

# Antioxidant and Antibacterial Properties of Methanol Extract of *Gletang* Flower (*Tridax procumbens*)

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## Abstract

In a previous study, the methanol extract of *Tridax procumbens* flowers (commonly known as *gletang*) at high concentrations was reported to exhibit strong antibacterial activity against *Streptococcus mutans* and *Enterococcus faecalis*. This study aimed to examine the secondary metabolite content, antioxidant activity, and antibacterial activity of the methanol extract of *gletang* flowers. The antioxidant activity was evaluated using two methods, namely DPPH and ABTS assays. The antibacterial activity was assessed using the Kirby-Bauer method at concentrations of 1000, 5000, and 10000 ppm against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, and *Enterococcus faecalis*. Phytochemical screening revealed that the methanol extract of *gletang* flowers contains phenolic compounds, flavonoids, alkaloids, and triterpenoids. The antioxidant activity tests showed very strong activity in both DPPH and ABTS assays, with IC<sub>50</sub> values of 6.8478 ± 1.335 and 12.8608 ± 0.579 ppm, respectively. The antibacterial activity tests showed that the methanol extract of *gletang* flowers exhibited strong activity against *E. faecalis* at concentrations of 5000 and 10000 ppm, with inhibition zone diameters of 3.00 ± 0.14 and 3.20 ± 0.14 mm, respectively.

**Keywords:** antibacterial; antioxidant; *gletang*; *Tridax procumbens*.

## INTRODUCTION

Infectious diseases and oxidative stress are two major health problems that remain a global concern. Infections caused by pathogenic bacteria can lead to various diseases, while oxidative stress resulting from an imbalance between free radicals and the body's antioxidant defense system contributes to the development of degenerative diseases such as cancer, diabetes, and cardiovascular disease (Ingole et al., 2022). Efforts to overcome these problems generally involve the use of synthetic antibiotics and artificial antioxidants. However, long-term use often causes side effects and may induce bacterial resistance. Therefore, the search for natural sources of antibacterial and antioxidant agents from plants continues to be developed (Alkowni et al., 2023).

One of the potential plants is *Tridax procumbens* (commonly known as *gletang*), a member of the *Asteraceae* family that grows widely in tropical regions, including Indonesia (Wijayanti et al., 2025). Traditionally, this plant has been used by local communities to treat wounds, reduce fever, and as a

traditional remedy for various diseases (Pradana et al., 2024). Several studies have reported that *T. procumbens* contains secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which are known to possess important biological activities, including antibacterial and antioxidant properties (Dattaray, 2022).

The flowers of *Tridax procumbens* (*gletang*), as one of the plant's parts, are believed to contain a significant concentration of active metabolites; however, scientific studies on the antibacterial and antioxidant potential of its flower extract remain limited. Considering the need for safe natural antibacterial and antioxidant agents, research on the biological activities of *T. procumbens* flower extract is therefore important to conduct.

Widiyawati et al. (2022) reported that the flower extract of *T. procumbens* has potential antibacterial activity against *E. faecalis* and *S. mutans* at concentrations of 20%, 40%, 60%, and 80% (Widiyawati et al., 2022). These concentrations are still considered very high, indicating the need for further investigation at lower concentrations. The presence of phenolic and flavonoid compounds reported in the *gletang* flower

extract also suggests potential antioxidant properties that warrant further exploration (Andriana et al., 2019).

Research on the whole *Tridax procumbens* plant has been widely conducted; however, studies specifically focusing on its flowers are still very limited. Therefore, this study aims to explore the antibacterial and antioxidant potential of *T. procumbens* flower extract, in order to support the development of natural herbal medicines that may be beneficial in the health sector.

## MATERIALS AND METHODS

### Material

The sample used in this study was the flower part of *Tridax procumbens* (gletang). The chemicals used included methanol (pro analysis grade), distilled water, DPPH, ABTS, ascorbic acid, Trolox, nutrient agar, nutrient broth, 0.2% chlorhexidine, 0.01% streptomycin, and 70% ethanol. The equipment used included a macerator, glassware, test tubes, micropipettes, a UV-Visible spectrophotometer, a laminar air flow cabinet, cotton swabs, a caliper, and an incubator.

### Sample Extraction

The flower samples of *T. procumbens* were collected from Gerogol District, Cilegon City, Banten, Indonesia. A total of 1 kg of fresh samples were collected. The fresh samples were then air-dried at room temperature for two weeks. About 250 g of the dried gletang flower samples were extracted using the maceration method with 1.5 L of 96% methanol. The extract was then concentrated using a rotary evaporator, yielding 62.8 g of concentrated methanol extract of gletang flowers.

### Phytochemical Screening

A total of 1 g of the concentrated flower extract of *T. procumbens* was weighed and subjected to qualitative phytochemical screening. The phytochemical screening was conducted to detect the presence of alkaloids, phenolics, flavonoids, triterpenoids, and steroids (Bialangi et al., 2024).

### Antioxidant Activity Test Using DPPH

Fifty mg of the *T. procumbens* flower extract sample was dissolved in methanol and transferred into a 50 mL volumetric flask to obtain a stock solution with a concentration of 1000 ppm. From this 1000 ppm stock solution, a series of concentrations (10, 20, 30, 40, and 50 ppm) was prepared. DPPH was weighed (10 mg) and dissolved in 62.5 mL of methanol to obtain a DPPH solution with a concentration of 0.05  $\mu$ M. For the assay, 2.4 mL of each extract concentration was mixed with 0.6 mL of the DPPH solution, then incubated at room temperature in the dark for 30 minutes. The absorbance was measured at a wavelength of 517 nm using a UV-

Visible spectrophotometer (Situmeang, Swasono, et al., 2025). All sample tests were performed in triplicate.

### Antioxidant Activity Test Using ABTS

Various concentrations (10, 20, 30, 40, and 50 ppm) of the methanol extract of *T. procumbens* flowers were prepared. A total of 1.8 mL of each sample was placed into a test tube, followed by the addition of 0.3 mL of ABTS solution. The mixture was incubated for 10 minutes, and the absorbance was then measured at a wavelength of 715 nm using a UV-Visible spectrophotometer (Kabr  et al., 2023). The same procedure was applied to Trolox as a positive control. All sample tests were performed in triplicate.

### Antibacterial Activity Test

The antibacterial activity of the methanol extract of *T. procumbens* flowers was tested against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*. The extract was tested at concentrations of 1000, 5000, and 10,000 ppm. streptomycin was used as the positive control for *E. coli* and *S. aureus*, while chlorhexidine was used as the positive control for *S. mutans* and *E. faecalis*. The antibacterial activity was evaluated by measuring the diameter of the clear inhibition zones formed around the paper discs (Satari et al., 2019).

### Data analysis

The one-way ANOVA test was used in statistical evaluation and data representation using Microsoft excel and origin 9 software. The data were reported as the mean  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

### Phytochemical Screening

The results of the phytochemical screening showed that the methanol extract of *T. procumbens* flowers tested positive for phenolic compounds, flavonoids, alkaloids, and triterpenoids, but negative for steroids. These findings are consistent with the study by Widyawati et al. (2022), which reported that the flower extract of *T. procumbens* contains alkaloids, phenolics, flavonoids, and triterpenoids.

### Antioxidant test Result

The results of testing the methanol extract of *T. procumbens* flowers against DPPH and ABTS radicals showed that the percentage of radical inhibition increased with increasing extract concentrations. At the lowest concentration (10 ppm), antioxidant activity had already reached approximately 50% (DPPH) and 46% (ABTS). At the highest concentration (50 ppm), the percentage of inhibition exceeded 70% for both methods. This pattern is consistent with the fundamental mechanism of antioxidant activity, in which a higher availability of

antioxidant compounds leads to a greater ability to neutralize DPPH and ABTS free radicals. The calculated % inhibition and IC<sub>50</sub> values of the methanol extract of

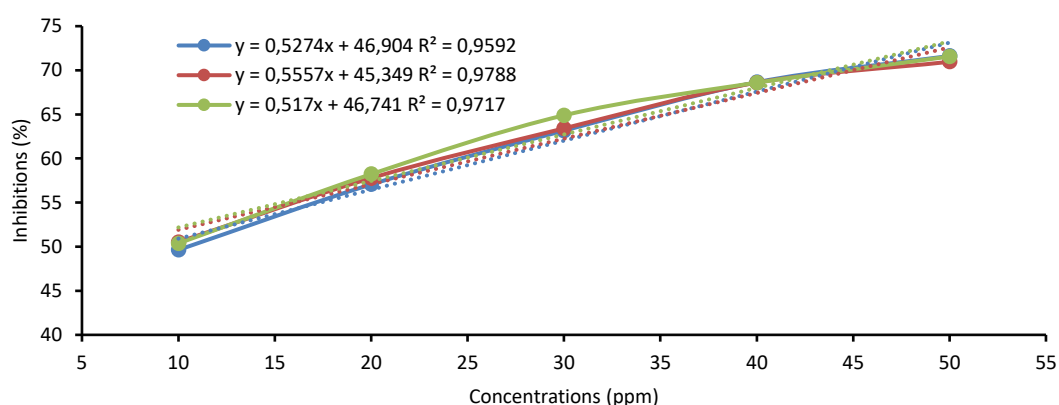
gletang flowers obtained from the DPPH and ABTS methods are presented in Table 1.

**Table 1.** % inhibition and antioxidant activity result using DPPH and ABTS method of methanol extract.

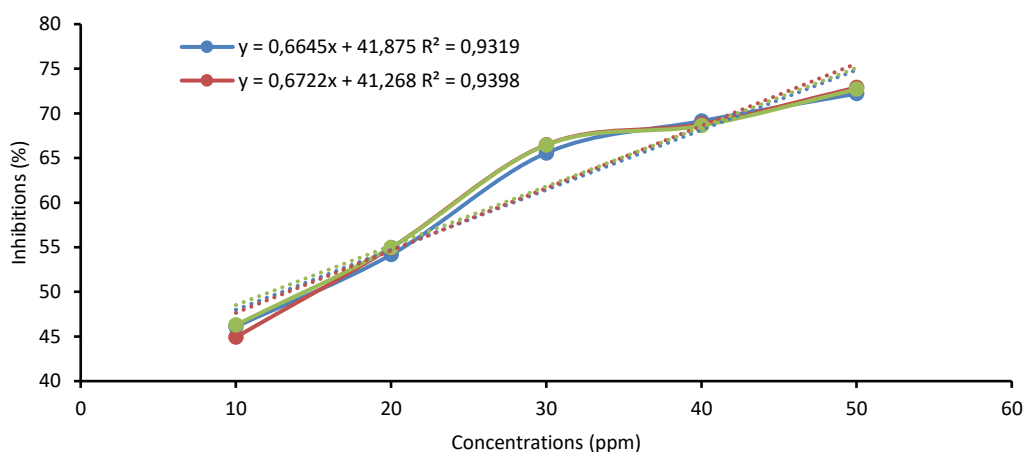
| Methods | Concentrations (ppm) | Inhibition (%) replications |         |         | IC <sub>50</sub> (ppm) ±SD |
|---------|----------------------|-----------------------------|---------|---------|----------------------------|
|         |                      | 1                           | 2       | 3       |                            |
| DPPH    | 0                    | 0                           | 0       | 0       | 6.8478±1.335               |
|         | 10                   | 49.6285                     | 50.5185 | 50.3703 |                            |
|         | 20                   | 57.0579                     | 57.7777 | 58.2222 |                            |
|         | 30                   | 63.1500                     | 63.4074 | 64.8888 |                            |
|         | 40                   | 68.6478                     | 68.5925 | 68.5925 |                            |
|         | 50                   | 71.6196                     | 70.9629 | 71.5555 |                            |
| ABTS    | 0                    | 0                           | 0       | 0       | 12.8608±0.579              |
|         | 10                   | 46.0947                     | 44.9293 | 46.3010 |                            |
|         | 20                   | 54.1613                     | 54.9422 | 54.9744 |                            |
|         | 30                   | 65.5569                     | 66.4955 | 66.4540 |                            |
|         | 40                   | 69.1421                     | 68.8061 | 68.6224 |                            |
|         | 50                   | 72.2151                     | 72.9139 | 72.7040 |                            |

The IC<sub>50</sub> values were obtained from the linear regression equations of each concentration plotted against the percentage of inhibition. The linear regression curves are shown in Figure 1 for DPPH and Figure 2 for ABTS. The correlation coefficients (R values) obtained

from the linear regression equations in both the DPPH and ABTS methods were greater than 0.9. This indicates that the relationship between concentration and percentage of inhibition is linear (Situmeang et al., 2025).

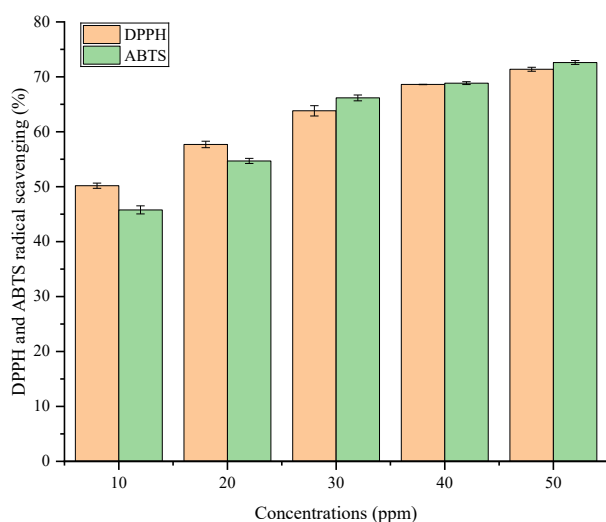


**Figure 1.** Regression linear curve of DPPH method.



**Figure 2.** Regression linear curve of ABTS method.

The higher the percentage of inhibition (% inhibition), the lower the  $IC_{50}$  value, indicating a stronger ability of the sample to scavenge free radicals in both the DPPH and ABTS assays. As shown in Figure 3, increasing the extract concentration resulted in a higher percentage of free radical inhibition. This pattern demonstrates that the antioxidant activity of the extract is concentration-dependent (dose-dependent), meaning that higher extract concentrations provide more antioxidant molecules capable of donating electrons or hydrogen atoms to neutralize DPPH radical and ABTS radical cation (Jiangseubchatveera et al., 2023). The positive relationship between concentration and radical scavenging activity confirms that the flower extract of *T. procumbens* contains bioactive compounds, such as phenolics and flavonoids, which are effective as antioxidants.



**Figure 3.** Correlation of DPPH and ABTS radical scavenging with various concentrations of methanol extract.

The  $IC_{50}$  value represents the concentration of extract required to inhibit 50% of free radicals. A lower  $IC_{50}$  value indicates stronger antioxidant activity. In the DPPH method, an  $IC_{50}$  of 6.85 ppm demonstrates that the methanol extract of *Tridax procumbens* flowers possesses very strong antioxidant activity. In the ABTS method, an  $IC_{50}$  of 12.86 ppm also indicates very strong activity, although slightly lower compared to DPPH. This difference may occur because each method has a distinct radical scavenging mechanism. The DPPH method involves electron transfer from antioxidants to the stable DPPH $\cdot$  radical, while the ABTS method involves both electron and hydrogen atom transfer to the ABTS $^+$  radical cation (Liu et al., 2023). The phenolic and flavonoid compounds present in *T. procumbens* flowers are likely to have a high electron-donating ability, making them more effective in the DPPH method.

These results indicate that the methanol extract of *T. procumbens* flowers contains bioactive compounds, such as flavonoids and phenolics, which act as potent antioxidants. This strong antioxidant activity has the

potential to be utilized in the development of natural ingredients to prevent oxidative damage in food products, cosmetics, or herbal medicines.

### Antibacterial test result

Antibacterial activity was evaluated using the Kirby-Bauer method. The antibacterial activity was determined based on the diameter of the inhibition zones formed, which reflects the ability of the methanol extract of *T. procumbens* flowers to inhibit bacterial growth. The results showed that the methanol extract exhibited varying antibacterial activity against the four tested bacterial strains *E. coli*, *S. aureus*, *S. mutans*, and *E. faecalis* with responses depending on the extract concentration used. The results of the antibacterial activity test are presented in Table 2.

**Table 2.** Antibacterial activity of *Tridax procumbens* methanol flower extract, negative control, and positive control.

| Bacteria           | Sampel/concentration  | Inhibition zone (mm) average $\pm$ SD | Category |
|--------------------|-----------------------|---------------------------------------|----------|
| <i>E. coli</i>     | Extract 1000 ppm      | 0.00                                  | None     |
|                    | Extract 5000 ppm      | 0.25 $\pm$ 0.07                       | Weak     |
|                    | Extract 10000 ppm     | 0.55 $\pm$ 0.07                       | Weak     |
|                    | Methanol 96%          | 0.00                                  | None     |
|                    | Streptomycin 200 ppm  | 8.45 $\pm$ 0.07                       | Strong   |
| <i>S. aureus</i>   | Extract 1000 ppm      | 0.00                                  | None     |
|                    | Extract 5000 ppm      | 0.00                                  | None     |
|                    | Extract 10000 ppm     | 0.00                                  | None     |
|                    | Methanol 96%          | 0.00                                  | None     |
|                    | Streptomycin 200 ppm  | 12.2 $\pm$ 0.14                       | Strong   |
| <i>S. mutans</i>   | Extract 1000 ppm      | 0.35 $\pm$ 0.07                       | Weak     |
|                    | Extract 5000 ppm      | 0.35 $\pm$ 0.07                       | Weak     |
|                    | Extract 10000 ppm     | 0.55 $\pm$ 0.07                       | Medium   |
|                    | Methanol 96%          | 0.00                                  | None     |
|                    | klorheksidin 2000 ppm | 4.15 $\pm$ 0.07                       | Strong   |
| <i>E. faecalis</i> | Extract 1000 ppm      | 2.35 $\pm$ 0.07                       | Medium   |
|                    | Extract 5000 ppm      | 3.00 $\pm$ 0.14                       | Strong   |
|                    | Extract 10000 ppm     | 3.20 $\pm$ 0.14                       | Strong   |
|                    | Methanol 96%          | 0.00                                  | None     |
|                    | klorheksidin 2000 ppm | 4.15 $\pm$ 0.07                       | Strong   |

For *E. coli*, the extract exhibited weak activity, producing inhibition zones of  $0.25 \pm 0.07$  mm at 5000 ppm and  $0.55 \pm 0.07$  mm at 10,000 ppm, while showing no activity at 1000 ppm. These results indicate that *E. coli* is relatively more resistant to the active compounds in the extract, likely due to the more complex structure of its Gram-negative cell wall, which contains a lipopolysaccharide layer that impedes the penetration of antibacterial compounds (Aldayel, 2023). In comparison, the positive control Streptomycin at 200 ppm produced an inhibition zone of  $8.45 \pm 0.07$  mm (strong category), demonstrating that the activity of the extract against *E. coli* is still much lower than that of the standard antibiotic.

For *S. aureus*, the extract showed no antibacterial activity at all tested concentrations. The absence of inhibition zones indicates that the antibacterial

compounds present in the *Tridax procumbens* flower extract are either ineffective or present at insufficient concentrations to inhibit the growth of *S. aureus*. In contrast, the positive control Streptomycin produced an inhibition zone of  $12.2 \pm 0.14$  mm (strong category), confirming the ineffectiveness of the extract against this bacterium (Idowu et al., 2023).

For *S. mutans*, the extract exhibited weak to moderate antibacterial activity. The inhibition zones were  $0.35 \pm 0.07$  mm at concentrations of 1000 and 5000 ppm, and increased to  $0.55 \pm 0.07$  mm (moderate category) at 10 000 ppm. These results indicate a moderate antibacterial potential against *S. mutans* that increases with higher concentrations, although it is still much lower than the positive control Chlorhexidine (2000 ppm), which produced an inhibition zone of  $4.15 \pm 0.07$  mm (strong category). This suggests that the extract begins to show activity at high concentrations, but it is not yet as effective as the standard antibacterial agent.

For *E. faecalis*, the extract exhibited moderate to strong antibacterial activity. The inhibition zone increased with higher concentrations, measuring  $2.35 \pm 0.07$  mm (moderate category) at 1000 ppm,  $3.00 \pm 0.14$  mm (strong category) at 5000 ppm, and  $3.20 \pm 0.14$  mm (strong category) at 10 000 ppm. Although slightly lower than the positive control Chlorhexidine ( $4.15 \pm 0.07$  mm), these results indicate that the extract has considerable antibacterial potential against *E. faecalis*. This activity is likely attributed to bioactive compounds present in the extract, such as flavonoids, phenolics, or terpenoids, which may disrupt the bacterial cell membrane or interfere with the metabolism of *E. faecalis*.

Overall, these results indicate that the methanolic flower extract of *T. procumbens* exhibits specific antibacterial activity that is more effective against Gram-positive bacteria than Gram negative bacteria, with the strongest effect observed against *E. faecalis*. The pattern of increasing inhibition zones with higher concentrations also confirms that the antibacterial activity of the extract is dose-dependent.

## CONCLUSIONS

Phytochemical screening revealed that the methanolic flower extract of *T. procumbens* contains phenolic, flavonoid, alkaloid, and triterpenoid compounds. The antioxidant activity assay showed very strong activity in both DPPH and ABTS methods, with  $IC_{50}$  values of  $6.8478 \pm 1.335$  and  $12.8608 \pm 0.579$  ppm, respectively. The antibacterial activity assay demonstrated that the methanolic flower extract exhibited strong activity against *Enterococcus faecalis* at concentrations of 5000 and 10 000 ppm, producing inhibition zones of  $3.00 \pm 0.14$  mm and  $3.20 \pm 0.14$  mm, respectively.

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**Authors' Contributions:** Boima Situmeang and Weny JA Musa designed the study and wrote the manuscript. Sriwijayanti and Dian Susvira carried out the laboratory work. Nurhayati Bialangi and Holisha Widiyanto analyzed the data. All authors read and approved the final version of the manuscript.

**Competing Interests:** The authors declare that there are no competing interests.

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