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Metabolite Profiling and Bioprospecting of *Acrolejeunea fertilis* (Reinw., Blume & Nees) Schiffn. from Kebun Raya Cibodas, West Java

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Abstract

Background: *Acrolejeunea fertilis* (liverwort) is known for having various potential natural products. However, its abundance is limited, and its secondary metabolites have not been extensively investigated. The *in vitro* culture technique might enhance its biomass. **Methods:** This study aimed to investigate the metabolite profile of *A. fertilis* from Kebun Raya Cibodas grown *in situ* and *in vitro*. The bioactivity, including antioxidant, total phenolic, and flavonoid content and antibacterial activity, was also evaluated. The *in vitro* culture of *A. fertilis* used ½ MS media with the addition of 0,1 mg/L of 2,4-D and 1 mg/L of Kinetin. Methanol and n-hexane were used for extraction. Gas Chromatography-Mass spectrometry (GC-MS) is used for metabolite profiling. **Results:** The optimum IC50 value from n-hexane extract is 68,18±2,65 mg/L. The highest yield of total phenolic and flavonoid content from *in situ* methanol extract, which resulted in 130,68±0,002 µgGAE/gr and 5,97±0,01 µgQE/gr, respectively. Antibacterial activities were evaluated by measuring the zone of inhibition for *S. aureus* and *E. coli*. The optimum area measured from *in situ* n-hexane extract was 23,91±1,54 and 13,08±0,23 cm, respectively. **Conclusions:** These findings carry important implications for the further development of natural products obtained from liverwort regarding its potential as a bioactive compound.

Keywords: *Acrolejeunea fertilis*; antimicrobial; antioxidant; in situ; in vitro culture

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Introduction

Secondary metabolites are naturally occurring compounds from microorganisms, animals, and plants. These natural products are widely used in daily life. Scientific studies have demonstrated the antibacterial, antifungal, and anticancer properties of compounds such as vincristine, vinblastine, thalicarpine, epidophyllotoxin derivatives, and camptothecin extracted from plants and cyanobacteria *in vitro* (Broniatowska et al., 2011; Kim & Park, 2002). Among these organisms, plants have been extensively used in traditional medicine to treat various ailments, such as open wounds, sunburn, and itching. Plants are particularly preferred for quick regeneration and well-known medicinal properties (Cragg & Suffness, 1988). Interestingly, nonvascular plants, such as bryophytes, have also been found to contain secondary metabolites with various bioactivity (Commisso et al., 2021; Dziwak et al., 2022). This highlights the potential of natural ingredients from plants and bryophytes to serve as sources for developing various bioactive compounds.

Liverworts are distinguished from other bryophytes by the presence of oil bodies, a unique characteristic. They contain a diverse range of secondary metabolites, such as terpenoids, quinones, phenylpropanoids, and flavonoids, which are crucial in helping them adapt to extreme environmental changes and biotic or abiotic stress (Joshi et al., 2023).

As a result, liverworts are sensitive to changes in their surroundings, and they have been identified as potential indicators of environmental conditions (Peters et al., 2018; Thakur & Kapila, 2017; Wang et al., 2017). The synthesis of secondary metabolites is

induced by both biotic and abiotic factors, with biotic factors involving interactions among plants, animals, and microorganisms and abiotic factors comprising environmental conditions such as salinity, temperature fluctuations, radiation, light intensity, humidity, climate, and precipitation (Ramakrishna & Ravishankar, 2011).

Flavonoids are a group of compounds that are useful in dealing with drought due to high temperatures and water availability, as well as the UV index factor (Ramakrishna & Ravishankar, 2011; Scher et al., 2002; Sonwa & König, 2003). While flavonoids are present in almost all liverworts of the *Marchantiaceae* family, they are found less frequently in the liverworts of the *Lejeuneaceae* family (Siregar et al., 2020). Although the reason for this is unknown, it is suggested that this could be due to differences in the habitats where the family members grow. The liverworts of the *Lejeuneaceae* family grow on tree trunks with shade, which results in lower surrounding temperatures, while colonies of the liverworts of the *Marchantiaceae* family grow on terrestrial areas, roadsides, and rocks, resulting in higher temperatures (Scher et al., 2002).

Liverwort species' secondary metabolites are known for their potential as therapeutic agents, including antibacterial, antifungal, antioxidant, and anti-inflammatory, rather than just for adaptation to various abiotic factors (Commisso et al., 2021; Dziwak et al., 2022). To explore these metabolites and their bioactivity potential, Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) can be used (Dey & De J.N., 2010; Makajanma et al., 2020). The *Marchantia* genus of thalloid liverworts has been extensively studied according to Makajanma et al. (2020), while from the Acrolejeunea genus of leafy liverwort, the species *Acrolejeunea securifolia* subsp. caledonica and *Acrolejeunea aulacophora* are potential sources of new secondary metabolite compounds in the *Lejeuneaceae* family (Sukkharak et al., 2011). Although their significant compounds have been identified as isolepidozene and pinguisane, further research is required to determine their bioactivity potential.

Previous studies on liverwort species' bioprospecting have typically used samples harvested directly from nature (Makajanma et al., 2020). However, this approach leads to limited biomass and the mixing of samples from different species in one colony. Our research proposes an *in vitro* culture technique to solve these problems. Specifically, we analyzed the secondary metabolites of the *Acrolejeunea fertilis* species from the *Lejeuneaceae* family, both grown *in situ* and through *in vitro* culture. This study also evaluated their potential for bioprospecting, including antioxidant activity, by evaluating their IC50 value and antibacterial effects.

Methods

Explant Collection and Identification

The leafy liverwort *A. fertilis* for *in vitro* culture explant was collected from the *Syzygium* sp. tree (Figure 1) in the Kebun Raya Cibodas (KRC), Jawa Barat, Indonesia, and confirmed through identification in Herbarium Depokensis (DEP) at Department of Biology, Faculty of Mathematics and Science, Universitas Indonesia. The KRC Committee has permitted all sampling procedures. Its identification was based on the presence and amount of a teeth-like structure on its lobule. The presence of its Massula-type oil body is also crucial for the identification of *A. fertilis*. These structures were observed under a light microscope [Leica DM500].

(A) (B)

Figure 1. Sampling location of *Acrolejeunea fertilis* for explants (A); *A. fertilis* grows epiphytically on *Syzygium* sp. tree barks (B)

Explant Sterilization

The sterilant method used in this study consisted of detergent 0.4% (v/v), disinfectant (alcohol 35% (v/v), and commercial bleach [Natrium Chloride (NaOCl)] 1.25% (v/v)), fungicide (dithane) 2% (w/v), and antibiotics (Tetracycline) 0.5% (w/v). The anatomy of the explant was observed after sampling, washing with 0.4% detergent, and after completion of the entire sterilization process to evaluate the effect of each sterilization process on the cell of the explant since its structure is small and fragile. The sterilization process was performed in the Laminar air flow cabinet (LAFC) [Type HS087], and only sterilization using detergent was conducted outside the LAFC. In the first step of the sample sterilization process, whole explants were washed under tap water for three minutes to remove tree bark and dirt. Before washing with detergent, the explants needed to be cut to a size of ± 1 cm using a scalpel [Renz No. 3]. The explants were washed using detergent and then sonicated [Cole-Parmer Instrument Company PC620E-1] for five minutes. Then, the explants were placed in the LAFC and sterilized in alcohol for 30 seconds, disinfectant for 3 minutes, fungicide for 5 minutes, and antibiotics for 5 minutes, respectively. After each step of the sterilization process, samples were rinsed using sterile water three times for five minutes each. This was done to ensure that no chemical compounds were on or inside the samples. After the entire sterilization process, samples were placed on a sterile petri dish and cut to precisely 1 mm using the millimeter block as a measurement tool. After that, samples were placed in sterile water to be planted in culture media.

Acrolejeunea fertilis' in vitro culture

All explants were then planted in ½ MS culture medium with macro and micronutrients, amino acids, vitamins, and mineral salts [PhytoTechnology Laboratories], and 0.1 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) and one mg/L Kinetin were added. The culture media was mixed with agar [Swallow Globe] to compact the texture. Explants were then cultured for 150 days. The placement of the explant in the culture media is depicted in Figure 2.

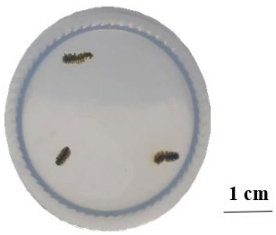

Figure 2. Position of the *A. fertilis* explant in ½ MS culture media

Sample Extraction and Metabolite Profiling

The extraction process for *A. fertilis* liverwort begins with cleaning the sample from remaining impurities, which can be tree bark (for *in situ* sample) or culture media (as in agar, for *in vitro* sample). The liverwort was washed under tap water and rinsed with sterile distilled water (ddH2O). The *A. fertilis* samples were air-dried for ±3 days until their dry weight was constant. The sample was then weighed at 0.1 grams and put into a falcon tube into which 100 ml of methanol and n-hexane was added, yielding a concentration of 1 mg/ml, equivalent to 1000 ppm. The *A. fertilis* samples were crushed using a Tissue Ruptor [Qiagen]. The sample was then sonicated for 2 ×10 minutes to ensure the moss sample had been destroyed.

Furthermore, the sample was centrifuged at 3000 rpm for 20 minutes, and the supernatant was evaporated. After that, 0.01 grams of methanol extract was diluted in 10 ml to obtain the final concentration of 100 ppm. The supernatant was then transferred to a 25 ml vial tube and stored at 4°C for further use.

Gas Chromatography-Mass Spectrometry (GC-MS) Assay

The method for GC-MS used is adopted from (Sukkharak et al., 2011), which is specific to the *Lejeuneaceae* family. The stages for analysis using GC-MS begin with the filtering process of 2 ml of n-hexane extract that was diluted with methanol using a syringe and placed into a specific vial. The GC-MS machine was tuned before injecting the sample for the analysis process, which includes an oven temperature of 50°C, an initial hold time of 3 minutes, and then setting the temperature at 250°C, with an increase of 5°C per minute and a hold process of 15 minutes after reaching a temperature of 250°C. The sample was injected at a temperature of 280°C and a helium flow rate of 1 ml per minute. The electron impact mode was 70eV with three scans. The temperature is 230°C, with a mass range (m/z) of 40–500. In reading, the slope used is 500. The machine running process is ± 2 hours long, and the results obtained will be analyzed based on its retention time and spectrum data using the latest version of the Wiley Library.

Total Phenolic Content

The method used to calculate total phenolic compounds (TPC) is the Folin-Ciocalteu method, and the number of repetitions was five. First, 200 µl of liverwort extract *A. fertilis* methanol and n-hexane extract (concentration 12.5 ppm, 25 ppm, 50 ppm, 100 ppm) mixed with 120 µl of Folin-Ciocalteu reagent and left at room temperature for 6 minutes. After that, 80 µl of Na_2CO_3 was added to the mixture. The mixture was then incubated in the dark room for 120 minutes, and then the absorbance was measured using a spectrophotometer [Thermofisher Scientific] at a wavelength of 750 nm. T total phenolic compounds were calculated and expressed as micrograms of gallic acid equivalent per gram of dry weight (µgGAE/g) (Tungmunnithum et al., 2018). The following formula was used to calculate total phenolic compounds:

$$TPC = \frac{C.V.df}{gr}$$

C = Phenolic concentration (µg/ml) df = Dilution factor
V = extract's volume (ml) gr = weight of the extract*Data analysis*

Total Flavonoid Content

The total flavonoid compounds (TFC) were measured by mixing 200 µl of liverwort *A. fertilis* methanol and n-hexane extract. The concentrations varied from 12.5 ppm, 25 ppm, 50 ppm to 100 ppm with 215 µl of 80% methanol, 5 µl of 1 M CH_3COOK, and 5 µl $AlCl_3.6H_2O$ 10% in each well. The mixture was then incubated at room temperature for 40 minutes. After that, the wavelength was measured using a spectrophotometer [Thermofisher Scientific] at a wavelength of 415 nm, with five repetitions. The total

flavonoid compounds are expressed as micrograms of quercetin equivalent per gram of dry weight (µgQE/g) (Tungmunnithum et al., 2018). The following formula was used to calculate TFC:

$$TFC = \frac{C.V.df}{gr}$$

C = Falvonoid concentration (µg/ml) df = Dilution factor
V = extract's volume (ml) gr = weight of the extract*Data analysis*

Antioxidant Activity

The method used to determine the antioxidant activity of *A. liverwort* extract is the DPPH method, and the number of repetitions was five. The process began by mixing the extract (*in situ* and *in vitro* extracts both in methanol and n-hexane) with 2,2-Diphenyl-1-picrylhydrazyl (DPPH), varying concentrations ranging from 125 ppm, 250 ppm, 500 ppm, to 1000 ppm with 0.5 ml of 0.1 mM DPPH. The mixture was then incubated in a dark room for 60 minutes. Then, absorbance was measured at a wavelength of 517 nm using a spectrophotometer [Thermofisher Scientific]. The blank was methanol, and a mixture of 0.5 ml methanol and 0.5 ml 0.1 mM DPPH was used to control it. Finally, the percentage of antioxidant activity and IC_{50} value was calculated. The IC_{50} value was determined by analyzing the linear regression curve. The expected R^2 coefficient value was above 0.99, and its categorization is by (Reviana et al., 2021).

Antibacterial Assay

The antibacterial assay was performed using the diffusion well method, in which the inhibition zone on the agar media was observed. *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were used as preculture for one night using nutrient broth (NB) [Himedia] media. An antibacterial test was performed using the Mueller Hinton Agar (MHA) [Difco] medium. As a positive control, tetracycline was used in a concentration of 7 µg/ml, while as a negative control, dimethylsulfoxide (DMSO) was used; for the treatment, each extract was used in a concentration of 100 ppm. The bacterial inoculum was adjusted to 0.5 McFarland standard as a reference to change the turbidity of the bacterial suspension, equivalent to 1.5 x 10^8 CFU (Kralik et al., 2012). After that, 10 µl of the inoculum was added to 10 ml of the medium and mixed gently. Then, the medium with the inoculum was poured over the previously hardened medium and allowed to dry before use. In all three treatments, the medium was perforated using a 6-mm diameter cylinder to form a well, and then 100 µl of the extract was loaded into each well. The culture was then incubated in the dark at 32°C for 16 hours. After that, the clear zone was observed and measured using a caliper. The measurement results were then categorized according to Sarker et al. (2014).

Data Analysis

All data obtained in this research included the metabolite profile of *A. fertilis*' extract, IC50 value, and the total phenolic and flavonoid compounds; moreover, the diameter of the inhibition zone was measured and descriptively analyzed. All quantitative data were then analyzed using a T-test conducted using SPSS (version 27).

Result

According to Table 1, it is observed that the methanol extract of *A. fertilis* KRC extract had the highest yield of phenol content, resulting in 130.68 ± 0.02 µgGAE/gr compared to the *in vitro* culture extract with 26.29 ± 0.02 µgGAE/gr. Meanwhile, the n-hexane extract had a lower yield of total phenolic content, which is 44.62 ± 0.02 µgGAE/gr and 4.78 ± 0.02 µgGAE/gr for *in situ* and *in vitro*, respectively. Meanwhile, the total yield of flavonoid

compounds obtained for the methanol fractions of *A. fertilis* KRC extract is consistent with the total yield of phenolic compounds. The total flavonoid compounds identified for the methanol extract of *A. fertilis* KRC *in situ* and *in vitro* extract were 5.97 ± 0.01 µgQE/gr and 4.00 ± 0.01 µgQE/gr, respectively. The total yield of flavonoid content for *A. fertilis* n-hexane extract was 5.03 ± 0.01 µgGAE/gr and 2.33 ± 0,01 µgGAE/gr for *in situ* and *in vitro* extract.

Table 1. Total phenolic and flavonoid content of *A. fertilis*

Extract	Conc. (ppm)	Total Phenolic Content (µgGAE/g)	Total Flavonoid Content (µgQE/g)
KRC MeOH *in situ*	100	130.68 ± 0.02	5.97 ± 0.01
KRC MeOH *in vitro*		26.29 ± 0.02	4.00 ± 0.01
KRC Hex *in situ*		44.62 ± 0.02	5.03 ± 0.01
KRC Hex *in vitro*		4.78 ± 0.02	2.33 ± 0.01

Based on the result obtained in this study, the antioxidant activity is presented by determining the IC50 value. As presented in Table 2, the IC50 value from the n-hexane extract of A. fertilis KRC in situ was categorized as vital, slightly better than the methanol extract. Meanwhile, all *A. fertilis in vitro* extracts for both n-hexane and methanol were categorized as moderate. These results show that in vivo extracts require a concentration of at least 68.18±2.65 mg/L to inhibit 50% of DPPH compounds. In contrast, *in vitro,* extracts require a higher concentration of 120.86±2.14 mg/L to achieve the same level of inhibition.

Table 2. IC50 value and antioxidant categorization

Extract	IC50 Value (ppm)	Categorization
KRC MeOH *in situ*	77.01±1.83	Strong
KRC Hex *in situ*	68.18±2.65	Strong
KRC MeOH *in vitro*	134.67±3.31	Moderate
KRC Hex *in vitro*	120.86±2.14	Moderate

The n-hexane extract of *A. fertilis in situ* extract from KRC contained the primary compound of Drim-7-en-11-ol, as presented in Table 3. The compound had an abundance of 54.61%. Moreover, the n-hexane extract from *A. fertilis* in vivo KRC contained 36 secondary metabolites' compounds with Stigmast-5-en-3-ol, with oleate as the primary compound. The results of compound identification differed significantly between the *in situ* and *in vitro A. fertilis* extracts. The chromatograms of the *in vitro* culture extracts revealed a more significant number of secondary metabolites compared to the *in situ* samples. Additionally, the type of substantial compound identified varied between the four extracts. The primary compound in the *A. fertilis in situ* methanol extract was cycloheptasiloxane, tetradecamethyl, with an abundance of 84.6%, as presented in Table 3.

Table 3. Primary compound characterized using GC-MS from *A. fertilis* KRC extracts

Explant Source		Solvent	Number of Compounds Identified	Major Compound	Major Compound Abundance
KRC	*In situ*	Methanol	3	Cycloheptasiloxane, tetradecamethyl	84.6%
		n-hexane	11	Drim-7-en-11-ol	54.61%
	In vitro	Methanol	0	-	-
		n-hexane	36	Stigmast-5-en-3-ol, oleate	46.01%

As mentioned in Table 4, the antibacterial activity test using the preculture of *S. aureus* and *E. coli* produced varying results. Table 4 presents the diameter of the

observed inhibition zones, which vary in category. The categorization of the diameter of the inhibition zone was determined based on guidelines by Sarker et al. (2014), using tetracycline (7 µg/ml) as a positive control. If the diameter of the inhibition zone is ≤ 11 mm, the isolate is categorized as resistant to the extract. If the diameter of the inhibition zone is 12 mm–15 mm, the isolate is classified as moderately sensitive to the extract. However, if the observed inhibition zone has a diameter of ≥ 15 mm, the isolate is categorized as sensitive to the tested extract.

Table 4. Zone inhibition diameter from *A. fertilis'* methanol and n-hexane extract, *in situ* and *in vitro* culture

Treatments	*S. aureus* (mm)	Zone Inhibition Category	*E. coli* (mm)	Zone Inhibition Category
Tetracycline	24.95	Sensitive	15.93	Sensitive
A. fertilis KRC, MeOH extract	19.75±0.56	Sensitive	11.61±0.55	Moderately Sensitive
A. fertilis KRC, Hex extract	23.91±1.54	Sensitive	13.05± 0.79	Moderately Sensitive
A. fertilis in vitro KRC, MeOH extract	0	No Inhibition	0	No Inhibition
A. fertilis in vitro KRC, Hex extract	13.85±0.82	Moderately Sensitive	0	No Inhibition

Discussion

Results obtained in this study are being compared to those of the literature study. The total phenolic and flavonoid content yields are by the exposure of light intensity and UV index of *A. fertilis* in the habitat, which affects the yield of phenolic content, with higher levels of flavonoids produced in response (Martínez-Silvestre et al., 2022; Yildirim, 2020). Furthermore, the low levels of total flavonoid compounds from *in situ* samples agree with the study by Siregar et al. (2020), in which the detection of flavonoid compounds is problematic in the *Lejeuneaceae* family. These results support the assumption that the UV index of *A. fertilis* habitat affects the secondary metabolite content, with higher levels of flavonoids produced in response to the adaptation to a high UV environment (Martínez-Silvestre et al., 2022; Yildirim, 2020). The UV index recorded is 11–12, considered extreme compared to an *in vitro* culture with no UV light exposure. According to the World Health Organization (WHO), a UV index is explained as the levels of UV radiation; therefore, the index's value varies daily. Based on WHO categorization, the UV index in the range of 1–2 is categorized as low, 3–5 as moderate, 6 – 7 as high, 8–10 as very high, and 11 and above as extreme. The extreme UV light leads to a higher accumulation of the phenolic compound, which functioned as a source of adaptation.

The IC50 value from the extract is also due to its potential for antioxidants. Stimulating the antioxidant potential in plants is particularly important in coping with ROS toxicity under drought stress. The first line of enzymatic defense against ROS is SOD, which actively converts O–2 to OH, followed by the conversion of H_2O_2 to H_2O with the help of other antioxidant enzymes, such as catalase (CAT) and ascorbate peroxidase (APX). The coordination among antioxidant components is critical to ensure better cell protection against oxidative damage caused by excessive ROS. These findings are consistent with research conducted by (Ghosh et al., 2021) on the liverwort *Marchantia polymorpha*, which is found in dry habitats; this liverwort produces antioxidant enzymes in self-defense (Khaleghi et al., 2019; Laxa et al., 2019). Based on this data, it is stated that there is a high correlation between the phenolic content and the antioxidant activity (Platzer et al., 2021). However, *A. fertilis in vitro* extracts both methanol and n-hexane and shows moderate antioxidant activity. It is presumably that the antioxidant enzyme is not produced as the microclimate conditions (temperature and humidity) were controlled daily.

The GC-MS analysis from *A. fertilis*' in situ and in vitro methanol extracts also revealed significant differences. All compounds identified in the GC-MS machine were mostly grouped as terpenoids, with some essential oils detected. Moreover, in this study, the use of GC-MS supports the theory that most of the compounds detected in liverworts are in the terpenoid group. According to Table 3, the result differed between *in situ* and *in vitro* extract. The availability of nutrients between nature and the media may cause this.

Furthermore, abiotic factors such as light intensity, humidity, and photoperiodism may lead to the diversity of secondary metabolite produced. This study compared to previous studies (Siregar et al., 2020). GC-MS is most suited for detecting volatile compounds produced by oil bodies in liverwort. However, since there might be non-volatile or polar compounds produced by liverwort, LC-MS may be required to complete this analysis for further study.

According to the result of the antibacterial assay, we referred its mechanism of action to Nogueira et al. (2021). Based on previous research conducted by Nogueira et al., 2021 on the mechanism of bacterial growth inhibition using terpenoid compounds, it was suggested that these compounds could damage the bacterial cell membrane and affect its permeability. Moreover, terpenoid compounds could alter the morphological structure of the cell membrane in both *S. aureus* and *E. coli* bacteria without causing cell lysis in both Gram-negative and positive bacteria (Mahizan et al., 2019). The activity of terpenoid compounds is believed to be linked to inhibiting the synthesis of crucial components necessary for the bacterial cell membrane function. This hypothesis is supported by the electron micrograph results obtained by (Nogueira et al., 2021).

Phenolic compounds possess antibacterial properties by interacting with various compounds synthesized in biological organisms, making them molecular targets for the biological activity of phenolics. According to the current database, several phenolic compounds—including rutin, quercetin, diosmin, hesperetin, and genistein—can interact with many compounds involved in bacterial metabolism. Polyphenols can act against bacteria at different levels and metabolic pathways by affecting the structure and formation of nucleic acids, cell walls, and various enzymes (Lobiuc et al., 2023; Shamsudin et al., 2022). Polyphenols can inhibit nucleic acid synthesis through topoisomerase inhibition and play an essential role in their antimycobacterial activity. Docking studies have proved that quercetin effectively binds to the subunit B of DNA gyrase through interaction with residues in the Toprim domain of the protein. The mechanism of action for affecting the cell wall is the hyperacidification at the plasma membrane interphase, which is a consequence of the dissociation of phenolic acids, as one of the possible mechanisms of the antimicrobial action of phenolic acids. This hyperacidification alters cell membrane potential, making it more permeable and causing irreversible alterations in the sodium-potassium ATPase pump, leading to cell death (Makarewicz et al., 2021). Despite all this, the solid and moderate categories of *A. fertilis* in vivo extract can still be utilized as effective antioxidant and antibacterial agents.

Conclusions

The study highlights the variation in secondary metabolite profiles and bioactivity of *A. fertilis* extracts obtained from different sources (*in situ and in vitro*) at Kebun Raya Cibodas, Jawa Barat. The study suggests that natural environmental conditions, such as temperature and light intensity, may contribute to the differences observed in the composition and bioactivity of the extracts. These findings have important implications for developing natural products for medicinal and industrial applications, followed by further research on its possible application as antiaging for humans.

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NTR, WH, Y, and AP designed the study. NTR conducted the experiments under the supervision of WH, and Y. NTR prepared the first draft of the manuscript. WH, Y, and AP finalized the manuscript. All authors read and approved the final manuscript.

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Declaration statement

The authors reported no potential conflict of interest.

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