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RESEARCH ARTICLE

Bioactivity of *Mikania micrantha* extract in controlling *Erwinia chrysanthemi*: a natural antibacterial approach

Nur Annisa Fadjrini[1*], Eyska Amanda[1], Sandra Juniarsi[1], Israwati Harahap[1], and Elsie[1]

[1]Department of Biology, Faculty of Mathematics, Natural Sciences and Health, Muhammadiyah University of Riau, 28294 Riau, Indonesia.

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☒* **CORRESPONDING AUTHOR**
Nur Annisa Fadjrini
Department of Biology,
Faculty of Mathematics,
Natural Sciences and Health,
Muhammadiyah University of Riau,
28294 Riau, Indonesia.
Email: 170202005@student.umri.ac.id

ABSTRACT

Crop losses caused by soft rot disease induced by *Erwinia chrysanthemi* pose a persistent threat to global agricultural productivity. Excessive use of synthetic chemical pesticides to control such bacterial diseases has raised serious environmental and health concerns due to their residual toxicity and ecological impact. As an alternative, plant-derived biopesticides have gained attention for their biodegradability, safety, and sustainability. This study aimed to evaluate the antibacterial activity of *Mikania micrantha* Kunth (sembung rambat) leaf extract against *E. chrysanthemi* and to determine the most effective concentration for bacterial inhibition. The antibacterial assay was performed using the Kirby–Bauer disc diffusion method with five treatments and four replications: three concentrations of *M. micrantha* leaf extract (25%, 50%, and 80%), a positive control (1% chloramphenicol), and a negative control (sterile distilled water). Data were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at a 95% confidence level.The results showed that *M. micrantha* extract exhibited moderate antibacterial activity, producing inhibition zones of 7.51 mm, 8.02 mm, and 8.40 mm for 25%, 50%, and 80% concentrations, respectively. Although inhibition increased with concentration, the differences among extract treatments were not statistically significant ($p > 0.05$). The positive control exhibited a much larger inhibition zone (30.95 mm), while the negative control showed none.These findings indicate that *M. micrantha* leaf extract possesses moderate antibacterial potential against *E. chrysanthemi* and may serve as a promising plant-based biocontrol agent for sustainable disease management.

1. INTRODUCTION

Losses in agricultural production caused by plant pathogens remain a persistent and serious obstacle for farmers across the globe. These losses not only diminish crop yield and quality but, in severe cases, may wipe out an entire harvest within a single growing season. Such large-scale failures can have cascading effects, disrupting food supply systems at multiple levels from local marketplaces to global commodity chains and ultimately threatening food security. Similar to pest infestations, plant diseases caused by pathogens can occur at various stages of the agricultural cycle, including during germination, crop growth in the field, and postharvest storage (Sutarman, 2017; Jawetz et al., 2001; Talaro, 2008).

Among these pathogens, *Erwinia chrysanthemi* is recognized as one of the most important bacterial agents causing soft rot disease in a wide range of economically valuable crops. This bacterium is classified as a pectinolytic plant pathogen due to its ability to secrete extracellular enzymes capable of degrading pectin and other structural components of plant cell walls, leading to tissue maceration and collapse (Collmer & Bauer, 1994). Under favorable environmental conditions particularly high temperature and humidity infections caused by *E. chrysanthemi* can progress rapidly, resulting in severe yield and quality losses. In horticultural crops, soft rot disease has been reported to cause yield reductions of up to 60% during severe outbreaks, while in major potato-producing regions, economic losses associated with soft rot and blackleg diseases reach hundreds of millions of dollars annually (Alvarez, 2014).

The host range of *E. chrysanthemi* is extensive, with documented infections occurring in roots, tubers, stems, cuttings, and leaves of numerous plant species (Jensen, 1986). In addition to preharvest damage, this pathogen has also been implicated in postharvest losses, including fruit collapse in pineapple (*Ananas comosus*) during storage and distribution (Prasetyo & Aeni, 2016). Due to its destructive potential and broad host range, *E. chrysanthemi* is classified as a quarantine organism in several regions, and disease

outbreaks may lead to trade restrictions and export bans that further exacerbate economic losses for producers (European & Mediterranean Plant Protection Organization, 1982).

Conventional management of bacterial plant diseases relies heavily on synthetic chemical pesticides. Although these compounds can effectively suppress pathogen populations, their prolonged and indiscriminate use raises serious environmental and health concerns. Chemical residues may accumulate in soil and water systems, disrupt beneficial microbial communities, and negatively affect non-target organisms, while repeated applications may also promote the development of pathogen resistance (Suharjo & Aeny, 2016; Pratiwi, 2008). Such impacts reduce the long-term sustainability of chemical-based disease control strategies.

These limitations have stimulated growing interest in alternative, environmentally friendly approaches, particularly the use of botanical pesticides derived from plants with natural antimicrobial properties. Botanical pesticides are generally biodegradable, pose lower risks to human health, and leave minimal toxic residues in the environment. Moreover, they can often be prepared from locally available plant materials, making them economically feasible for smallholder farmers (Aeni, 2016). Many plant species synthesize secondary metabolites including alkaloids, flavonoids, tannins, polyphenols, saponins, and steroids—that function as natural defense compounds against microbial pathogens (Kusmayati & Agustini, 2007; Pratiwi, 2008).

Mikania micrantha Kunth, locally known as sembung rambat, is a fast-growing perennial vine widely distributed across tropical and subtropical regions. Although commonly regarded as an invasive weed due to its aggressive growth and ability to smother cultivated plants, this species possesses a rich phytochemical profile. Phytochemical analyses have revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, saponins, polyphenols, tannins, and steroids in *M. micrantha* leaves, which are associated with antioxidant, anti-inflammatory, and antimicrobial activities (Fernandes et al., 2018; Perawati et al., 2018). Ecological and biological studies also highlight the widespread distribution and biological impacts of *M. micrantha* in agricultural ecosystems (Day et al., 2016; Integrated Taxonomic Information System, 2021).

Despite its abundance and reported bioactive potential, studies investigating the antibacterial activity of *M. micrantha* against economically important plant pathogens remain limited. Previous research has primarily focused on its ecological impacts and natural enemies, with relatively few studies addressing its potential use in plant disease control, particularly against bacterial pathogens such as *E. chrysanthemi* (Barreto & Evans, 1995; Abraham et al., 2002; Matawali et al., 2016).

Therefore, the present study aims to evaluate the antibacterial activity of *M. micrantha* leaf extract against *E. chrysanthemi*. Specifically, this research seeks to determine the effect of different extract concentrations on bacterial growth inhibition and to identify the concentration that produces the strongest inhibitory response. The results of this study are expected to provide preliminary scientific evidence supporting the potential application of *M. micrantha* as a botanical pesticide, contributing to sustainable plant disease management and reducing dependence on synthetic chemical control strategies.

2. MATERIALS AND METHODS

2.1. Study area

This study was conducted from June to August 2021. The *Erwinia chrysanthemi* isolate was obtained from the Research Center for Biology, Indonesian Institute of Sciences (LIPI). Extraction of *Mikania micrantha* leaves was carried out at the Chemistry Laboratory, while the antibacterial activity assays were conducted at the Microbiology Laboratory, Faculty of Mathematics, Natural Sciences, and Health, Universitas Muhammadiyah Riau.

2.2. Equipment and materials

The main equipment used included a blender, distillation apparatus, rotary vacuum evaporator, laminar air flow cabinet, oven, incubator, autoclave, electric stove, and Bunsen burner. Glassware consisted of Boston round bottles, 100 ml beakers, 250 ml Erlenmeyer flasks, measuring cylinders, Petri dishes, test tubes, and glass stir rods. Additional tools included filter paper, wrapping paper, plastic wrap, micropipettes with tips, inoculating loops, analytical balance, cotton swabs, forceps, vernier calipers, and sterile paper discs.

The materials used were fresh *Mikania micrantha* leaves, *E. chrysanthemi* bacterial cultures, nutrient agar (NA) medium (Merck), sodium chloride (NaCl), sterile distilled water, chloramphenicol (Merck), and ethanol as the extraction solvent.

2.3. Experimental design

This laboratory-based experimental study employed a completely randomized design (CRD) with five treatments and four replications. The *M. micrantha* leaf extract was obtained through the maceration method, and antibacterial activity was assessed using the Kirby–Bauer disc diffusion technique.

2.4. Experimental procedures

2.4.1. Preparation of *Mikania micrantha* leaf samples

Both young and mature leaves of *M. micrantha* were collected from Jalan Bina Krida, Simpang Baru, Pekanbaru. The plant identification was carried out independently with reference to a standard plant taxonomy guidebook and the *Tropical Plants Database* (Ken Fern, tropical.theferns.info).

2.4.2. Extraction of *M. micrantha* leaves

A total of 2 kg of fresh *Mikania micrantha* leaves was washed thoroughly with running water to remove impurities and air-dried at room temperature (25–30°C) for seven days in a shaded, well-ventilated area. The dried leaves were ground into fine powder using a mechanical grinder and sieved through a 60-mesh screen to obtain uniform particle size. Approximately 500 g of powdered leaves was macerated in 2 L of ethanol at 25–30°C for 72 hours, with manual stirring every 24 hours using a sterile glass rod to facilitate solvent penetration. After maceration, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate (macerate) was collected. The residue was re-macerated twice under the same conditions to ensure maximum extraction of bioactive compounds. All filtrates were pooled and concentrated under reduced pressure using a rotary evaporator at 45°C until the solvent was completely evaporated. The resulting thick extract (17 g) was transferred into an amber sterile container and stored at 4°C until use. The extraction yield was calculated as the ratio of dried extract weight to the initial fresh leaf weight, following the modified method of Elin et al. (2006).

2.4.3 Preparation of test solutions

Three concentrations of *M. micrantha* extract were prepared: 25%, 50%, and 80% (w/v). Each was made by weighing 0.25 g, 0.50 g, and 0.80 g of extract, respectively, and dissolving in 1 ml of ethanol (Alfiah et al., 2015). The positive control (1% chloramphenicol) was prepared by dissolving 0.01 g of chloramphenicol in 1 ml of sterile distilled water. The negative control consisted of 1 ml sterile distilled water without extract. All treatments were applied according to the experimental design.

2.4.4 Bacterial culture revitalisation

Pure cultures of *E. chrysanthemi* were streaked aseptically on nutrient agar plates using an inoculating loop and incubated at 37°C for 24 hours.

2.4.5 Preparation of bacterial suspensions

Bacterial suspensions were prepared by transferring colonies into 0.9% NaCl solution under aseptic conditions. Turbidity was visually adjusted to match the 0.5 McFarland standard, equivalent to 1×10^7–1×10^8 CFU/ml (Clinical & Laboratory Standards Institute, 2009).

2.4.6 Antibacterial assay of *M. micrantha* extract against *E. chrysanthemi*

Sterile paper discs were immersed in microplate wells containing the respective extract concentrations. The discs were then placed on the surface of nutrient agar plates previously inoculated with the bacterial suspension. Plates were incubated at 37°C for 24 hours, after which the inhibition zones were measured using vernier calipers.

2.5. Data collection and analysis

The diameter of the inhibition zones was measured 24 hours after incubation. Data from each treatment were analysed using analysis of variance (ANOVA). If a significant difference was detected, Duncan's Multiple Range Test (DMRT) was performed at a 5% significance level (α = 0.05). Results were presented in tabular form and described based on inhibition zone measurements. The inhibition zone diameter was calculated using the following formula:

$$\frac{(DV - DC) + (DH - DC)}{2}$$

Where:

D_V = Vertical diameter

D_H = horizontal diameter

D_c = disc diameter

1. RESULT AND DISCUSSION

3.1 Results of the antibacterial activity test against *E. chrysanthemi*

The antibacterial activity assay in this study consisted of five distinct treatments designed to evaluate and compare the inhibitory potential of different agents against *Erwinia chrysanthemi*. The treatments comprised a positive control, a negative control, and three experimental groups treated with *Mikania micrantha* leaf extract at concentrations of 25%, 50%, and 80%. These concentrations were selected to observe the possible dose–response relationship between extract strength and antibacterial efficacy. Following a 24-hour incubation period, the mean diameters of the inhibition zones formed around each treatment disc were measured and are summarized in Table 1. The table clearly illustrates the variation in antibacterial activity among the different treatments.

Table 1: Antibacterial activity of *M. micrantha* leaf extract against *E. chrysanthemi.*

Treatment	Inhibition Zone Diameter (mm)	Response Category
25% extract	7.51	Moderate
50% extract	8.02	Moderate
80% extract	8.40	Moderate
Positive control (1% chloramphenicol)	30.95	Very strong

The classification of inhibition categories applied in this research follows the criteria established by Davis and Stout (1971). According to this system, inhibition responses are classified as weak (≤ 5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (≥ 20 mm). This standardized approach ensures that the observed inhibition zone diameters can be meaningfully compared to previous studies.

The results reveal a distinct and consistent trend: as the concentration of *M. micrantha* extract increased, the inhibition zone diameter also tended to increase. The smallest inhibition zone was observed at the lowest concentration of 25% (7.51 mm), whereas the largest among the extract treatments occurred at the highest concentration of 80% (8.40 mm). While the numerical differences between the extract concentrations appear relatively small, the overall pattern indicates a concentration-dependent response. This suggests that even slight increases in extract strength can modestly enhance antibacterial activity, likely due to a greater abundance of active phytochemicals in the higher-concentration treatments.

In sharp contrast, the positive control—1% chloramphenicol—produced an inhibition zone of 30.95 mm, which is more than three times greater than the highest inhibition zone produced by any plant extract treatment. This dramatic difference confirms the potent bactericidal nature of chloramphenicol, a well-established synthetic antibiotic. The negative control (sterile distilled water) produced no measurable inhibition zone (0.00 mm), validating its role as an inert baseline and confirming that it lacks any intrinsic antibacterial properties.

Previous phytochemical investigations have demonstrated that *Mikania micrantha* harbors a wide spectrum of secondary metabolites, including phenolic acids (e.g., caffeic acid, *p*-coumaric acid), thymol derivatives, flavonoids, sesquiterpene lactones, terpenoids, and essential oils. Zhang et al. (2017) reported two novel phenolic compounds together with caffeic and *p*-coumaric acid derivatives exhibiting pronounced antioxidant activity. Likewise, Lee et al. (2022) identified germacrane sesquiterpene dilactones with remarkable antibacterial properties, even against resistant strains such as MRSA, with MIC values substantially lower than those of crude extracts.

In the present study, the progressive increase in inhibition zone diameter across extract concentrations corroborates the established principle that higher concentrations of plant extracts generally contain proportionally greater amounts of bioactive constituents. Compounds such as phenols, flavonoids, and tannins are well documented for their antibacterial efficacy, acting through multiple mechanisms, including disruption of cell wall and membrane integrity and interference with essential bacterial metabolic pathways. The superior activity of the 80% extract, which yielded the largest inhibition zone among the plant-based treatments, is therefore likely attributable to the higher effective concentration of these phytochemicals.

Nevertheless, the inhibition zones observed in this study (~7–8 mm, classified as moderate) were considerably smaller compared with the synthetic antibiotic chloramphenicol. This discrepancy may reflect the relatively low concentration of active molecules in crude extracts, potential diffusion limitations, or the complex nature of the extract matrix. In line with earlier reports on the isolation of bioactive fractions from *M. micrantha* (Devkota & Sahu, 2021), further fractionation and purification, coupled with MIC determination, are warranted to identify the specific compounds responsible for antibacterial activity and to optimize their inhibitory potential beyond that achieved with crude extracts.

Microscopic examination of the inhibition zones provided visual confirmation of the quantitative measurements. Well-defined and clear bacterial clearance zones were observed around the discs impregnated with 50% and 80% extracts (Figure 1). The clarity of these zones indicates complete inhibition of bacterial growth within the affected area, strongly supporting the assertion that *M. micrantha* leaf extract contains active metabolites capable of suppressing *E. chrysanthemi* proliferation.

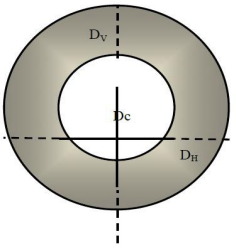

Figure 1: inhibition zone

These observations are consistent with the findings of Prescott (2005), who reported that inhibition zone diameter is directly influenced by the concentration of the antimicrobial agent being tested. The slight variations noted among replicates in this study may be attributed to differences in disc absorption capacity and the rate of compound diffusion through the agar medium. Factors such as immersion time of the discs in the extract, the polarity of the active compounds, and their molecular weight can all influence how quickly and effectively the compounds spread from the disc into the surrounding medium (Dewi, 2010).

Phytochemical profiling of *M. micrantha* has revealed the presence of a diverse array of secondary metabolites,

including phenols, flavonoids, terpenoids, alkaloids, saponins, polyphenols, tannins, and steroids (Alridiwirsah et al., 2020). Among these, phenolic compounds—especially flavonoids and tannins—are the most extensively documented for their antibacterial properties. Phenols can function as protoplasmic poisons, penetrating and destabilizing bacterial cell walls, which leads to protein denaturation and eventual cell death. Cowan (1999) explained that phenolic compounds can also alter the permeability of the cytoplasmic membrane, causing leakage of essential cellular contents. This leakage disrupts normal cellular functions and can be fatal to bacteria—a mechanism that likely contributes to the growth inhibition observed in this study.

The susceptibility of *E. chrysanthemi* to *M. micrantha* extract may also be linked to its structural and physiological characteristics. This bacterium, known primarily for attacking fleshy plant tissues such as roots, tubers, stem cuttings, and leaves, may possess membrane structures that are particularly vulnerable to disruption by phenolic compounds. Pelczar et al., (1988) described how lipid extraction from bacterial membranes can enlarge pore sizes, thereby increasing membrane permeability. Such structural alterations can lead to uncontrolled leakage of vital intracellular components, impair essential metabolic processes, and ultimately cause cell death or severe growth suppression.

Taken together, these findings provide both quantitative and qualitative evidence that *M. micrantha* leaf extract possesses measurable antibacterial activity against *E. chrysanthemi*. Although its inhibitory effect is classified only as "moderate" and is significantly lower than that of a synthetic antibiotic like chloramphenicol, the extract still demonstrates potential as a natural antibacterial agent. Given its plant-based origin, it may offer an environmentally friendly alternative to chemical pesticides for the management of bacterial plant diseases. Further research, including optimization of extraction methods and possible combination with other natural antimicrobials, could enhance its effectiveness and broaden its applicability in sustainable agricultural practices.

3.2. Statistical Analysis

The results of the Analysis of Variance (ANOVA) presented in Table 2 indicate that the calculated F-value (114.58) is substantially higher than the critical F-value (3.05) at a significance level of $\alpha = 0.05$. This large difference between the calculated and critical F-values confirms that the type of treatment had a statistically significant effect on the mean diameter of the inhibition zones observ7ed in the experiment. In other words, the antibacterial activity against *E. chrysanthemi* varied significantly depending on whether the treatment involved *M. micrantha* leaf extract at different concentrations, the positive control (1% chloramphenicol), or the negative control (sterile distilled water).

Table 2: ANOVA Analysis Results of the Antibacterial Activity of *M. micrantha* Leaf Extract

Source of Variation	SS	Df	MS	F	P-value	F crit
Type of Treatment	2188,29	4	547,07	114,58	4,72	3,05
Residual	71,61	15	4,77			
Total	2259,90	19				

To determine which treatments differed from each other in terms of their antibacterial effectiveness, Duncan's Multiple Range Test was applied as a post-hoc analysis. The results of this test are summarized in Table 3.

Table 3. Duncan's Test Results for the Antibacterial Activity of *M. micrantha* Leaf Extract

Treatment	Mean Inhibition Zone (mm)	Inhibition Response Category
1% Chloramphenicol	30.95[a]	Very Strong
80 % Extract concentration	8.40[b]	Moderate
50 % Extract concentration	8.02[b]	Moderate
25 % Extract concentration	7.51[b]	Moderate
Sterile Distilled Water	0[c]	-

According to Duncan's test, all three concentrations of *M. micrantha* leaf extract (25%, 50%, and 80%) fell within the same statistical grouping, as indicated by the shared superscript letter "b". This means that, although the inhibition zone diameters slightly increased with higher concentrations, the differences among the plant extract treatments were not statistically significant at the 5% level. In contrast, the positive control (1% chloramphenicol) formed a separate statistical group ("a") with a significantly larger inhibition zone, reflecting its superior antibacterial potency. The negative control (sterile distilled water) showed no inhibition and was placed in its own distinct group ("c"), confirming the absence of antibacterial activity.

Overall, these findings demonstrate that *M. micrantha* leaf extract exhibits measurable antibacterial activity against *E. chrysanthemi*, with inhibition responses classified as "moderate" according to Davis and Stout's (1971) criteria. However, its effectiveness is considerably lower than that of the synthetic antibiotic chloramphenicol, which produced a "very strong" inhibition response. Despite this lower potency, the presence of consistent inhibitory effects across all tested extract concentrations suggests that *M. micrantha* leaf extract contains bioactive compounds with potential application as a biological control agent. Such plant-based alternatives could provide eco-friendly solutions for managing bacterial plant pathogens, reducing reliance on chemical pesticides.

4. CONCLUSION

The findings of this study clearly indicate that the leaf extract of *Mikania micrantha* exhibits notable antibacterial activity against *Erwinia chrysanthemi*, a pathogenic bacterium known to cause significant plant diseases. The observed inhibition of bacterial growth suggests the presence of bioactive compounds within the extract that interfere with essential bacterial cellular processes, ultimately restricting proliferation. Moreover, the variation in inhibition zone diameters across different extract concentrations underscores the concentration-dependent nature of the antibacterial effect. While all tested concentrations demonstrated measurable inhibitory activity, each falling within the classification of moderate inhibition, the magnitude of inhibition increased progressively with higher extract concentrations. Among the concentrations evaluated, the 80% leaf extract produced the largest inhibition zone, signifying the most potent antibacterial response in the tested range. This finding implies that higher concentrations of *Mikania micrantha* extract may contain greater amounts of active phytochemicals, such as flavonoids, tannins, or terpenoids, which are potentially responsible for the bacteriostatic or bactericidal effects observed.

However, the results of the ANOVA test showed that there was no significant difference ($p > 0.05$) among the three extract concentrations (25%, 50%, and 80%) in relation to the inhibition zone. This finding indicates that increasing the extract concentration does not necessarily correlate with a proportional increase in antibacterial activity. Therefore, the use of a 25% extract concentration is considered the most efficient, as it provides an effect comparable to higher concentrations while requiring less material. Collectively, these results highlight the potential of *Mikania micrantha* as a natural antibacterial agent and suggest its possible application in the development of environmentally friendly plant protection strategies to mitigate bacterial wilt diseases caused by *E. chrysanthemi*.

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