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Antioxidant, Anti-Aging and Antibacterial Activity from Dewa Leaves Ethanolic Extract (*Gynura japonica* (Thunb.) Juel)

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ABSTRACT. Due to their phytochemical constituents, Dewa leaves (*Gynura japonica* (Thunb.) Juel.) are often used in traditional medicinal herbs. However, *in vitro* and *in vivo* of antioxidative and anti-aging studies of Dewa leaves on yeast as a eukaryotic cell model have not been widely carried out. This study aims to determine the antioxidant, anti-aging, and antibacterial activities derived from *G. japonica* leaves extract. Extraction was conducted using 70% and 96% ethanol solvents, total phenolic content (TPC) was assayed using *Folin-Ciocalteu* method, flavonoid contents (TFC) was assayed using aluminum chloride method, antioxidant activity was tested using DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-azinobis-3-Ethylbenzothiazoline)-6-sulfonic acid) radicals. Subsequently, an anti-aging activity test was performed on the yeast *Saccharomyces cerevisiae* as a model organism following antibacterial activity. The antibacterial activity test was carried out using well diffusion agar, and the Minimal Inhibitory Concentration (MIC) was determined using the microplate method. We obtained that *G. japonica* leaves extracted from 70% and 96% methanol solvents, have TPC of 13.14 and 22.11 mg GAE/gr extract, and TFC of 8.04 and 14.09 mg QE/gr extract, respectively. As for DPPH and ABTS antioxidant activity, D70 showed the best activity with IC_{50} values of 1411.36±56.35 µg/mL and 2516.10±18.77 µg/mL, respectively. The anti-aging test showed that both 70% and 96% ethanol extracts were able to maintain the yeast cell viability under H_2O_2 oxidative stress. Further, 70% and 96% ethanol extract also showed antibacterial activity at the best value against *Staphylococcus aureus* with a MIC value of 390.62 µg/mL, it means that Gynura leaves extract has a potency as antibacterial agent. The results of this study indicate that the ethanol extract of *G. japonica* leaves can be developed for further investigation as an antioxidant and antibacterial therapeutic agent.

Keywords: Anti-aging, antibacterial, antioxidant, *Gynura japonica, Saccharomyces cerevisiae*

INTRODUCTION

The use of native Indonesian plants as herbal preparations that are rich in benefits has started to become a trend and has continued to increase for several decades. One such plant is Daun Dewa (*Gynura japonica* (Thunb.) Juel) and also known as *Gynura segetum.* This plant is one of the Indonesian medicinal plants which has long been used for generations to treat various diseases such as cancer, fever (antipyretic), diabetes, high blood pressure, and skin diseases (external medicine) (Bari et al., 2021; Meng et al., 2021; Tan et al., 2016). To improve the quality, safety, and benefits of *G. japonica* plant as Indonesian natural medicine, it is necessary to standardize the raw materials, both in the form of simplicial and in the form of extracts or galenic preparations.

Pharmacological studies show that *G. japonica* leaves are proven to have various biological activities that strengthen the scientific basis for their use as traditional medicine. *G. japonica* leaves extract showed a significantly greater anti-angiogenic effect in inhibiting the growth and metastasis of tumor cells (Seow et al., 2011). This extract has also been shown in experimental animals to reduce blood cholesterol levels, lower uric acid levels, and be anti-inflammatory (Nazri et al., 2019; Tan et al., 2020; Seow et al., 2014). In vitro tests on *G. japonica* leaf extract have been shown to contain phenolic compounds that can inhibit DPPH free radicals (Hsieh et al., 2020). This evidence reinforces the notion that many benefits of *G. japonica* leaves in medicine are due to their chemical content which is efficacious as an antioxidant. The content of phenolic

compounds and the antioxidant power of *G. japonica* leaves are influenced by some conditions including the drying and extraction method, and the solvent used in the extraction process (Rivai et al., 2012). In addition, *G. japonica* leaves did not cause congenital abnormalities or defects in the organogenesis phase of the experimental rats. Based on the results of this research, *Gynura* leaves are safe to use as medicine during pregnancy (Kamaruzaman & Mat Noor, 2017).

Of note, *G. japonica* leaves are reported to contain some phytochemicals compounds such as alkaloids, saponins, flavonoids, essential oils, and tannins (Bari et al., 2021). Interestingly, essential oils have antibacterial and antifungal properties. Tannins are ingredients found in medicinal plants and have a physiological action in inhibiting bacteria. On the other hand, flavonoids are a class of phenolic compounds which have bactericidal and fungicidal activity. *G. japonica* leaf extract effective in inhibiting the growth of *Staphylococcus aureus, Bacillus subtilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabili,* and *Candida albicans* (Seow et al., 2012; Nasiruddin & Sinha, 2020).

Moreover, cellular manifestations of the aging process are also influenced by the Reactive Oxygen Species (ROS) factor produced in cells. Suppose there is an imbalance between oxidants and antioxidants in the body due to an increase in ROS and a decrease in antioxidants from the body. In that case, it causes cell damage and affects aging (Prastya et al., 2020). Ethanolic extract of *G. japonica* leaves exhibited strong antioxidant activity, because of rich flavonoid content (Kaewseejan et al., 2015). Antioxidants from synthetic materials provide side effects that are quite harmful to health, especially causing cancer. Therefore, natural sources of antioxidants that are safer to develop are sought. Chemical compounds belonging to the group of antioxidants and can be found in plants, among others, come from the group of polyphenols, bioflavonoids, vitamin C, vitamin E, beta carotene, catechins, and resveratrol (Xu & Zhang, 2017). The results of previous research have identified the content of antioxidant compounds in Gynura plants and carried out in vitro tests (Kim et al., 2021). However, this research will also test the effect of administering Gynura leaf extract on the model organism *Saccharomyces cerevisiae*, where this organism has a known genome map, has an mRNA splicing process that is similar to the metazoan group, and has a trait inheritance mechanism that is similar to mammalian cells (Vanderwaeren et al., 2022). Therefore, it is hoped that the results of this research can be the basis for developing therapeutic agents for *G. japonica* leaves as antioxidants, antiaging and antibacterial.

EXPERIMENTAL SECTION

Material

The materials used in this study were samples of *G. japonica* leaf plants, ethanol, *Folin-Ciocalteu* reagent, gallic acid, quercetin standard, ammonium chloride, $AlCl_3$, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox, ascobic acid, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate powder ($K_2S_2O_8$), EAST model *Saccharomyces cerevisiae* (InaCC B612), YPD medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 20 g L^{-1} glucose, and 1 L distilled water). Bacterial culture for antibiotic assay (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Bacillus subtilis*).

Plant Extraction

The plant used in this research is Daun Dewa (*Gynura japonica (*Thunb.) Juel) taken from Solo District (GPS location: 7°32'55.9"S 110°50'51.2"E). The leaves are dried in the oven and then crushed into powder and then weighed. The sample was then macerated with ethanol as the solvent. Maceration was carried out by immersing the sample in a 70% ethanol solution with a ratio of 1: 10 and 96% ethanol with a ratio of 1: 5, stored in a dark place at room temperature for 2-5 days without stirring. The sample is then separated by filtering and separated from the solvent by evaporation using a rotary evaporator at a temperature of 50-60 °C at a speed of 50-90 rpm to obtain a concentrated extract (Putri et al., 2024).

The sample extract solution with a test concentration of 10.000 ppm was prepared by weighing 10 mg of the sample and dissolved in 1 mL of 96% ethanol then dissolved until homogeneous. The solution is put in a tube which is then stored at 4 °C to avoid evaporation. The positive controls for antioxidant activity were Ascorbic Acid (AA) and Trolox (Tr) made in a stock concentration of 1000 μg/mL by weighing 1 mg AA and Tr and dissolved in 1 mL of 96% ethanol then dissolved until homogeneous. The solution is put in a tube covered with aluminum foil and stored at 4 °C to prevent evaporation (Astuti et al., 2021).

Determination of Total Phenolic Content (TPC)

The total phenol test was carried out using the standard Folin-Ciocalteu Reagent (FCR) method (Abo El-Maati et al., 2012)]. 500 μL concentration of 1000 μg/mL sample mixed with 3.5 mL of distilled water and 250 μL of FCR reagent. The mixture was homogenized and allowed to stand for 8 minutes. The solution was then added with 750 μL of 20% sodium carbonate. The mixture was homogenized again and then incubated for 2 hours at room temperature in the dark. Sample absorbance was measured at a wavelength of 765 nm using an ELISA reader (Thermoscientific Varioskan-Flash). Gallic acid stock concentration of 1000 μg/mL was used as a positive control. Gallic acid standard curves were

made in concentrations of 5, 10, 15, 20, and 25 μg/mL. Total phenol was calculated based on the equivalent number of mg gallic acid (EAG)/g extract.

Determination of Total Flavonoid Content (TFC)

The total flavonoid test was carried out by the aluminum chloride method using the quercetin standard. Samples with a concentration of 1000 μg/mL were used from a stock sample dilution of 10.000 μg/mL and dissolved in 96% ethanol. 500 μL of sample solution was mixed with 2.45 mL of distilled water and 150 μL of 5% $NaNO_2$. The mixture was homogenized and allowed to stand for 2 minutes. The solution was then added with 150 μL $AlCl_3$ and incubated for 8 minutes. Then 2 mL of NaOH 1 M was added to the solution. Sample absorbance was measured at a wavelength of 415 nm using an ELISA reader (Thermoscientific Varioskan-Flash). Quercetin stock concentration of 1000 μg/mL was used as a positive control. Quercetin standard curves were made in concentrations of 10, 20, 30, 50 and 70 μg/mL. Total phenol was calculated based on the equivalent number of mg quercetin/g extract (Prastya et al., 2019).

Antioxidant Activity Assays with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radicals

Both DPPH and ABTS radical's antioxidant activity were performed following methods as described elsewhere (Prastya et al., 2018). For DPPH activity, several sample solutions were made with the highest concentration of 10,000 μg/mL then diluted 8 times. Samples were pipetted 100 μL for 3 repetitions and 100 μL 96% ethanol was added to each well. 100 μL of 2.5 μM DPPH solution was added to the 96-well plate. The mixture was incubated for 30 minutes at 37 °C in the dark, then the absorbance was measured with ELISA microplate reader at a wavelength of 515 nm. Measurements were carried out 2 times with Trolox and Ascorbic Acid as positive controls and treated the same as the samples. The negative control was 100 μL ethanol added with 100 μL DPPH and the blank was 200 μL ethanol. As for ABTS methods, a total of 19.2 mg of ABTS powder and 3.3 mg of Potassium Persulfate powder ($K_2S_2O_8$) were dissolved in 5 mL of ethanol each. The two solutions were mixed in a 1:1 ratio and then incubated for 12-16 hours at room temperature in the dark. ABTS solution was diluted with 96% ethanol to obtain an absorbance value of 0.6 – 0.7 at a wavelength of 734 nm. Several sample solutions were made with the highest concentration of 10.000 ppm and then diluted 8 times. Samples were pipetted 50 μL for 3 repetitions and 50 μL 96% ethanol was added to each well. 150 μL of ABTS solution was added to the 96-well plate. The mixture was incubated for 30 minutes at 37 °C in the dark, then the absorbance was measured with an ELISA microplate reader at a wavelength of 734 nm. Measurements were carried out 2 times with Trolox and Ascorbic Acid as positive controls and treated the same as the samples. The negative control was 50 μL ethanol added with 150 μL ABTS and the blank was 200 μL ethanol. Inhibition value of both DPPH and ABTS method following formula:

$$\% \text{ Inhibition} = \left(\frac{AC - AS}{AC}\right) \times 100\%$$

Note:
AC = Absorbance Control
AS = Absorbance Sample

The percentage inhibition value is represented by the IC_{50} value calculated by the formula above. The inhibition value was regressed in the linear regression equation to obtain the 50% Inhibitory Capacity (IC_{50}) antioxidant capacity of each sample.

Anti-aging Activity Assays with Streak Method

Anti-aging activity was conducted following method as described (Astuti et al., 2021). Briefly, 100,000 μg/mL sample extract solution dissolved in 10% DMSO, and then filtered using a Millipore membrane 0.22 μm (Sartorius). Inoculation of 375 μL (OD_{600} 0f 0.05) *Saccharomyces cerevisiae* (InaCC B612) was carried out in 3 mL of liquid YPD medium supplemented with 200 uL of 2% glucose sterile solution and added 30 μL of plant extract with a concentration of 500 ppm. The test tubes were tightly closed and then incubated for 24 hours in a shaker at 200 rpm at room temperature (30 °C). Specifically for the control (-) only glucose and yeast were added, while the DMSO control was replaced with 10% DMSO of 30 μL. After that the tubes were incubated for 4 days (96 hours) in a shaker at 200 rpm at room temperature (30 °C). The media was poured into a petri dish and waited for it to solidify. Subsequently, streak the results of the inoculum on the test tube, onto YPD media containing 6 mM H_2O_2, 3 mM H_2O_2 and media without H_2O_2. After etching, the petri dishes were tightly closed and then incubated for 2 days (48 hours) at room temperature. The lines were observed for yeast growth and documented. The results showed that yeast only grew on solid YPD media treated with 6 mM H_2O_2. Samples showing anti-aging activity were observed.

Determination of Antibacterial Activity Using Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) Method

The initial antibacterial activity test conducted with an antibacterial screening on Gynura leaf ethanolic extract (70% and 96%) using the well diffusion agar method. The test bacteria used included *E. coli, P. aeruginosa, S. aureus,* and *B. subtilis,* Each of bacteria was grown on Mueller Hinton Agar medium. Subsequently, the test bacteria were adjusted to a 0.5 McFarland standard (equivalent to 1.5 x 10^8 CFU mL^{-1}) and then

inoculated. Using a micropipette, the test bacteria were extracted and then used the spread plate technique (spread method) to inoculate onto solid media. There was a waiting interval following inoculation to give the bacteria time to absorb into the solid media. To make it easier to insert the test samples, the negative control (DMSO solution in distilled water), and the positive control (chloramphenicol antibiotics), holes were made in the solid media. After that, the test medium's bacterial culture was concentrated for 24 hours at 37°C in an incubator. The last phase involved measuring and observing the bacterial inhibition zone, which was shown by the clear zone, carried out in triplicate.

The stock sample solution was prepared in 100,000 μg/mL at 10% DMSO. The control used was 2000 ppm tetracycline. Bacterial inoculation was carried out using MHB liquid medium. The MIC test was carried out using the dilution method. Beginning by filling all wells with 100 µL MHB media. Then added with 100 µL of two-fold diluted sample with two repetitions as well for the control. The extracted sample was replaced with 2000 ppm tetracycline and 10% DMSO 100 µL, then diluted. Next, add 100 µL of the bacteria which is set to Mc Farland standard of 0.5 which is equivalent to 1×10^8 CFU/mL. Place the cap on the microplate and incubate for one day (24 hours) in a shaker at 200 rpm at room temperature (30 °C). After 24 hours of incubation, it was observed whether there was bacterial growth. If there is bacterial growth, the concentration is expressed as the MIC value. Samples at concentrations where there was no bacterial growth were then tested for MBC, namely at the concentrations in the table and concentrations one level above it. The MBC test was carried out by taking 10 µL of the selected extract concentration and then dropping it on a petri dish containing NA media. Then incubated for a day (24 hours) at room temperature. After 24 hours of incubation, it was observed whether there was bacterial growth. If there is no bacterial growth, the concentration is expressed as the MBC value (Priyanto et al., 2022).

RESULTS AND DISCUSSION

G. japonica is a plant that is often found in the yard and is easy to cultivate. Indonesian people know this plant as "Daun Dewa" because it has many health benefits such as anti-inflammatory, antioxidant, treating high blood pressure, anti-diabetic, and anti-cancer (Wu et al., 2011) (Xu & Zhang, 2017). *G. japonica* leaves are also used as a source of natural antioxidant compounds that can inhibit the adverse effects of oxidative stress. Traditional medicinal plants such as *G. japonica* leaves have free antiradical compounds such as polyphenols, flavonoids, and phenolic compounds (Tan *et al.*, 2020). In this study, the antioxidant, anti-aging and antimicrobial activities of the ethanol extract of *G. japonica* leaves were tested. The results of this research imply that *G. japonica* leaves extract can be developed as a health supplement candidate to scavenge free radicals, anti-aging, as well as an alternative therapy for bacterial infections, especially against antibiotic-resistant bacteria.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Detection of total phenolic content in 70% ethanol extract and 96% ethanol extract of *G.japonica* leaves were 13.14±1.46 mg GAE/g and 22.11±2.63 mg GAE/g extract, respectively. While the total flavonoid content of 70% ethanol extract and 96% ethanol extract of *G. japonica* leaves were 8.04±1.22 mg QE/g and 14.70±1.05 mg QE/g extract, respectively (**Table 1**). The more concentrated of the solvent used the greater the phenolic content detected. Phenolics are one of the most abundant compounds and main active constituents obtained from plants of the genus Gynura (Meng et al., 2021). Phenolic compound were suggested to be responsible mainly for the antioxidant (Chen et al., 2015), antibacterial activities of Gynura plants (Saha et al., 2023). The use of ethanol solvents also greatly influences the extraction of phenolic compounds where ethanol has high polarity and good solubility (Abo et al., 2012). Ethanol is a safe solvent for natural products extraction and is used in the food and drug industry. Absolute ethanol and dilute ethanol are widely used for the extraction of antioxidant compounds derived from phenol from natural products with good results. The presence of phenol and flavonoid compounds in *G. japonica* leaves extract indicates that this plant contains natural antioxidants that can reduce or inhibit cell oxidative activity.

Antioxidant Activity of *G. japonica* Leaves Extract

Based on the results of the in vitro antioxidant test using the DPPH method on samples of *G. japonica* leaves extracted with 70% and 96% ethanol solvents, they showed antioxidant activity with an IC_{50} values of 1411.36±56.35 μg/mL and 1470.00±41.82 μg/mL respectively (**Table 2**). In this method, ascorbic acid and trolox were used as positive controls. The IC50 values of the two solution concentrations are not much different. This indicates that differences in solvent concentration have no effect on the antioxidant activity of plant samples on DPPH free radicals. The test results for the antioxidant activity of dewa leaf extract are still relatively low. The type of plant organ extracted also influences antioxidant activity. (Krishnan et al., 2015) stated that the antioxidant activity of leaf extracts is much lower than that of root extracts. The IC_{50} value is used to interpret the results of the DPPH method and is a substrate concentration that can cause a

Table 1. The total content of phenolic and flavonoid compounds of *G. japonica* leaves extract

Sample	Phytochemical contents	
	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
G. japonica leaf with 70% ethanol	13.14±1.46	8.04±1.22
G. japonica leaf with 96% ethanol	22.11±2.63	14.70±1.05

50% reduction in DPPH activity as a free radical (Proestos et al., 2013). The smaller the IC_{50} value, the less amount of substrate needed to inhibit the DPPH free radical, thus showing higher antioxidative abilities such as positive control Trolox (2.17±0.06 μg/mL) and ascorbic acid (1.06±0.28 μg/mL). DPPH is a free radical that can accept electrons or hydrogen radicals to become stable molecules. DPPH is known as a stable lipophilic free radical model. The chain reaction of lipophilic radicals is initiated by lipid autooxidation. The presence of natural exogenous antioxidants can react with DPPH to reduce the number of available hydroxyl groups which is indicated by a color change from purple to yellow when read with a spectrophotometer at 517 nm (Proestos et al., 2013). The higher the ability of a plant extract to turn DPPH purple color to yellow, due to the formation of 1,1-diphenyl-2-picrylhydrazine, the higher its antioxidant power (**Figure 1).**

The results of this study show that the 70% and 96% ethanol extracts of *G. japonica* leaves are detected to have antioxidant activity by the DPPH method, which means that there are compound components in the *G. japonica* leaf extract that can capture free radicals through electron or hydrogen donation mechanisms. Even
though the IC_{50} value is still high compared to the control, the results of this study make it possible that the *G. japonica* leaf can be used as a therapeutic agent to capture free radicals that cause cell damage.

The antioxidant activity of *G. japonica* leaves tested using the ABTS method showed an IC_{50} value of 2516.10 + 18.77 μg/mL in samples extracted with 70% ethanol and 3598 + 27.98 μg/mL in samples extracted with 96% ethanol. The use of extract solutions of different concentrations also affects the antioxidant activity of the samples. Ethanol, as a polar solvent, is effective in extracting polar and semi-polar compounds. Compounds such as flavonoids, alkaloids, tannins and glycosides dissolve well in ethanol. This is because ethanol is able to penetrate cell walls and dissolve these compounds efficiently (Yulianti et al., 2021). Plants extracted with ethanol solvent at a lower concentration showed lower antioxidant activity when compared to plant samples extracted with ethanol solvent with a higher concentration. On the other hand, the use of different concentration of ethanol solvent, revealed the same antioxidant activity (Spigno et al., 2007). The IC_{50} values detected by the ABTS method tend to be higher than those detected by the DPPH method (Abo et.al, 2012). The use of DPPH method in detecting antioxidant activity is more often used than ABTS. Proton radical scavenging is very important in antioxidant activity which can reduce the binding of ABTS free radicals by plant extracts indicating that there are compound components in the extract that are able to bind free radicals via the electron/hydrogen donation mechanism and can protect cells from degradation caused by free radicals (Batubara et al., 2020).

Anti-aging of *G. japonica* Leaves Extract with the Streak Method

Based on the anti-aging test using the streak method, the viability of *S. cerevisiae* cells can be seen based on their ability to grow on the surface of the agar medium. Adding H_2O_2 to the medium creates environmental stress for yeast cells and tests their ability to tolerate oxidative stress. For the negative control, yeast cells were supplemented with 1% DMSO without adding extracts.

Figure 2. showed that *S. cerevisiae* cells added with *G. japonica* leaves extract with 70% and 96% ethanol can grow on YPD plate medium with the addition of 3 mM H_2O_2 and 6 mM H_2O_2 after 48 hours of incubation. The difference is that in **Figure 2.** the cells without the addition of extract were then streaked on YPD medium with 6 mM H_2O_2 which did not grow **(Figure 2a)**, whereas at 3 mM H_2O_2 the cells could still grow **(Figure 2b).** This indicates that the addition of *G. japonica* leaf extract which was previously detected to have antioxidant activity was able to maintain the number of *S. cerevisiae* cells

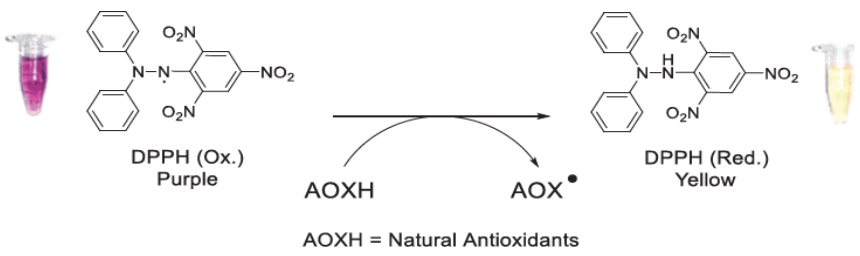

Figure 1. DPPH reaction with natural antioxidant (Arce-Amezquita et al., 2019)

Table 2. Data on antioxidant activity using the DPPH and ABTS methods

Sample	Antioxidant Activities	
	DPPH IC_{50}±SD (μg/mL)	ABTS IC_{50}±SD (μg/mL)
G. japonica leaf with 70% ethanol	1411.36±56.35	2516.10±18.77
G. japonica leaf with 96% ethanol	1470.00±41.82	3598.41±27.98
Ascorbic acid	1.06±0.28	4.30±0.40
Trolox	2.17±0.06	14.30±6.28

under oxidative stress of H_2O_2 up to a concentration of 6mM. Meanwhile, *S. cerevisiae* cells that were not treated with *G. japonica* leaves extract could still grow on YPD medium with the addition of 3 mM H_2O_2 (**Figure 2c**). This shows that giving *G. japonica* leaf extract extracted with 70% or 96% ethanol solvent can extend cell life and prevent cell death due to H_2O_2 oxidative stress response. H_2O_2 is considered as a harmful molecule that impairs the integrity and functionality of cells. In oxidative stress, it is a crucial redox signaling molecule that is hazardous to a variety of organisms (Saputra et al., 2024). Several potential mechanisms for the antioxidant activity of Gynura are suggested as follows: suppression of ROS, suppression of lipid peroxidation, modification of enzymatic antioxidant synthesis or activities, and manipulation of GSH-related parameters are some of the hypothesized mechanisms underlying Gynura's antioxidant action (Tan et al., 2020).

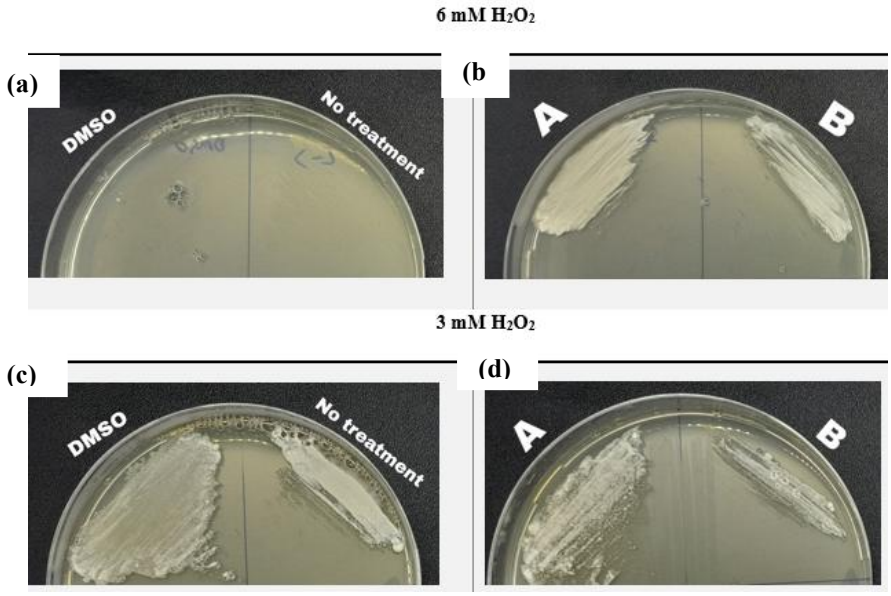

Figure 2. *G. japonica* leaves extract from 70% and 96% ethanol extract showed anti-aging activity in the model organism of *S. cerevisiae* after culture was incubated for 4 days in aging assay; (**a**). *S. cerevisiae* without addition extract streaked on YPD plate medium containing 6 mM H_2O_2, (**b**) *S. cerevisiae* with addition extract streaked on YPD plate medium containing 6 mM H_2O_2, (**c**) *S. cerevisiae* without addition extract streaked on YPD plate medium containing 3 mM H_2O_2, and (**d**) *S. cerevisiae* with addition extract streaked on YPD plate medium containing 3 mM H_2O_2. A. *G. japonica* with 70% ethanol solvent extraction; B. *G. japonica* with 96% ethanol extraction

Table 3. Screening for antibacterial activity in several ethanol extracts of herbal leaves using the disc method

*Extract Concentration (%)	Bacterial Inhibition Zone (mm)								
	S. aureus		*E.coli*		*P. aeruginosa*		*B. subtilis*		
	*Ethanol 70%	Ethanol 96%	Ethanol 70%	Ethanol 96%	Ethanol 70%	Ethanol 96%	Ethanol 70%	Ethanol 96%	Control
100	22.5± 2.5	10.0± 4.8	22.5 ± 4.1	25.0±6.5	10.0 ± 2.5	12.5±3.4	10.0±2.9	20.0±3.7	+
75	10.0 ± 3.1	5.0± 1.8	22.5± 1.9	5.0±2.1	-	10.0±3.7	10.0±1.8	10.0±4.6	+
50	15.0± 3.2	-	3.5 ±1.3	-	-	-	10.0± 3.5	5.0±1.5	+

* Extract concentration in percent; **Concentration of ethanol solution; tetracycline used for control (+)

Table 4. MIC and MBC values of *G. japonica* leaves extracts

	MIC/MBC values (μg/mL)			
Extract	*S. aureus*	*E. coli*	*P. aeruginosa*	*Bacillus subtilis*
70% ethanol	390.62/>781.25	> 25,000/> 25,000	>25,000/> 25,000	>25,000/> 25,000
96% ethanol	390.62/>781.25	> 25,000/> 25,000	>25,000/> 25,000	>25,000/> 25,000

Screening of Antibacterial Activity

The antibacterial activity of the ethanol extract of *G. japonica* leaves was screened using the well diffusion method. Based on the screening results, it was found that the *G. japonica* leaf extract with 70% and 96% ethanol had bacterial inhibitory activity against all test bacteria at extract concentrations of 100%, 75%, and 50% (w/v) (**Table 3**). The inhibition zone formed by the 70% ethanol extract tends to be higher than that of the 96% ethanol extract. The inhibition test was then confirmed by the Minimum Inhibition Concentration (MIC) test using microplate well 96 in MHB medium.

MIC test results showed that *G. japonica* leaves extract with 70% or 96% ethanol solvent could inhibit the growth of *S. aureus* at a minimum concentration of 390.62 μg/mL. Meanwhile, the concentration of other inhibiting bacteria in *E. coli*, *P. aeruginosa*, *B. subtilis* was still high (> 25,000 μg/mL) (**Table 4**). This indicates that *G. japonica* leaves extract has antibacterial activity which is sensitive to *S. aureus*. On the other hand, the Minimum Bacterial Concentration (MBC) value of *G. japonica* leaves extract against *S. aureus* is 781.25 μg/mL. The same thing was also reported by (Ashraf et al., 2020) who states that the ethanol extract of Gynura leaves is more effective at inhibiting *S. aureus* bacteria than other types such as Methicillin-resistant *Staphylococcus aureus*. *G. japonica* leaves extract was also reported to have antibacterial activity against *S. aureus* and *P. aeruginosa* (Jiangseubchatveera et al., 2015). The antibacterial activity of Gynura leaves is caused by the presence of several flavonoid compounds and phenolic compounds contained in its constituents (Wan et al., 2011). The results of this research indicate that the ethanol extract of *G. japonica* leaves can be developed herbal medicines or phytopharmaceuticals to prevent diseases caused by oxidative stress. The results of the antiaging activity test of *G. japonica* leaf extract on *Saccharomyces cerevisiae* cells showed the potential to protection from cells damage due to exposure to free radicals H_2O_2. The antibacterial test results of the ethanol extract of Gynura leaves have the potential to be developed as an alternative natural antibiotic.

CONCLUSIONS

Gynura japonica ethanol extract was detected to contain phenolic and flavonoid compounds which are responsible for antioxidant activity. The results of the antioxidant activity test on DPPH and ABTS free radicals showed that Dewa Leaf extract at both types of solvent concentrations showed antioxidant activity that was not much different in vitro. The results of the anti-aging test using *S. cerevisiae* as a model organism showed that the addition of leaf extract was able to maintain the number of *S. cerevisiae* cells under H_2O_2 oxidative stress up to a concentration of 6 mM. The results of the antimicrobial test showed that the ethanol extract of *G. japonica* leaves showed antibacterial activity against *S. aureus*. The results of this study imply that the ethanol extract of *G. japonica* leaves has the potential to be developed as a therapeutic agent, especially as an antioxidant and antimicrobial agent.

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