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Original Research Article

The Potency of *Adenostemma platyphyllum* and *A. madurense* as Antioxidant and Anti-aging Agents

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ABSTRACT

Adenostemma platyphyllum and *Adenostemma madurense* are medicinal plants belonging to the same genus as *A. lavenia.* These plants are characterized by similar bioactivity. Therefore, this research is aimed at determining the antioxidant and anti-aging properties of *A. platyphyllum* and *A. madurense,* as well as identifying the potentially bioactive compounds in these plants. The plant extracts were obtained by successive maceration in *n*-hexane, ethyl acetate, and methanol in order to increase polarity. The total phenolic, total flavonoid, and total terpenoid contents were quantified using standard methods. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline sulfonate (ABTS) radical scavenging assays. The anti-aging activity was assessed using *Schizosaccharomyces pombe* yeast cells. The results showed that the methanol extract of *A. platyphyllum* had the highest phenolic content of 120.06 mg gallic acid equivalent (GAE)/g extract, while the ethyl acetate extract had the highest flavonoid and terpenoid contents of 58.62 mg quercetin equivalent (QE)/g extract and 6.88 mg nerol equivalent (NE)/g extract, respectively. The methanol extracts of the plants showed the highest antioxidant activity of 38.42 mg ascorbic acid equivalent (AAE)/g extract with IC_{50} value of 91.83 µg/mL. In addition, the methanol extracts showed the highest yeast cell viability, indicating anti-aging potential. The total phenolic content was strongly correlated with the antioxidant activity, suggesting that these substances were essential in the radical scavenging properties of the extracts.

Keywords: Anti-aging, Antioxidants, Flavonoids, Phenolics, Terpenoids.

Introduction

The Asteraceae family is the second largest within the plant kingdom,[1] comprising over 23,000 species distributed worldwide.[2] These plants have been widely used in conventional medicine, with *Adenostemma* characterized by numerous health benefits.[3] *Adenostemma lavenia* (L.) O. Kuntze, also called legetan warak, is one species within the genus that has been reported to possess antimelanogenic, anti-inflammatory, antioxidant,[4] and anti-aging properties.[5] Therefore, other species within the same genus, such as *A. platyphyllum* Cass and *A. madurense* DC., may also be considered to possess anti-aging and antioxidant potentials.[6] Generally, aging occurs due to the decline in cellular activity caused by an increased presence of free radicals, specifically reactive oxygen species (ROS).[7] Free radicals are reactive molecules that are rapidly oxidized in a chain reaction. To prevent radical-induced reactions, it is imperative to convert free radicals to stable molecules by providing compounds with antioxidant activity to donate an electron to these free radicals, thereby facilitating their stability.[8,9]

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Previous research has shown that *A. madurense* and *A. platyphyllum* possess potential antioxidant and anti-aging properties. To fully harness these benefits, there is a need to extract the metabolites in these species using suitable extraction solvents and investigate their biological activities. Among the various methods for investigating antioxidant activity of plants, *in vitro* tests are commonly used, particularly the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, which is significantly effective due to its high sensitivity and selectivity.[10] The 2,2'-azino-bis-3-ethylbenzothiazoline sulfonate (ABTS) radical scavenging assay is also a recommended, and highly sensitive method for assessing the antioxidant capacity of natural product extracts.[11] The effect of plant extracts on cellular processes is often investigated by analyzing their anti-aging properties. In this context, the yeast *Schizosaccharomyces pombe* serves as a widely used model organism, replicating the biological reactions and metabolic processes of higher species. The cells of *Schizosaccharomyces pombe* have a relatively short lifespan of 2-3 days and easily reproduce due to their existence as single cells.[12] The lifespan of the yeast can be influenced by metabolites in plants with antioxidant activity, which can activate the yeast cell mitochondria.[13] The type of solvents used for the extraction of metabolites from plants can affect the concentration and composition of these metabolites, resulting in differences in their antioxidant activity.[14] In this research, three solvents with varying levels of polarity, namely *n*-hexane, ethyl acetate, and methanol were used to extract metabolites from the leaves of *A. platyphyllum* and *A. madurense.* The total concentration of phenolics, flavonoids, and terpenoids in these extracts were analyzed along with their antioxidant and anti-aging properties. Furthermore, *A. lavenia* which belong to the same genus *Adenostemma* with its compound ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid (11αOH-KA) characterized by antioxidant and anti-aging properties was included in the study for the basis of comparison.

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By varying the solvents used for extraction of the putative bioactive metabolites, this research focused on determining the antioxidant and anti-aging properties of the leaf extracts of *A. platyphyllum* and *A. madurense*.

Materials and Methods

Solvents, reagents, and equipment

Ethanol, methanol, ethyl acetate, n-hexane, potassium persulfate, Folin-Ciocalteu reagent, sodium carbonate, aluminum chloride hexahydrate, potassium acetate, chloroform, sulfuric acid, dimethyl sulfoxide (DMSO), quercetin, ascorbic acid, ABTS, gallic acid, DPPH, nerol, yeast *Schizosaccharomyces pombe*, yeast extract powder, glucose, histidine, leucine, adenine, uracil, arginine, and agar. All chemicals had a purity of ProAnalyst (PA) and were purchased from Merck (Germany) or Sigma-Aldrich (USA). The equipment includes laboratory glass wares, analytical balance (Ohaus Pioneer PX124, USA), oven (Memmert UM 40, Germany), rotary evaporator (Buchi, Swiss), microplate reader (BioTek Epoch, USA), UV-Vis spectrophotometer (Hitachi, Japan), autoclave (GEA, China), shaker incubator (Labao, China), laminar airflow cabinet (LAFC) (Gelaire BSB 4A, Australia), Minitab 17.0 software, GraphPad Prism 10.2.3 software, and R-Studio 4.2.2 software.

Plant collection and identification

Fresh leaves of *A. platyphyllum*, *A. madurense*, and *A. lavenia* were collected from the Biopharmaca Conservation and Cultivation Station, Tropical Biopharmaca Research Center, IPB University, Indonesia (6°32'25.47" N; 106°42'53.22" E; 142.60 m altitude) on December 26, 2022. The plant materials were identified and authenticated with voucher specimen numbers; BMK0542032024, BMK0541032024, and BMK0540032024 for *A. platyphyllum*, *A. madurense*, and *A. lavenia*, respectively.

Plant sample preparation and moisture content determination

The leaves of *A. platyphyllum*, *A. madurense*, and *A. lavenia*, were cleaned and dried at 40°C. the dried leaves were pulverized to a coarse powder of about 80 mesh particle size.[1] The moisture content was determined using gravimetric method described by the Association of Official Analytical Chemists (AOAC) standards. In this method, the powdered plants samples were placed in clean dry crucibles, and then heated in an oven at 105°C for four hours. The moisture content was determined from the difference in weight between the wet weight and dry weight of the sample.[15]

Extraction

The extraction was done according to the procedure described by Rafi *et al*.[16] with slight modifications and in compliance with the Indonesian Herbal Pharmacopoeia standard (2nd edition). Briefly, 40 g of the leaf powder was macerated in 400 mL of *n*-hexane at 25°C for 6 h, with occasional stirring, and thereafter left to stand for 18 hours. The extract was filtered, and the filtrate was concentrated in a rotary evaporator at 50°C. The residue was subjected to another extraction by maceration in ethyl acetate followed by methanol. The entire extraction process was carried out three times using *n*-hexane, ethyl acetate, and methanol in the order of increasing polarity. The yield of the different concentrated extracts was determined.

Determination of total phenolic content

The total phenolic content was determined following the method described by Budiarti *et al.*[1] In a 96-well microplate, 20 µL of the plant extract was mixed with 110 µL of 10% Folin-Ciocalteu reagent in each well. Subsequently, 70 µL of 7.5% sodium carbonate solution was added to the mixture, and then incubated at 25°C for 30 min. The absorbance of the resulting mixture was measured at 765 nm. A standard solution of gallic acid was prepared in different concentrations (200, 100, 70, 50, 30, 10, 5, and 2.5 µg/mL), and used to prepare a calibration curve. The total phenolic content was obtained from the equation of the curve. The total phenolic content was reported as mg gallic acid equivalent (GAE)/g extract.

Determination of total flavonoid content

The total flavonoid content was determined following the method described by Adelegan *et al.*[17] and Budiarti *et al.*[1] Briefly, the extract solution (10 µL) was mixed with 60 µL of methanol, 10 µL of 10% aluminum chloride, 10 µL of 1 M potassium acetate, and 110 µL of water in the wells of 96-well microplates. The mixture was maintained at room temperature for 30 min, and the absorbance was determined at 415 nm. Subsequently, standard solutions of quercetin (500, 250, 100, 70, 50, 25, 12.5, 6.25, and 3.125 µg/mL) were prepared, and used to make a calibration curve. The total flavonoid content was obtained from the equation of the curve, and expressed as mg quercetin equivalent (QE)/g extract.

Determination of total terpenoid content

The total terpenoid content was determined according to the method described by Łukowski *et al.*[18] Briefly, 200 µL of the extract was mixed with 1.5 mL of chloroform. The mixture was allowed to stand for 3 min and 100 µL of sulfuric acid was added. The sample and standard solutions were incubated in the dark for 2 h 5 min. After the formation of a reddish-brown precipitate, the liquid mixture was separated, and 1.5 mL of methanol was added to the residue, and mixed thoroughly. Subsequently, the solution was transferred to a cuvette and the absorbance was measured at 520 nm. Standard solutions of quercetin (500, 250, 100, 70, 50, 25, 12.5, 6.25, and 3.125 µg/mL) were prepared, and used to plot a calibration curve. Total terpenoids were presented in terms of mg nerol equivalent (NE)/g extract.

Evaluation of antioxidant activity using the DPPH radical scavenging assay

The antioxidant activity of the extracts was evaluated using the method described by Prastya *et al.* [19] and Ijoma *et al.*[20] Briefly, 100 µL of DPPH (125 µM) was mixed with 100 µL of the test sample, reference sample (ascorbic acid) in 96-well microplates. The mixture was incubated at room temperature for 30 min in the dark, and the absorbance was measured at 515 nm using a microplate reader (BioTek Epoch). The antioxidant potential of the extracts was estimated as half-maximal inhibitory concentration (IC_{50}).

Evaluation of antioxidant activity using the ABTS radical scavenging assay

The antioxidant capacity was determined using the ABTS strategy described by Prastya *et al.*[18] In this assay, 10 mL of 7 mM ABTS solution was oxidized by reacting it with 5 mL of 2.45 mM potassium persulfate, and the reaction mixture was incubated in the dark for 16 h. Standard solutions (ascorbic acid) were prepared in different concentrations (75, 50, 25, 12.5, 6.25, and 3.125 µg/mL). To measure the antioxidant capacity, a volume of 180 µL of the ABTS radical was combined with 20 µL of the extract or standard for six minutes in a 96-well microplate. Subsequently, the absorbance was measured at 734 nm, and the antioxidant capacity was expressed as mg ascorbic acid equivalent (AAE)/g extract.

Evaluation of anti-aging activity using the spot test method

The spot test method was performed according to the procedures described by Astuti *et al*.[21] and Batubara *et al*.[5] To prepare a solid yeast extract supplement (YES) medium, 1.5 g of yeast extract powder, 9 g glucose, 0.128 g histidine, 0.128 g leucine, 0.128 g adenine, 0.01 g uracil, 0.128 g arginine, and 6 g agar were dissolved in distilled water and made up to in 300 mL. Subsequently, the mixture was sterilized using an autoclave at 100°C for 10 min. The liquid YES medium was prepared with the same composition as the solid YES medium but without adding agar powder. As an inoculum, *S. pombe* yeast was cultured in 3 mL of liquid YES medium for 24 h and adjusted to an absorbance of 0.05 at 600 nm wavelength (OD_{600} of 0.05). The extract at concentrations of 1 and 5 times the IC_{50} value was dissolved in DMSO and added to the culture. Subsequently, positive controls were prepared by culturing *S. pombe* yeast in liquid YES medium with 0.3% glucose. While the negative controls comprised cultures growing in liquid YES medium with 3.0% glucose. All culture treatment was kept in a shaker incubator at 300 rpm for 11 days, with spotting carried out

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on days 7 and 11. The OD_{600} values were adjusted to 1 for each treatment and diluted to 10^4 times. The spot test medium was incubated for three days to observe yeast viability based on colony growth density and each culture treatment was performed in duplicate.

Statistical analysis

The experimental data were expressed as the mean value with the corresponding standard deviation. Subsequently, Minitab 17.0 software was used to determine significant difference between the test samples and the reference. The data was subjected to a two-way analysis of variance (ANOVA), followed by Tukey post hoc test at 95% confidence interval. The association between antioxidant activity and the total phenolic, flavonoids, and terpenoid contents was examined using Pearson's correlation, facilitated by the Performance Analytics package in R-Studio version 4.2.2.

Results and Discussion

Extraction yield

There was a significant variation in the yield following the extraction of *A. platyphyllum* and *A. madurense* leaves using different solvents (*n*-hexane, ethyl acetate, and methanol). From the results obtained, the yield ranged from 1.18 ± 0.03% for *A. madurense n*-hexane extract to 11.95 ± 0.83% for *A. madurense* methanol extract. However, the yields of these two species were not significantly different from that of *A. lavenia*. This showed that the use of solvents with different polarities substantially affected the yield of the extract as confirmed by the Tukey test. The results emphasized the significance of solvent polarity in the extraction of compounds from *A. platyphyllum* and *A. madurense*. The extract yield increased as the polarity of the solvent increased in the following order; methanol > ethyl acetate > *n*-hexane (Figure 1). This indicates that the plant samples contained more of polar components. Additionally, methanol solvent has greater potential to extract protein and carbohydrate components.[22]

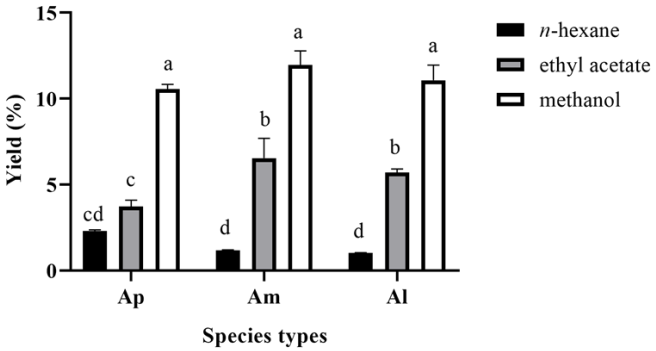

Figure 1: Extract yields of *A. platyphyllum* (Ap), *A. madurense* (Am), and *A. lavenia* (Al). Data are presented as mean ± standard deviation (SD). The letters a-d indicate significant difference (two-way ANOVA, Tukey test, $p < 0.05$, and n = 3)

Total phenolic content

The total phenolic content of *A. platyphyllum* and *A. madurense* differed significantly, showing intriguing relationship with the extraction solvent (Table 1). The total phenolic content was estimated from the standard gallic acid calibration curve, and the results were expressed as mg GAE/g extract. The methanol extract of *A. platyphyllum* produced the highest phenolic content, while the lowest phenolic content was obtained for the *n*-hexane extract of *A. madurense*. The variation in the results can be attributed to the polarity of phenolic compounds, making polar solvents such as methanol more effective for extraction.[23] In comparison, the total phenolic content obtained for the methanol extract of *A. platyphyllum* was significantly higher than that of the methanol extract of *A. madurense*. Although *A. lavenia* was used as a reference species, the total phenolic content obtained in this study exceeded that

Table 1: Total Phenolic, total flavonoid, and total terpenoid contents of *A. platyphyllum*, *A. madurense,* and *A. lavenia*

Sample	Extract	Total phenolics (mg GAE/ g extract)	Total flavonoids (mg QE/ g extract)	Total terpenoids (mg NE/ g extract)
A. platyphyllum	*n*-Hexane	5.16 ± 1.89^{fg}	21.44 ± 2.23^{ef}	1.13 ± 0.34^{d}
A. madurense	*n*-Hexane	1.43 ± 0.68^{g}	28.83 ± 1.71^{d}	1.59 ± 0.27^{d}
A. lavenia	*n*-Hexane	8.69 ± 1.46^{f}	24.80 ± 1.65^{de}	1.17 ± 0.04^{d}
A. platyphyllum	Ethyl acetate	46.33 ± 3.24^{d}	58.62 ± 2.85^{b}	6.88 ± 0.10^{a}
A. madurense	Ethyl acetate	36.73 ± 1.48^{e}	43.62 ± 0.84^{c}	2.84 ± 0.27^{c}
A. lavenia	Ethyl acetate	55.55 ± 0.34^{c}	74.41 ± 1.43^{a}	4.84 ± 0.56^{b}
A. platyphyllum	Methanol	120.06 ± 6.11^{a}	16.20 ± 1.23^{f}	2.86 ± 0.38^{c}
A. madurense	Methanol	52.41 ± 1.02^{c}	9.32 ± 0.47^{g}	1.53 ± 0.19^{d}
A. lavenia	Methanol	91.04 ± 2.90^{b}	20.05 ± 0.33^{ef}	0.84 ± 0.13^{d}

Data represent the mean ± standard deviation (SD). Different superscript letters ($^{a-g}$) signify significant differences among the values in the same column (two-way ANOVA, Tukey test, $p < 0.05$, and n = 3).

reported by Budiarti *et al.*[1] where 12.83 mg GAE/g extract was obtained. This disparity could be due to variations in the extraction methods, for example, the tiered maceration method produces more specific compounds irrespective of the solvent used.[24]

Phenolic compounds comprise one or more hydroxyl groups in their aromatic ring and are commonly found in several plants. Examples of phenolic compounds are quercetin, resveratrol, epigallocatechin gallate, kaempferol, chlorogenic acid, curcumin, catechin, apigenin, ferulic acid, and salicylate. Furthermore, phenolic compounds possess discrete bioactivities, such as antioxidant, anti-inflammatory, antitumor, and rejuvenating properties. This suggests that phenolic compounds from *A. platyphyllum* and *A. madurense* may contribute to their antioxidant and anti-aging activities by inhibiting digestive enzyme activity and protecting cells from oxidative damage.[25]

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Total flavonoid content

The flavonoid contents of *A. platyphyllum* and *A. madurense* showed significant variation from that of *A. lavenia*, with the solvents playing a role in the observed variations. The values obtained were estimated from the standard quercetin calibration curve and the results were presented in mg QE/g extract. The highest total flavonoid content was found in the ethyl acetate extracts of both species, although the total flavonoid content for *A. platyphyllum* and *A. madurense* were lower compared to that of *A. lavenia* (Table 1).

The high flavonoid content in the ethyl acetate extracts suggests that these species are rich in semipolar flavonoid components. Generally, polar components such as carbohydrates are coextracted, resulting in low flavonoid content in the methanol extract.[26,27] The flavonoid content obtained in this study were higher than the values obtained in the study of Budiarti *et al.*[1], where the total flavonoid content of *A. lavenia* was 0.48 mg QE/g extract. The flavonoid content in this study were slightly higher compared to that obtained from the study of Nair *et al.*[28], where 19.82 mg QE/g extract was obtained. Generally, flavonoid compounds belong to the phenolic compound category, with a carbon framework of diphenyl propane; i.e. two benzene rings connected through a carbon bridge. Different types of flavonoids, such as quercetin, kaempferol, and flavonol, are recognized for their neuroprotective effects, while catechin, epicatechin, and flavanol possess antihypertensive and nephroprotective properties.[29] These compounds may be responsible for the antioxidant activity observed in *A. platyphyllum* and *A. madurense*.

Total terpenoid content

The results from this study showed that the total terpenoid contents in ethyl acetate and methanol extracts of *A. platyphyllum* and *A. madurense* varied significantly from that of *A. lavenia*. However, the values obtained for the *n*-hexane extracts of each species did not show significant differences. The total terpenoid contents were calculated from the standard nerol calibration curve and the results were expressed as mg NE/g of extract. As shown in Table 1, the highest total terpenoid content was found in the ethyl acetate extract, with *A. platyphyllum* having a higher content than *A. lavenia*, while *A. madurense* had a lower content.

The terpenoid compounds in certain species have hydroxyl functional groups and are semipolar in nature.[30] This assertion is in congruence with the research by Maeda *et al.*[4], where *A. lavenia* was found to contain terpenoid compounds, including ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid, 11αOH-KA, 9,11α-dehydroxy-15-oxo-kaur-16-en-19-oic acid (adenostemmoic acid B), and 11α,15-dihydroxy-16-kauren-19-oic acid, with antioxidant activity.

Antioxidant activity

The results obtained from the present study showed that the antioxidant activity of the extracts derived from *A. platyphyllum* and *A. madurense* was comparatively weaker against DPPH radical. However, the ethyl acetate extract of *A. platyphyllum* showed higher levels of antioxidant activity compared to *A. lavenia* (Table 2). The study of Budiarti *et al.*[1] showed significant antioxidant activity for *A. lavenia*, with IC_{50} reported as 121.82 µg/mL. The variation observed could be attributed to variations in the solvents used for extraction and extraction method. Based on these results, *A. platyphyllum* was classified as a strong antioxidant with IC_{50} ranging from 50 to 100 µg/mL similar to *A. lavenia.* Meanwhile, *A. madurense* was considered a weak antioxidant with IC_{50} between 151 and 200 µg/mL. The antioxidant activity of all the extracts did not compare with that of ascorbic acid which was used as the positive control, and was classified as a powerful antioxidant with $IC_{50} < 50$ µg/mL.[31]

Table 2: Antioxidant activity of *A. platyphyllum*, *A. madurense*, and *A. lavenia* extracts

Sample	Extract	Antioxidant activity	
		DPPH IC_{50} (µg/mL)	ABTS (mg AAE/g extract)
A. platyphyllum	*n*-Hexane	1556.89 ± 36.25^{a}	10.77 ± 1.28^{d}
A. madurense	*n*-Hexane	1540.44 ± 46.74^{a}	7.54 ± 1.37^{d}
A. lavenia	*n*-Hexane	1439.59 ± 19.52^{b}	8.31 ± 1.83^{d}
A. platyphyllum	Ethyl acetate	137.43 ± 2.23^{de}	16.99 ± 0.75^{c}
A. madurense	Ethyl acetate	305.86 ± 11.15^{c}	10.00 ± 1.21^{d}
A. lavenia	Ethyl acetate	203.17 ± 5.17^{d}	15.36 ± 1.85^{c}
A. platyphyllum	Methanol	91.83 ± 1.90^{ef}	38.42 ± 0.62^{a}
A. madurense	Methanol	190.03 ± 7.36^{d}	28.03 ± 0.81^{b}
A. lavenia	Methanol	59.67 ± 1.51^{f}	35.79 ± 1.37^{a}
Ascorbic acid*		4.80 ± 0.07	-

Data represent the mean ± standard deviation (SD). Different superscript letters ($^{a\text{-}f}$) signify significant differences among the values in the same column (two-way ANOVA, Tukey test, $p < 0.05$, and n = 3). *Ascorbic acid is a positive control.

The ABTS method is a sensitive and simple method used to assess the antioxidant capacity of various samples. In the present study, the ABTS radical scavenging activity of *A. platyphyllum* and *A. madurense* was evaluated with reference to ascorbic acid standard. The values were expressed as mg ascorbic acid equivalent (AAE)/g extract. The highest ABTS radical scavenging activity was observed in the methanol extract of *A. platyphyllum*, and was comparable to that of *A. lavenia*, while the methanol extract of A. madurense exhibited the lowest activity. The work of Budiarti *et al*. showed that *A. lavenia* have high antioxidant activity with a value of 3.38 mg trolox equivalent (TE)/g extract, which was higher than what was obtained in this study. The differences in the results might be attributed to the use of different extraction solvents.

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Anti-aging activity

The anti-aging properties of *A. platyphyllum* and *A. madurense* was evaluated using the spot test method. In this method, the effect of the test sample on the viability of *S. pombe* yeast cells grown on YES medium is used as a measure of antiaging properties. The results showed that the methanol extract of *A. platyphyllum* had the highest cell viability compared to the other samples. As shown in Figure 2, cell viability was represented by colony density at each dilution, with a high colony density signifying high cellular viability. The administration of *A. platyphyllum* methanol extract at a concentration of 459.20 µg/mL (5 times IC_{50}) was more effective than 91.83 µg/mL concentration (1-time IC_{50}) as shown by the cell viability on day 11. These results were similar to those obtained for *A. lavenia*. It is important to state that *A. playphyllum* methanol extract maintained yeast cell viability for 11 days, identical to the effect of calorie restriction therapy (positive control). Calorie restriction therapy is capable of promoting cell longevity by inducing the sirtuin pathway and autophagy.[32,33] This phenomenon is conserved in many models of organisms, including yeast, worms, fish, mice, and human cells.[34,35] Previous research has reported the potential of the compound 11αOH-KA extracted from *A. lavenia* as an anti-aging agent. Therefore, further investigation is required to isolate and characterize compound(s) with anti-aging activity in *A. platyphyllum* methanol extract.

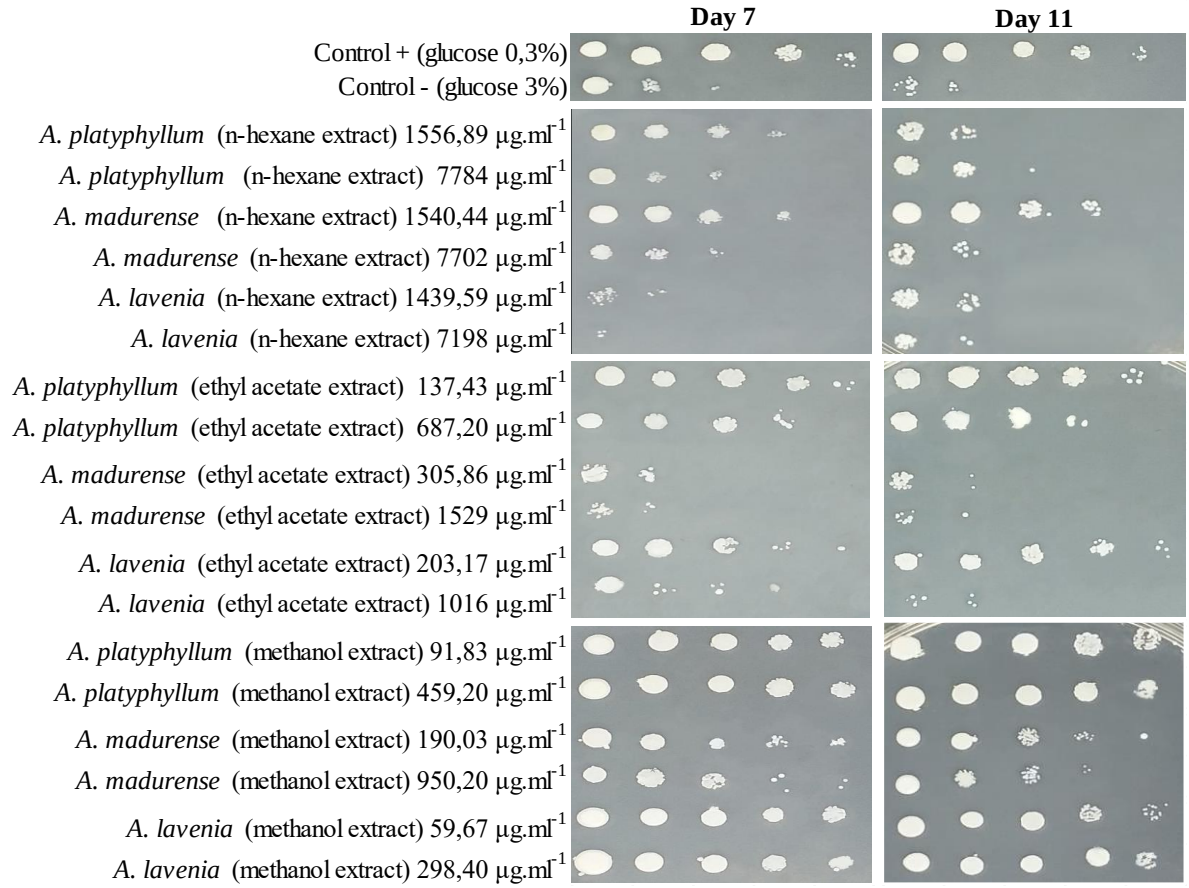

Figure 2: Effect of extracts on the viability of *S. pombe* yeast cells grown on YES medium on day 7 and day 11. The concentration used is 1 and 5 times the IC_{50} values in the DPPH analysis. The species *A. lavenia* is used for comparison.

Correlation matrix of test parameter

Figure 3 shows the correlation coefficient between the antioxidant activity, and the total phenolic, total flavonoid, and total terpenoid contents in *A. platyphyllum*, *A. madurense*, and *A. lavenia*. The correlation coefficient between IC_{50} and antioxidant activity (DPPH and ABTS radical scavenging activities) was -0.70, reflecting a strong negative correlation. This indicates that the variables are inversely proportional, where lower IC_{50} value translate to higher antioxidant activity.[1] From the correlation analysis, total phenolic content was positively correlated with the antioxidant and anti-aging properties of the methanol extract of *A. platyphyllum*. Whereas in the ethyl acetate extract of *A. platyphyllum*, the total flavonoid and total terpenoid were positively correlated with the antioxidant and anti-aging activities. It was found that the correlation coefficients between DPPH radical-scavenging activity and total phenolic, total flavonoid, and total terpenoid contents were -0.82 (a strong negative correlation at a significance level of 0.01), -0.24 (a weak negative correlation), and –0.48 (a moderate negative correlation), respectively. Meanwhile, the correlation coefficients between ABTS antioxidant capacity and total phenolic, total flavonoid, and total terpenoid contents were 0.92 (a robust positive correlation at a significant level of 0.001), -0.40 (a moderate negative correlation), and -0.073 (a negligible negative correlation), respectively. These observations indicate that the phenolic compounds had the most significant impact on the antioxidant activity of each species. Pearson's correlation is a statistical analytical method that is used to determine the association between two parameters. The significance of this relationship is evaluated by the *p*-value, which should be less than a certain level. The correlation coefficient (r) shows the effectiveness of the relationship between two variables and is classified into five different categories: robust (0.90-1.00), strong (0.70-0.89), medium (0.40-0.69), weak (0.10-0.39), and neglected correlation (0.00-0.10).[36]

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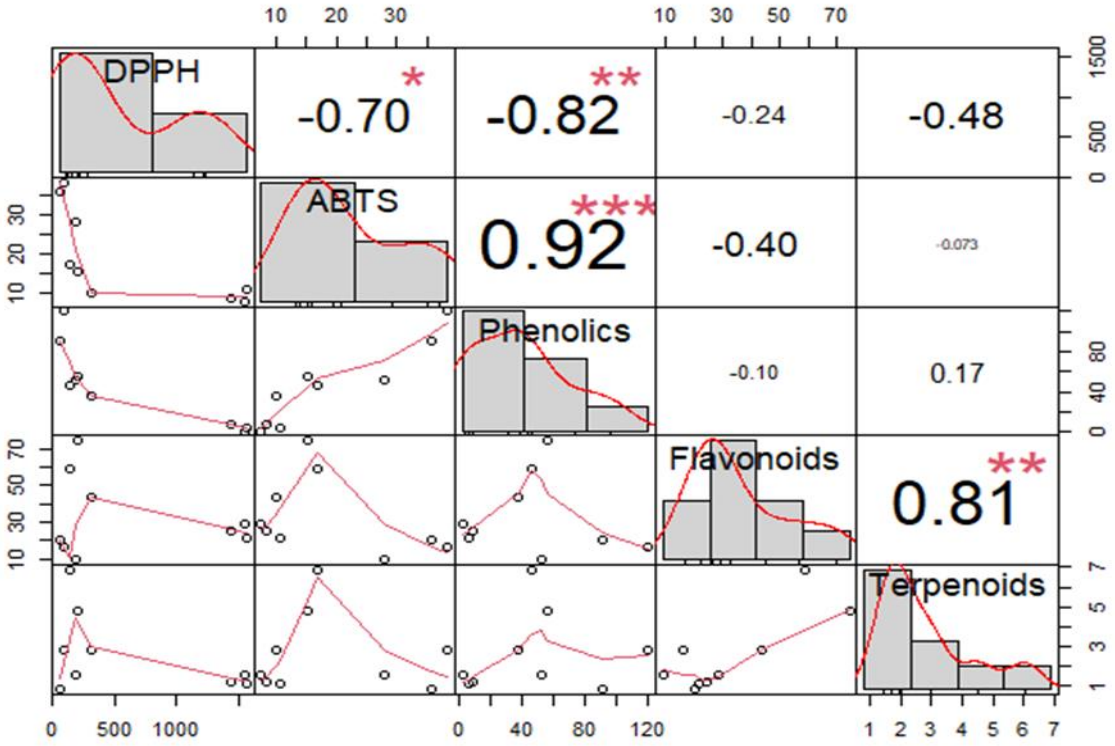

Figure 3: Diagonal matrix of correlation between antioxidant activity, total phenolic content, total flavonoid, and total terpenoid content of *A. platyphyllum*, *A. madurense*, and *A. lavenia*. The upper diagonal signifies the Pearson correlation coefficient, while the lower diagonal signifies the score plot. The *, **, and *** signs represent significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Conclusion

The findings from this study have shown that the plant species A. platyphyllum and A. madurense has variable antioxidant activities depending on the polarity of the solvent used for extraction. *A. platyphyllum* is a more potent antioxidant compared to *A. madurense* which had similar antioxidant activity as *A. lavenia*. Phenolic content was highest in the methanol extract of *A. platyphyllum*. Whereas the ethyl acetate extract of the same plant contained the highest amount of flavonoids and terpenoids. The methanol extract of *A. platyphyllum* also showed the potential to increase the viability of yeast cells, indicating potential as an anti-aging agent. Correlation analysis showed that phenolic molecules were strongly associated with the antioxidant activity. Therefore, among all the extracts of the different plant species, the methanol extract of *A. platyphyllum* possessed the strongest anti-aging and antioxidant properties which could be attributed to the presence of phenolic compounds.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Budiarti E, Batubara I, Ilmiawati A. The potency of Asteraceae plants extracts as antioxidant and antiglycation agent. Jurnal Jamu Indon. 2019; 4(3):103–111.
2. Oktarina R and Salamah A. Species identification of Asteraceae family at Universitas Indonesia, Depok. J Pro-Life. 2017; 4(1): 241-249.
3. Jeong KS, Heo TI, Lee KH, Choi K, Kim HJ. A new record *Adenostemma madurense* DC. (Asteraceae) in Korea. Korean J Plant Res. 2017; 30(3):331-334.
4. Maeda M, Suzuki M, Fuchino H, Tanaka N, Kobayashi T, Isogai R, Batubara I, Iswantini D, Matsuno M, Kawahara N, Koketsu M, Hamamoto A, Takemori H. Diversity of *Adenostemma lavenia*, multi-potential herbs, and its kaurenoic acid composition between Japan and Taiwan. J Nat Med. 2022; 76(1):132–143.
5. Batubara I, Astuti RI, Prastya ME, Ilmiawati A, Maeda M, Suzuki M, Hamamoto A, Takemori H. The anti-aging effect of active fractions and ent-11α-hydroxy-15-oxo-kaur-16-en-19-oic acid isolated from *Adenostemma lavenia* (L.) O. Kuntze at the cellular level. Antioxid. 2020; 9(8):1–14.
6. Fauzan A, Praseptiangga D, Hartanto R, Pujiasmanto B. Characterization of the chemical composition of *Adenostemma lavenia* (L.) Kuntze and *Adenostemma platyphyllum* Cass. IOP Conf Ser.: Earth Environ Sci. 2018; 102(1): 012029.
7. Nur S, Hanafi M, Setiawan H, Nursamsiar N, Elya B. Molecular docking simulation of reported phytochemical compounds from *Curculigo latifolia* extract on target proteins related to skin anti-aging. Trop J Nat Prod Res. 2023; 7(11):5067-5080.
8. Sayakti PI, Anisa N, Ramadhan H. Antioxidant activity of methanol extract of cassava leaves (*Manihot esculenta* Crantz) using CUPRAC method. J Ilm Farm. 2022:97-106.

9. Andarina R and Djauhari T. Antioksidan dalam dermatologi. J Kedokt dan Kesehat. 2017; 4(1):39-48.
10. Maesaroh K, Kurnia D, Al Anshori J. Comparison of DPPH, FRAP, and FIC antioxidant activity test methods against ascorbic acid, gallic acid and quercetin. Chim Nat Acta. 2018; 6(2):93-100.
11. Puspitasari AD, Susanti E, Khustiana A. Antioxidant activity and determination of vitamin C content of lemon juice (*Citrus limon* (L.) Osbeck) using the ABTS method. J Ilmiah Teknosains. 2019; 5(2):99–104.
12. Lin SJ and Austriaco N. Aging and cell death in the other yeasts, *Schizosaccharomyces pombe* and *Candida albicans*. FEMS Yeast Res. 2014; 14(1):119–135.
13. Lesmana D, Andrianto D, Astuti RI. Anti-aging properties of the ethanol fractions of clove (*Syzygium aromaticum* L.) bud and leaf at the cellular levels: Study in yeast *Schizosaccharomyces pombe*. Sci Pharm. 2021; 89(4):1-14.
14. Rafi M, Meitary N, Septaningsih DA, Bintang M. Phytochemical profile and antioxidant activity of *Guazuma ulmifolia* leaves extracts using different solvent extraction. Int J Pharm Pharm Sci. 2020; 31(3):171-180.
15. Association of Official Analytical Chemists [AOAC]. Official Method of Analysis of The Association of Official Analytical Chemists Nineteenth Ed. Arlington: AOAC International; 2016.
16. Ministry of Health Republic of Indonesia. Indonesian Herbal Pharmacopoeia. (2nd ed.). Jakarta: Ministry of Health Republic of Indonesia; 2017.
17. Adelegan AA, Dokunmu TM, Iweala EEJ. *In-vitro* antioxidant activity and cytotoxic effect of ethanol leaf extract and fractions of *Olax subcorpioidea* Oliv. (Olacaceae). Trop J Nat Prod Res. 2023; 7(8):3806-3812.
18. Łukowski A, Jagiełło R, Robakowski P, Adamczyk D, Karolewski P. Adaptation of a simple method to determine the total terpenoid content in needles of coniferous trees. Plant Sci. 2022; 314:111090.
19. Prastya ME, Astuti RI, Batubara I, Wahyudi AT. Bacillus sp. SAB E-41-derived extract shows anti-aging properties via ctt1-mediated oxidative stress tolerance response in yeast *Schizosaccharomyces pombe*. Asian Pac J Trop Biomed. 2018; 8(11):533–539.
20. Ijoma KI, Ajiwe VIE, Odinma ASC. The organic extreact from the leaves of *Ficus thonningii* Blume, *Jatropha tanjorensis* J.L Ellis and Saroja and *Justicia carnea* Lindley as potential nutraceutical antioxidants and functional foods. Trends Phytochem Res. 2023; 7(1):76-85.
21. Astuti RI, Prastya ME, Batubara I, Budiarti E, Ilmiyawati A. Anti-aging and antioxidant bioactivities of Asteraceae plant fractions on the cellular functions of the yeast *Schizosaccharomyces pombe*. Adv Pharmacol Pharm Sci. 2021; 2021(2):1-12.
22. Kurniawati I, Maftuch, Hariati AM. Determination of the best solvent and extract duration on the technique of *Gracilaria sp*. maceration as well as its influence on moisture content and yield. J Ilmu Perik. 2016; 7(2):72-77.
23. Yulianti W, Ayuningtyas G, Martini R, Resmeiliana I. Effect of extraction method and solvent polarity on total phenolic content of cherry leaves (*Muntingia calabura* L). J Sains Terapan. 2020; 10(2):41–49.
24. Hamka Z, Noena RAN, Azmin RAP. The effect of the multilevel maceration method on the yield value and thin layer chromatography (TLC) profile of basil leaf extract (*Ocimum basilicum* L.). Jurkes Yamasi Makassar. 2022; 6(1):154-162.
25. Zhu F. Interactions between starch and phenolic compound. Trends Food Sci Technol. 2015; 43(2):129-143.
26. Theodora CT, Gunawan IWG, Swantara IMD. Isolation and identification of flavonoid groups in ethyl acetate extract of gedi leaves (*Abelmoschus manihot* L.). J Chem. 2019; 13(2):131-138.
27. Verdiana M, Widarta IWR, Permana IDGM. The effect of solvent type in extraction using ultrasonic waves on the antioxidant activity of lemon peel extract (*Citrus limon* (Linn.) Burm F.). J Ilmu dan Teknologi Pangan. 2018; 7(4):213-222.
28. Nair NC, Sheela D, Cherian P. HPTLC analysis of flavonoids among selected members of Asteraceae. Int Res J Biol Sci. 2017; 6(6):31-37.
29. Mutha RE, Tatiya AU, Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Fut J Pharm Sci. 2021; 7(1):1-13.
30. Purnama A, Mustanir, Ginting B. Isolation and cytotoxic activity of terpenoid compounds from *n*-hexane extract of cacao pod husk (*Theobroma cacao* L.). AIP Conf Proc, Medan; 2021.
31. Molyneux P. The use of the stable free radical diphenylpicryl hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004; 26(2):211-219.
32. Chung KW and Chung HY. The effect of calorie restriction on autophagy: Role in aging intervention. Nutr. 2019; 11(12):1-18.
33. Taormina G and Mirisola MG. Calorie restriction in mammals and simple model organisms. Biomed Res Int. 2014; 308690:1-9.
34. Fontana L, Partridge L, Longo VD. Dietary restriction, growth factors and aging: from yeast to humans. Sci. 2010; 328(5976):321-326.
35. Ruetenik A and Barrientos A. Dietary restriction, mitochondrial function and aging: from yeast to humans. BBA – Bioenergetics. 2015; 1847(11): 1434-1447.
36. Schober P and Schwarte LA. Correlation coefficients: Appropriate use and interpretation. Anesth Analg. 2018; 126(5):1763–1768.