphenols | Alkaloids | Amino acid derivatives |
| Water extract | Andrographolide | Caffeic acid | Nicotinic acid | Choline |
| | | 2-Hydroxycinnamic acid | Hypoxanthine | |
| | | 7-Hydroxycoumarine | Pyridoxine | |
| | | Matairesinol | | |
| | | 4-Methoxycinnamic acid | | |
| | | Vanillin | | |
| | | Dibenzylamine | | |
| | | Acetophenone | | |
| | **Total = 1 compounds** | **Total = 8 compounds** | **Total = 3 compounds** | **Total = 1 compound** |
| 50% ethanol extract | Cafestol | 7-Hydroxycoumarine | Trigonelline | Choline |
| | Andrographolide | 4-Methoxycinnamic acid | Piperine | Betaine |
| | | Vanillin | Nicotinamide | |
| | | Shogaol | | |
| | | Quercetin-3β-D-glucoside | | |
| | | Phloretin | | |
| | | Acetophenone | | |
| | | Dibenzylamine | | |
| | **Total = 2 compounds** | **Total = 8 compounds** | **Total = 3 compounds** | **Total = 2 compounds** |
| 100% ethanol extract | Not detected | Benzophenone | 4-Picoline | Choline |
| | | Dibenzylamine | | Betaine |
| | | 7-Hydroxycoumarine | | |
| | | o-Toluidine | | |
| | | **Total = 4 compounds** | **Total = 1 compound** | **Total = 2 compounds** |

**Table 2**. The extraction process of seagrass constituents utilized different extraction techniques with the best solvent-applied.

| Extraction technique | Solvent | Material:Solvent ratio (g/mL) | Temperature (°C) | Time (min) | Process condition |
|---|---|---|---|---|---|
| Convenntional | 50% ethanol | 1:20 | Ambient (±30 °C) | 1440 [A] | Maceration on shaker-water bath (medium speed) |
| MAE | 50% ethanol | 1:20 | 50 (the lowest temperature can be set on the MAE equipment) | 5 [B] | Max. Pressure 18.0 bar; Max. Pressure increase rate 0.5 bar/s; Max. Microwave power 700 W; Stirrer = high |
| UAE-Probe | 50% ethanol | 1:20 | Without temperature setting | 5 [C] | Fixed amplitude at 70%; Fixed frequency at 20 kHz |
| UAE-Bath | 50% ethanol | 1:20 | Without temperature setting | 60 [C] | Fixed frequency at 39 kHz |

*Note:* A = using the optimum time for phytochemicals extraction with the maceration process according to (Albuquerque et al., 2017); B and C = modifying the maximum time of MAE and UAE-Probe for phytochemicals extraction is based on the result of (Albuquerque et al., 2017). D= using the maximum time of UAE-bath according to (Albuquerque et al., 2017) without any modification. Each treatment is repeated 3 times.

### *2.3 Phytochemicals screening and detection of bioactive compounds*

Phytochemicals content in seagrass extracts was screened with Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Q-exactive types of Thermo Fisher Scientific by (Susilo et al., 2020) method. Each seagrass extract (100 µL) was dissolved in 1400 µL adjusted solvents (water, 50% ethanol, or 100% ethanol). Samples that had been filtered with a 0.22 m RC minisart were injected into the LC-HRMS apparatus. A total of 10 ul samples will be processed automatically using a hypersil gold aQ 50 × 1 mm × 1.9 column at positive polarity conditions, flow rate 40 L/min, oven column temperature 30 °C with elution gradient as follows; 0-2 min 5% B, 15-22 min 60%-95% B, then let it stabilize at 95% B for 3 min and let it drop to 5% B at 30 min. For compound identification, the chromatogram data generated from the injection process will be analyzed using the Compound Discoverer 3.1 software based on the mzcloud online library. The PubChem page and the scientific papers were used to detect the bioactive compounds in the samples that have been screened (Susilo et al., 2021). Accordingly, the active compound types with several bioactivities on health were able to detect.

### *2.4 Scanning electron microscope (SEM)*

A half milligram of seagrass extracts from the different extraction techniques was placed on a cover glass that has been coated with a carbon tip with a size of 0.5 × 0.5 mm. The mounted tissues were gold-coated with a Q150R S sputter-coated unit (Quorum Technologies, Ltd) and viewed with a tabletop scanning electron microscope (SEM) TM 3000 Hitachi (Hitachi High Technologies Co., Ltd, Japan).

### *2.5 Antioxidant analysis*

The antioxidant activity of produced extracts from the different extraction techniques was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Adhikari et al., 2019). Freshly DPPH solution (0.2 mM) in absolute ethanol was used in this study. An equal volume (100 µL) of the DPPH solution and seagrass extracts were mixed in a microplate (Costar 96-well plate). The reaction mixture was incubated for 30 min at room temperature (25 °C) in dark. An equal proportion (100 µL) of DPPH solution and ethanol was mixed for the control. The absorbance value of the reaction mixtures was measured at 517 nm using a microplate spectrophotometer (SPECTROstar Nano - BMG LABTECH, Germany). The DPPH radical scavenging activity was calculated by the following Equation 1:

$$DPPH\ radical\ inhibition(\%) = \frac{Absorbance\ \ control - Absorbance\ sample}{Absorbance\ \ sample} x100 \quad (1)$$

Fifty value was interpolated to the linear regression equation ($R^2 \geq 0.99$) that has been obtained from the inhibition curve, it is used as $IC_{50}$ calculation.

### *2.6 Statistical data analysis*

The results of quantitative data were expressed as mean values ± standard deviation from triplicate experiments. Analysis of variance (ANOVA) was done by the general linear model in Minitab 18 software. Furthermore, the tukey test with $p \leq 0.05$ revealed a significantly different.

## 3. Result and discussion

### *3.1 Total phytochemical extraction with different solvent polarities*

The previous study by (Rengasamy et al., 2019) reported that methanolic extract was found to possess greater amount of phenolic than flavonoid compounds. However, in this study, 50% ethanol was used to get the maximum amount of phytochemicals compound. As can be seen at Table 1, the phytochemical groups are detected on all different extract polarities i.e. terpenes, polyphenols, alkaloids, and amino acid derivatives. The extracted seagrass with 50% ethanol exhibited the most content of total phytochemicals (2 terpene, 8 polyphenol, 3 alkaloid, and 2 amino acid-derivative compounds) when compared to both water and 100% ethanol extracts. The extracted seagrass with different ethanol concentrations also contained more amino acid-derivative compounds than pure water extract, wherein betaine was only contained in both 50% and 100% ethanol extracts. A 50% ethanol is semi-polar, therefore able to extract more phytochemical polarities in polar up to non-polar on *Syringodium isoetifolium*. (Silva et al., 2022; Vargas-Madriz et al., 2021) reported that the water/ethanol (v/v) mixture is capable to extract many secondary metabolites in different polarities. Comparing to non-polar solvent, the extraction of phytochemicals in bean (*Phaseolus vulgaris*) using high polarity solvents resulted in high extract yield but low of phenolic and flavonoid content. (Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Nawaz et al., 2020). However in this study, a semi polar solvent resulted in a high extracted phytochemicals content on *Syringodium isoetifolium*.

### *3.2 Extraction of bioactive components using different extraction techniques*

(Rengasamy et al., 2019) used conventional technique with methanol solvent to produce high phenolic content. In this study, the different extraction techniques (MAE, UAE-bath, UAE-probe, and conventional) using the best extraction solvent (50% ethanol) were employed to maximalize the extraction of bioactive components in *Syringodium isoetifolium*. From all extraction techniques, terpenes, polyphenols, alkaloids, and amino acid derivatives compound groups were detected as shown in Table 3. UAE-probe extraction showed more polyphenol compounds than the other methods. One of the interesting and dominant polyphenol compounds was quercetin. Many bioactivities advantages of quercetin such as an antioxidant, anti-inflammatory, anti-hypertensive, antiobesity, anti-hypercholesterolemic, and anti-atherosclerotic activities had been reported (Anand David et al., 2016).

In the extraction with the UAE-Probe was also known that the presence of dominant choline and betaine compounds. Choline was an essential nutrient that plays an important role in the human body, from cell structure to neurotransmitter synthesis.

**Table 3.** The bioactive components in seagrass extracts were extracted by different techniques.

| Extraction technique | Phytochemicals group | Bioactive compounds | Biological Activities |
|---|---|---|---|
| **Conventional** | Terpenes | Cafestol | Anti-inflammation, hepatoprotective, anticancer, antidiabetic, and anti-osteoclastogenesis activities (Ren et al., 2019) |
| | | Andrographolide | Antiviral (Adiguna et al., 2021). |
| | Polyphenols | 7-Hydroxycoumarine | Antiulcerogenic antidiarrheal and antibacterial (Cruz et al., 2020). |
| | | 4-Methoxycinnamic acid | Anticancer (Hasibuan et al., 2020). |
| | | Vanillin | Antibacterial (Retnosari et al., 2021). |
| | | Shogaol | Antimicrobial (Rampogu et al., 2018). |
| | | Quercetin-3β-D-glucoside | Anticarcinogenic agent (Razavi et al., 2009). |
| | | Phloretin | Antimicrobial (Barreca et al., 2014). |
| | | Acetophenone | Antioxidant agent (Emami et al., 2018). |
| | | Dibenzylamine | Antibiotic (Dinarvand & Spain, 2021). |
| | Alkaloids | Trigonelline | Hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and antitumor activities (Zhou et al., 2012). |
| | | Piperine | Antibacterial (Hikal, 2018). |
| | | Nicotinamide | As an adjunct in many dermatological diseases because of its anti-inflammatory, antioxidant, barrier repair and protective effects (Bains et al., 2018). |
| | Amino acid derivatives | Choline | Essential nutrient for human health (Zeisel & da Costa, 2009). |
| | | Betaine | Anti-inflammatory (Zhao et al., 2018). |
| | *Total* | 15 compounds | |
| **MAE** | Terpenes | Cafestol | Antiinflammation, hepatoprotective, anticancer, antidiabetic, and anti-osteoclastogenesis activities (Ren et al., 2019). |
| | | Andrographolide | Antiviral (Adiguna et al., 2021). |
| | Polyphenols | 7-Hydroxycoumarine | Antiulcerogenic antidiarrheal and antibacterial (Cruz et al., 2020). |
| | | Benzophenone | Antifungal, anti-HIV, antimicrobial, antioxidant, antiviral and cytotoxic (Wu et al., 2014). |
| | | Vanillin | Antibiotic (Bezerra et al., 2017). |
| | | Acetophenone | Antioxidant agent (Emami et al., 2018). |
| | | 3,5-di-tert-Butyl-4-hydroxybenzoic acid | Antitumor (Ding et al., 2012). |
| | | Dibenzylamine | Antibiotic (Dinarvand & Spain, 2021). |
| | Alkaloids | Trigonelline | Hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and antitumor activities (Zhou et al., 2012). |
| | | Piperine | Antibacterial (Hikal, 2018). |
| | | Nicotinamide | As an adjunct in many dermatological diseases because of its antiinflammatory, antioxidant, barrier repair and protective effects (Bains et al., 2018). |
| | | Nicotinic acid | Antioxidant (Sinthupoom et al., 2014). |
| | Amino acid derivatives | Choline | Essential nutrient for human health (Zeisel & da Costa, 2009). |
| | *Total* | 13 compounds | |
| **UAE-Bath** | Terpenes | Andrographolide | Antiviral (Adiguna et al., 2021). |
| | Polyphenols | 7-Hydroxycoumarine | Antiulcerogenic antidiarrheal and antibacterial (Cruz et al., 2020). |
| | | Diaveridine | Antibacterial (Wang et al., 2021). |
| | | 4-Methoxycinnamic acid | Anticancer (Hasibuan et al., 2020). |
| | | Shogaol | Antibacterial (Rigane et al., 2018). |
| | | Acetophenone | Antioxidant agent (Emami et al., 2018). |
| | | Dibenzylamine | Antibiotic (Dinarvand & Spain, 2021). |
| | | 3,5-di-tert-Butyl-4-hydroxybenzoic acid | Antitumor (Ding et al., 2012). |
| | Alkaloids | Trigonelline | Hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities (Zhou et al., 2012). |
| | | Reserpine | Antibacterial (Negi et al., 2014). |
| | | Nicotinamide | As an adjunct in many dermatological diseases because of its antiinflammatory, antioxidant, barrier repair and protective effects (Bains et al., 2018). |
| | | Piperine | Antibacterial (Hikal, 2018). |
| | Amino acid derivatives | Choline | Essential nutrient for human health (Zeisel & da Costa, 2009). |
| | | Betaine | Anti-inflammatory (Zhao et al., 2018). |
| | *Total* | 14 compounds | |

**Table 3.** Continued...

| Extraction technique | Phytochemicals group | Bioactive compounds | Biological Activities |
|---|---|---|---|
| **UAE-Probe** | Terpenes | Cafestol | Antiinflammation, hepatoprotective, anticancer, antidiabetic, and anti-osteoclastogenesis activities (Ren et al., 2019). |
| | | Andrographolide | Antiviral (Adiguna et al., 2021). |
| | Polyphenols | 7-Hydroxycoumarine | Antiulcerogenic antidiarrheal and antibacterial (Cruz et al., 2020). |
| | | Phloretin | Antimicrobial (Barreca et al., 2014). |
| | | 4-Methoxycinnamic acid | Anticancer (Hasibuan et al., 2020). |
| | | Quercetin-3β-D-glucoside | Anticarcinogenic agent (Razavi et al., 2009). |
| | | Quercetin | Antioxidant (Song et al., 2020). |
| | | Acetophenone | Antioxidant agent (Emami et al., 2018). |
| | | Dibenzylamine | Antibiotic (Dinarvand & Spain, 2021). |
| | | Caffeic acid | Antioxidant, antiinflammatory and anticarcinogenic (Espíndola et al., 2019). |
| | | 2-Hydroxycinnamic acid | Antioxidant, antimicrobial, anticancer and antiinflammatory (Sova & Saso, 2020). |
| | | 3,5-di-tert-Butyl-4-hydroxybenzoic acid | Antitumor (Ding et al., 2012). |
| | Alkaloids | Reserpine | Antibacterial (Negi et al., 2014). |
| | | Trigonelline | Hypoglycemic, hypolipidemic, neuroprotective, antimigraine, sedative, memory-improving, antibacterial, antiviral, and anti-tumor activities (Zhou et al., 2012). |
| | | Nicotinamide | As an adjunct in many dermatological diseases because of its antiinflammatory, antioxidant, barrier repair and protective effects (Bains et al., 2018). |
| | | 4-Picoline | Anticancer (Altay et al., 2018). |
| | Amino acid derivatives | Choline | Essential nutrient for human health (Zeisel & da Costa, 2009). |
| | | Betaine | Anti-inflammatory (Zhao et al., 2018). |
| | *Total* | 18 compounds | |

Choline deficiency in the human body may cause diseases such as liver disease, atherosclerosis, and possibly neurological disorders (Zeisel & da Costa, 2009). Betaine was also essensial consumed through dietary intake. Betaine mainly functions as an osmolyte and a methyl group donor. The major physiological effects of betaine in whole-body health and its ability to protect against both liver- as well as non-liver-related diseases and conditions. Several studies show that betaine protects against the development of alcohol-induced hepatic steatosis, apoptosis, and accumulation of damaged proteins (Arumugam et al., 2021). In this study, UAE-Probe technique using the 50% ethanol produces more bubbles than water. The surface tension of the liquid is contributes to the formation of cavitational bubbles. In case liquids with lower surface tension, cavitational bubbles will be easily created due to the ultrasonic energy applied can more easily exceed the surface tension. The 50% ethanol is more effective to extract phenolic compound and having a greater force to plant tissues (Ghasemzadeh et al., 2015). Therefore, UAE-Probe was the most potential emerging technique to extract various bioactive compounds on *Syringodium isoetifolium*.

### *3.3 The disintegration of Syringodium isoetifolium microstructure on the impact of different extraction techniques*

Seagrass powder before and after being treated with different extraction methods was observed under SEM equipment on 1000x magnification. Seagrass cell damage showed in all extraction treatments. When compared with the conventional method, the cell damage by MAE, UAE-bath, and conventional techniques exhibited slightly similar. Cell wall disintegration and hole forming severely were exhibited by UAE-Probe (Figure 1). UAE-probe promotes damage to the cell wall due to mechanical effects induced by pressure gradients generated during the collapse of cavitation bubbles, by shear force, and also by a chemical attack during cavitation that leads to the disintegration of the cell wall (Gomes et al., 2021).

### *3.4 Antioxidant activity*

Figure 2, although the antioxidant activity on all produced extracts of different extraction techniques does not show significant differences. However, UAE-probe extract shows the strongest antioxidant activity. Moreover, the UAE-probe extract (1317.16 ± 94.79 ppm) was very stronger compared to the *Syringodium isoetifolium* extract from a previously reported study (5390.00 ± 90.00 ppm) (Rengasamy et al., 2019). The richest bioactive compounds on UAE-probe extract than on another extracts (Table 3), might be contributed to its antioxidant capacity. The bioactive compounds of UAE-probe extract (18 compounds i.e. cafestol, andrographolide, 7-hydroxycoumarine, phloretin, 4-methoxycinnamic acid, quercetin-3β-D-glucoside, quercetin, acetophenone, dibenzylamine, caffeic acid, 2-hydroxycinnamic acid, 3,5-di-tert-butyl-4-hydroxybenzoic acid, reserpine, trigonelline, nicotinamide, 4-picoline, choline and betaine) were also richer than compared to the *Syringodium isoetifolium* extract (14 compounds i.e. Caftaric acid, 3-(4-hydroxyphenyl)lactic acid, caffeic acid, caffeoyl-4'-O-phenyllactate, 3-phenyllactic acid, 3-methyladipic acid, 4-coumaric acid, loliolide, isololiolide, chicoric acid, hydroxydodecanoic acid, pheophytin B, pheophytin A and pheophytin A isomer) from (Rengasamy et al., 2019) result.

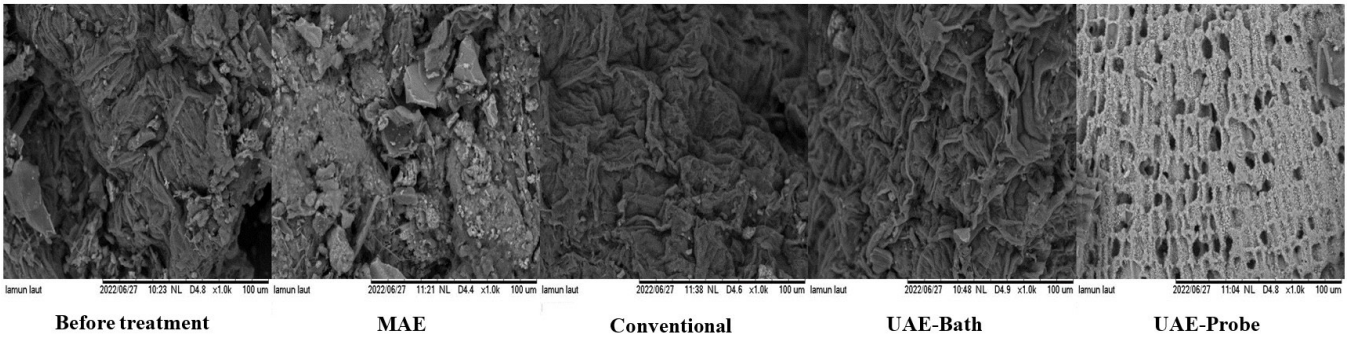


**Figure 1.** The impact of different extraction techniques on the microstructure disruption of seagrass was observed by SEM under 1000x magnification.

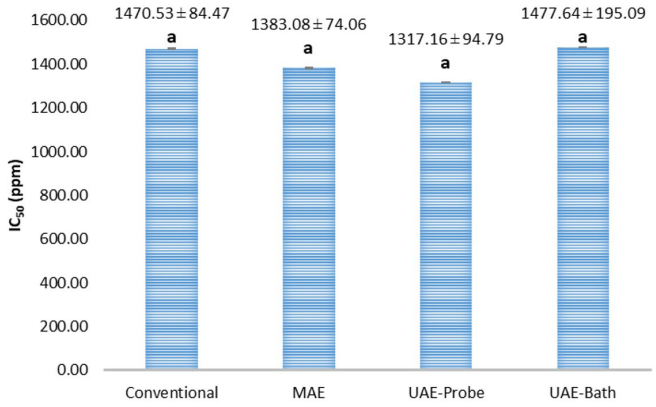


**Figure 2.** The impact of different extraction techniques on the antioxidant activity of seagrass extracts. The superscript with the same letter showed no significant difference ($p < 0.05$).

## 4 Conclusion

Phytochemical extraction on *Syringodium isoetifolium* with 50% ethanol solvent resulted in the most dominant terpene, polyphenols, alkaloid, and amino acid derivative groups. UAE-probe was capable to extract the richest bioactive compounds inasmuch as destroying the seagrass cell wall the most severely.

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