

Antibacterial activities

By Utmi Arma

ANTIBACTERIAL ACTIVITY TEST OF DIFFERENT PARTS OF GLETANG (*Tridax procumbens*) FROM WEST SUMATERA, INDONESIA

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ABSTRACT

The study evaluates the antibacterial activities of different parts of gletang (*Tridax procumbens*) against *Streptococcus mutans* and *Enterococcus faecalis* oral pathogens bacteria. The extracts of different parts were subjected to qualitative phytochemical screening and antibacterial activity. The antibacterial activity of extracts of the plants was investigated using the agar Kirby-Bauer method and chlorhexidine as a positive control. The Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) using the ELISA reader micro-dilution method in 96-well microplates. The part of *Tridax procumbens* was extracted with ethanol 96%. Phytochemical screening showed that the leaf extract contained flavonoids, terpenoids, tannin, and saponin, while stem extract contained flavonoids, terpenoids, tannin, and the flower alkaloids, flavonoids, tannins, and terpenoids. The results showed that % of the leaf, stem, and flower of *Tridax procumbens* with concentrations of 20, 40, and 60% had antibacterial activity against *S. mutans* and *E. faecalis* in the resistant to moderate category. The MIC values of stem, leaf, and flower *T. procumbens* against *S. mutans* were 2.5, 10, and 5%, respectively, and MBC values were 10, 20, and 20%, respectively. The MIC values of stem, leaf, and flower gletang against *E. faecalis* were 5, 10, and 10%, respectively, and MBC values were 10, 40, and 40%, respectively. The highest antibacterial activity was shown in the stem extract of gletang.

Keywords: Antibacterial; Gletang; *Enterococcus faecalis*; *Streptococcus mutans*; *Tridax procumbens*.

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INTRODUCTION

Dental caries and periodontal are diseases that destroy the tooth structure. Dental caries is a bacterial deposit, and its product attaches to the tooth surface caused by *Streptococcus mutans* and *Enterococcus faecalis* oral pathogens bacteria.¹⁻³ According to World Health Organization (WHO) reports, in developing countries, nearly 80 percent of the community uses traditional medicines for sustaining health and vitality. According to one estimation, in the entire world, 20.000 to 35.000 species of plants are used as medicines.⁴ The previous research showed that gletang plants have potential as antibacterial-active phytochemical compounds. *Tridax procumbens* is part of the *Asteraceae* family and, commonly called gletang, or gletang daisy is an annual weed. The gletang plants are completed with leaves, stem, and flower.⁵⁻⁶ Gletang is a weak herb with a length of about 10-30 cm, leaves about 4-6 cm and has two types of flowers. The stem of gletang is ascending and branched. The gletang plant is well known for cough, diarrhea, asthma, and epilepsy diseases.⁷⁻⁸ In Indonesia, gletang has been used as an antifungal, anticoagulant, and insecticide in traditional medicine.⁹⁻¹¹ According to Krishnaswamy and Christina (2015) gletang extracts showed higher inhibitory activity of various aerobic and anaerobic bacteria.¹² According to Kumar et al. (2016), the methanolic extract of the flower was found to inhibit *P.aragenosa*, *E.coli*, and *B.cereus* bacteria. The methanolic extract of the leaf of gletang gave a maximum inhibition zone against *P.fluorescence*.¹³ According to Das et al. (2017), the Anti-bacterial activity of methanolic extract of gletang leaf had more bactericidal activity against *P. aeruginosa* than *S. aureus*.¹⁴ Nguyen et al. in 2015 have successfully isolated two compounds from ethyl acetate extract of the whole plant of gletang, namely glucopyranoside and

glucopyranoside compounds³ *Streptococcus mutans* and *Enterococcus faecalis* play an essential role in forming oral dental diseases. *Streptococcus mutans* is the predominant microorganism discovered in dental plaque associated with¹⁵ caries lesion.^{16,17} Conventional antibiotic has been increasing in resistance to pathogens and bacteria. This study aimed to evaluate the antibacterial activities of different parts of gletang against oral pathogens bacteria. The part of *Tridax procumbens* was extracted with ethanol 96%. The antibacterial activity was investigated by¹ the Kirby-Bauer method and chlorhexidine as a positive control. The MIC and MBC use an ELISA reader micro-dilution method in 96-well microplates.

EXPERIMENTAL

Sample Preparation

A sample of gletang (*Tridax procumbens*) was collected from Padang, West Sumatra, Indonesia.¹ The specimen was determined and deposited (No. MP – 254/K-ID/ANDA/VII/2018) at the Laboratory of Plants Taxonomy, Andalas University, West Sumatra, Padang, Indonesia. The following chemicals used were ethanol 96%, aqua dest, alcohol 70 %, bunsen burner, chlorhexidine 0,1%, and Mueller Hilton medium. Instrumentation used laminar air flow, incubator, autoclave, anaerobic jar, and ELISA reader. The fresh sample of gletang flowers (500 g), leaf (500 g), and stem (500 g) were cut into small pieces. All samples were soxhlet using ethanol 96% as a solvent for 2x24 hours four times. All extracts were evaporated to give a residue of flower extract of ethanol (22 g), leaf extract of ethanol (21.5 g), and stem extract of ethanol (11,2 g).

Antibacterial¹³ Activity of Test

The bacteria *Streptococcus mutans* ATCC 25175 and *Enterococcus faecalis* ATCC 29212 were used for¹⁴ antibacterial activity test. *S. mutans* and *E. faecalis* were placed on Muller Hinton medium. Chlorhexidine was used as a positive control. The anaerobic jar was used for anaerobic² conditions. The ethanol 96% was used as a negative control.¹⁸ The MIC and MBC activities of the extract against *S. mutans* ATCC 25175 and *E. faecalis* ATCC 29212 following Satari et al. (2019). Ethanol 96% was used for dissolving extract. Ethanol is chosen because it does not affect bacteria. The test was performed in duplicates.²

RESULTS AND DISCUSSION

Determination was conducted at the Taxonomy Plants, Andalas University, and conducted that the sample used was *Tridax procumbens*. *T. procumbens* extraction with the soxhletation method yields a yield of flower extract of ethanol at 4.4%, the leaf extract of ethanol at 4.3%, and stem extract of ethanol at 2.24%. Variations in the yield of extract in *Tridax procumbens* can be caused by several factors, including the size of the simplicia and the content of secondary metabolites. According to Akintunde et al. (2017), the yield percentage produced from *Tridax procumbens* extraction using ethanol solvents ranges from 2-5%.⁶ The secondary metabolites phytochemical screening of gletang with different plant parts is shown in Table-1. The antibacterial activity of ethanol 96% extracts of different parts of the gletang plant against oral dental pathogenic bacteria is presented in Tables-2 and 3.

Table-1: The Phytochemical Screening of various Parts of an Extract *T. procumbens*

No	Secondary metabolites	Reagent	Leaves	Stem	Flower
1	Alkaloids	Dragendorf	-	-	+
2	Flavonoids	FeCl ₃ 5%	+	+	+
3	Tannins	FeCl ₃ 1%	+	+	+
4	Terpenoids	Acetic anhydride and H ₂ SO ₄ (p)	+	+	+
5	Saponins	Aquadest	+	-	-

The highest antibacterial activity against *Streptococcus mutans* ATCC 25175 was shown in stem extract,² while inhibition zone 25.40±0.20 mm at a concentration of 60% indicated a very strong interpretation. Compared to chlorhexidine as a positive control at 0.1% (16.67±0.25 mm), the stem extract was more active than the controls. All extracts of parts of *Tridax procumbens* have an inhibition zone. According to (Krishnaswamy and Christina, 2015), the alcoholic extracts of the leaves showed a better antibacterial activity compared to other parts of the plant.¹² The result of the inhibition zone against *Streptococcus*

mutans ATCC 25175 was not revealed compared to the previous study against *Escherichia coli*. The stem extract shows the highest antibacterial activity against *Enterococcus Faecalis* ATCC 29212, while the inhibition zone is 13.40 ± 0.40 mm. Compared to chlorhexidine as a positive control at 0.1% (16.33 ± 0.23 mm), the stem extract was less active than the controls. The result is revealed in the present study.¹⁹ According to the CLSI protocol's data, the categories of susceptibility on bacteria shown in the inhibition zone method are as follow susceptible (≤ 20 mm), intermediate (15-19 mm), and resistant (≤ 14 mm). It is found that among the inhibited bacteria, most are susceptible only to the ethanolic extracts, indicating that many of the active compounds are soluble only in polar organic solvents like ethanol.²⁰⁻²²

Table-2: Inhibition Zone of *Tridax procumbens* against *Streptococcus mutans* ATCC 25175

Parts	Concentration	Inhibition Zone (mm) Replication			Rate \pm SD (mm)	Interpretation
		1	2	3		
Leaf	20%	12.0	12.1	11.8	11.97 \pm 0.15	resistant
	40%	14.4	14.2	14.6	14.40 \pm 0.20	resistant
	60%	15.2	16.2	15.8	15.73 \pm 0.50	intermediate
Chx	0.1%	11.7	11.6	11.7	11.67 \pm 0.06	resistant
Ethanol	96%	0	0	0	0	resistant
Stem	20%	19.6	18.3	18.7	18.87 \pm 0.67	intermediate
	40%	22.9	22.8	23.2	22.97 \pm 0.21	susceptible
	60%	25.6	25.4	25.2	25.40 \pm 0.20	susceptible
Chx	0.1%	16.9	16.4	16.7	16.67 \pm 0.25	intermediate
Ethanol	96%	0	0	0	0	resistant
Flower	20%	12.9	12.4	13.2	12.83 \pm 0.40	resistant
	40%	18.9	18.2	18.1	18.40 \pm 0.44	intermediate
	60%	19.4	19.8	20.2	19.80 \pm 0.40	intermediate
Chx	0.1%	11.1	11.1	11.4	11.20 \pm 0.17	resistant
Ethanol	96%	0	0	0	0	resistant

Table-3: Inhibition Zone of *Tridax procumbens* against *Enterococcus Faecalis* ATCC 29212

Parts	Concentration	Inhibition zone (mm) Replication			Rate \pm SD (mm)	Interpretation
		1	2	3		
leaf	20%	9.6	9.9	9.3	9.60 \pm 0.30	resistant
	40%	10.3	10.4	9.9	10.20 \pm 0.26	resistant
	60%	12.4	12.0	12.7	12.37 \pm 0.35	resistant
Chx	0.1%	17.6	17.9	17.6	17.70 \pm 0.17	intermediate
Ethanol	96%	0	0	0	0	resistant
Stem	20%	9.1	9.4	9.4	9.30 \pm 0.17	moderate
	40%	12.8	12.4	12.9	12.70 \pm 0.26	resistant
	60%	13.0	13.8	13.4	13.40 \pm 0.40	resistant
Chx	0.1%	16.2	16.6	16.2	16.33 \pm 0.23	intermediate
Ethanol	96%	0	0	0	0	resistant
Flower	20%	7.5	7.7	7.7	7.63 \pm 0.12	Resistant
	40%	8.9	8.7	8.9	8.83 \pm 0.12	Resistant
	60%	9.1	9.1	9.3	9.17 \pm 0.12	resistant
Chx	0.1%	14.5	14.7	14.7	14.63 \pm 0.12	Resistant
Ethanol	96%	0	0	0	0	resistant

The MIC values of leaf, stem, and flower *T. procumbens* against *S. mutans* (Table-4) were 5, 10, and 2.5%, respectively, and MBC values were 10, 20, and 20%, respectively. The MIC values of leaf, stem, and flower *T. procumbens* against *E. faecalis* were 10, 10, and 5%, respectively, and MBC values were 40, 40, and 10%, respectively (Fig.-2). The result showed that flower extract is more active than stem and leaf extract. From the present study, the inhibition zone values of ethanolic extracts of stem of gletang against *S. mutans* and *E. faecalis* are more effective. The minimum inhibitory concentration results do not reveal that the ethanolic extract of the stem was more effective

than the ethanolic extract of the flower against *S. mutans* and *E. faecalis*. The MIC value revealed that almost all tested bacterial strains were sensitive to our research's 96% ethanolic extracts.

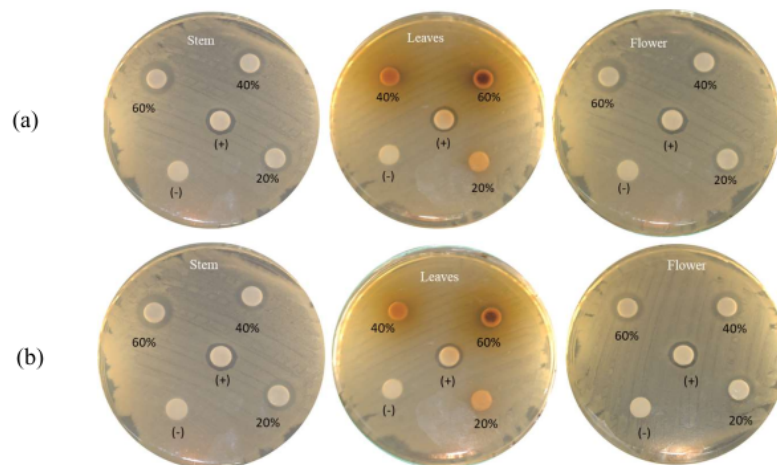


Fig-1: Activity Test Results of the Stem, Leaf, and Flower Extract of *T. procumbens* against bacteria (a) *S. mutans*, (b) *E. faecalis*

Table-4: The MIC and MBC Values of the Parts of the Gletang Plant

Bacteria	Parts	MIC (%)	MBC (%)
<i>S. mutans</i>	Leaf	10	20
	Stem	2.5	10
	flower	5	20
<i>E. faecalis</i>	Leaf	10	40
	Stem	5	10
	flower	10	40

MIC and MBC value of *S. mutans* and *E. faecalis*

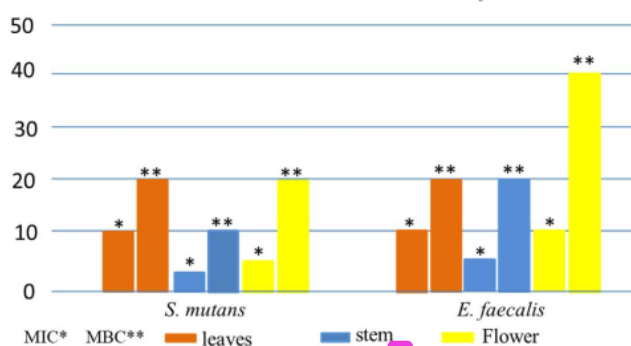


Fig.-2: MIC and MBC Value of *T. procumbens* against *S. mutans* and *E. faecalis*

CONCLUSION

The results showed that ethanol extract of 96% leaf, stem, and flower of gletang had antibacterial activity against *S. mutans* ATCC 25175 and *E. faecalis* ATCC 29212 in the resistant to moderate category. The MIC values of stem, leaf, and flower *T. procumbens* against *S. mutans* were 2.5, 10, and 5%, respectively, and MBC values were 10, 20, and 20%, respectively. The MIC values of stem, leaf, and flower *T. procumbens* against *E. faecalis* were 5, 10, and 10%, respectively, and MBC values were 10, 40, and 40%, respectively.

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