IGNATIAN
INTERNATIONAL JOURNAL FOR MULTIDISCIPLINARY RESEARCH
www.icceph.com
ISSN 2984-9942

ANTIBACTERIAL SUSCEPTIBILITY ASSESSMENT OF FRESH *CHROMOLAENA ODORATA* LEAF EXTRACT

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https://doi.org/10.5281/zenodo.15034789

ABSTRACT

Chromolaena odorata, commonly known as the "marasili" plant, has been recognized for its potential antibacterial properties, particularly in its crude extract form. This study evaluates the antibacterial susceptibility of fresh *C. odorata* leaf extract against *Staphylococcus aureus* using the agar well diffusion method. Three replicates across three different concentrations were tested. Results showed no observable zone of inhibition in all trials, indicating that the fresh extract lacks significant antibacterial activity against both bacterial strains. These findings contrast with previous studies demonstrating that crude extracts of *C. odorata* exhibit notable antibacterial effects, likely due to the higher concentration of bioactive compounds achieved through solvent extraction. The absence of inhibition in the fresh extract suggests that the method of extraction significantly influences the plant's antibacterial efficacy. Further research is recommended to optimize extraction techniques for maximizing the antimicrobial potential of C. odorata.

Keywords: *Chromolaena odorata, fresh extract, crude extract, Staphylococcus aureus, antibacterial susceptibility, agar well diffusion*

INTRODUCTION

The increasing prevalence of antibiotic-resistant bacterial strains has intensified the search for alternative antimicrobial agents, particularly those derived from medicinal

plants. Traditional medicinal knowledge has long recognized the therapeutic potential of plant-based compounds, which possess diverse pharmacological properties, including antimicrobial activity. Among these medicinal plants, Chromolaena odorata (L.) R.M. King & H. Rob., commonly known as the "marasili" plant, has been widely utilized for its wound-healing and antimicrobial properties. Indigenous communities have traditionally employed C. odorata for the treatment of wounds and skin infections, and scientific studies have corroborated its pharmacological effects, which include anti-inflammatory, antioxidant, and antimicrobial activities (Sirinthipaporn & Jiraungkoorskul, 2017). The plant's medicinal properties have been attributed to its diverse bioactive constituents, such as flavonoids, tannins, saponins, alkaloids, and terpenoids (Akinmoladun et al., 2020). However, the efficacy of C. odorata as an antimicrobial agent is highly dependent on the extraction method, which influences the concentration and bioavailability of its active compounds.

Several studies have reported the antibacterial activity of C. odorata crude extracts against pathogenic bacteria, particularly Staphylococcus aureus and Escherichia coli (Adeshina et al., 2010; Owoseni et al., 2021). For instance, Nandini et al. (2014) demonstrated that ethyl acetate and aqueous extracts of C. odorata leaves exhibited significant antibacterial activity against Proteus mirabilis, Bacillus megaterium, and Bacillus subtilis, with efficacy surpassing that of the conventional antibiotic streptomycin.

Similarly, Hanphanphoom and Krajangsang (2016) reported that ethanolic, methanolic, and hexane extracts of *C. odorata* effectively inhibited the growth of Gram-positive bacterial strains associated with skin infections, including Staphylococcus aureus and Streptococcus pyogenes. These findings suggest that C. odorata possesses substantial antimicrobial potential when appropriate solvent-based extraction techniques are employed. However, studies investigating the antibacterial efficacy of fresh C. odorata extracts remain limited. Given that solvent-based extractions typically enhance the bioavailability of active phytochemicals, it is necessary to determine whether fresh extracts exhibit comparable antimicrobial activity.

This study assesses the antibacterial activity of fresh C. odorata leaf extract against Staphylococcus aureus using the agar well diffusion method. This technique is widely utilized in antimicrobial research as it allows for the direct evaluation of inhibitory effects exerted by plant extracts on bacterial growth, while also providing insights into their minimum inhibitory concentration (MIC) (Balouiri, Sadiki, & Ibnsouda, 2016). The study aims to determine whether fresh C. odorata extracts demonstrate antibacterial properties comparable to those reported for crude extracts and to explore the implications of extraction methods on the bioactivity of C. odorata. The findings of this research will contribute to the existing body of knowledge on plant-based antimicrobial agents and provide valuable insights into optimizing extraction methods for potential medicinal applications.

Research Questions

This study aims to assess the antibacterial susceptibility of fresh *Chromolaena odorata* leaf extract against *Staphylococcus aureus* using the agar well diffusion method. Specifically, it seeks to answer the following research questions:

1. How can a pure culture of *Staphylococcus aureus* be grown on a solid medium for antibacterial susceptibility testing?
2. What is the antibacterial effect of fresh *Chromolaena odorata* leaf extract against *Staphylococcus aureus*?
3. Which concentration of fresh *Chromolaena odorata* leaf extract exhibits the highest inhibitory effect on the growth of *Staphylococcus aureus*?

METHODOLOGY

Research Design

This study employs a comparative-experimental research design to evaluate the antibacterial susceptibility of fresh *Chromolaena odorata* leaf extract against *Staphylococcus aureus* using the agar well diffusion method. The comparative aspect involves assessing different concentrations of the extract to determine the most effective formulation, while the experimental approach systematically tests the extract's ability to inhibit bacterial growth under controlled conditions.

Research Locale

The study is conducted at Saint Mary's University (SMU), where all experimental procedures, including bacterial culturing, extract preparation, and antimicrobial susceptibility testing, are performed under controlled laboratory conditions. The institution provides the necessary microbiological facilities, including an incubator, autoclave, laminar flow cabinet, and other sterile equipment required for the study.

Materials

The following materials and equipment are utilized for sample preparation, bacterial culturing, and antimicrobial susceptibility testing:

- Laboratory Equipment: Incubator, autoclave, micropipette, borer, Vernier caliper, glassware (sterile beakers, test tubes, and flasks), prepared inoculum, Petri dishes with agar medium.
- Reagents and Consumables: Distilled water, rubbing alcohol, aluminum foil/paper, sterile cotton swabs, tissue paper, labeling pad, labeling pen.
- Bacterial Strain: Staphylococcus aureus (pure culture).

Procedure

A. Collection and Preparation of Sample

Fresh leaves of *Chromolaena odorata* are collected and washed thoroughly to remove dirt and unwanted microorganisms. The leaves are then air-dried to minimize excess moisture before extraction. The dried sample is blended with **100 mL of distilled water** to obtain a fresh aqueous extract. The extract is then filtered using a gauze pad to remove solid residues, ensuring only the liquid extract is used for testing. The prepared extract is covered with aluminum foil or paper to prevent contamination.

B. Preparation of Concentrations

To determine the antibacterial efficacy of different concentrations, the extract undergoes a **two-fold serial dilution** to create three concentrations:

- **10^{-1} dilution**
- **10^{-2} dilution**
- **10^{-3} dilution**

Each dilution is prepared in sterile beakers and labeled accordingly to ensure accurate identification during testing.

C. Preparation of Agar Plates and Inoculation

1. Sterilized agar plates are prepared and labeled accordingly at the bottom to avoid confusion.
2. In a laminar flow cabinet, a pure culture of *Staphylococcus aureus* is inoculated onto the agar plates using a sterile cotton swab to ensure even bacterial distribution.
3. A sterile borer is used to create uniform wells in the agar medium, ensuring they do not interfere with labeling for clear visibility of results.
4. Using a micropipette, 0.1 mL of each extract concentration is introduced into the corresponding wells.
5. The plates are covered with aluminum foil or paper to minimize external contamination while ensuring proper bacterial growth.
6. The inoculated plates are incubated at 37°C for 24–48 hours to allow bacterial growth and interaction with the test extract.

D. Observation and Data Collection

1. After 24 hours, the agar plates are retrieved from the incubator.
2. The presence or absence of zones of inhibition (clear areas surrounding the wells where bacterial growth is inhibited) is observed.
3. If inhibition zones are present, their diameters are measured using a Vernier caliper to determine the antibacterial efficacy of each concentration.
4. The results are recorded, and comparisons are made to identify whether higher concentrations yield greater antibacterial activity.

RESULTS

A. Observed agar plates

Figure 1. Agar plates after 24 hours. Left to right (Replicate 1-Replicate 3)

B. Measured Zone of Inhibition

Table 1. Antibacterial Activity of Fresh *Chromolaena odorata* Leaf Extract Against *Staphylococcus aureus*: Zone of Inhibition Measurements

Test Substance	**ZONES OF INHIBITION (mm)**			**Mean**	**Qualitative Description**
Fresh Extract of *Chromolaena odorata* leaf	Replicate 1	Replicate 2	Replicate 3		
Well 1 (100%)	0	0	0	0	inactive
Well 2 (50%)	0	0	0	0	inactive
Well 3 (25%)	0	0	0	0	inactive
Negative control (distilled water)	0	0	0	0	inactive
Positive control (Amoxicillin Trihydrate)	21.71	21.73	22.26	21.9	Very active

Legend: ZOI of < 10 mm-inactive; ZOI of 10-13 mm-partially active; ZOI of 14-19 mm-active; and ZOI of > 19 mm is very active (Guevara, 2005)

DISCUSSION

The findings of this study indicate that the fresh *Chromolaena odorata* leaf extract exhibited no antibacterial activity against *Staphylococcus aureus*, as evidenced by the absence of any zone of inhibition in all three replicates across different concentrations (100%, 50%, and 25%). Table 1 presents the recorded inhibition zones, showing that even at full concentration, the fresh extract failed to inhibit bacterial growth, with results comparable to the negative control (distilled water). In contrast, the positive control,

Amoxicillin Trihydrate, demonstrated significant antibacterial activity, with inhibition zones averaging 21.9 mm, confirming the accuracy and validity of the experimental procedure. These results suggest that fresh *C. odorata* extract does not possess immediate antibacterial properties under the conditions tested.

The observations recorded in Figure 1 substantiate these findings. The agar plates labeled *S. aureus* 1, 2, and 3 represent three independent replicates of the antibacterial susceptibility assay using fresh *Chromolaena odorata* extract. The widespread bacterial lawn and the absence of inhibition zones around the wells indicate that S. aureus was able to grow uninhibited despite exposure to the plant extract. This aligns with the quantitative data from Table 1, reinforcing the conclusion that the fresh extract lacks significant antibacterial efficacy.

These findings contrast with previous studies that have reported the antimicrobial potential of *Chromolaena odorata*. For instance, Sirinthipaporn and Jiraungkoorskul (2017) and Odutayo et al. (2017) demonstrated that crude extracts of C. odorata exhibited significant antibacterial activity against various pathogens, including Staphylococcus aureus. This antimicrobial effect was attributed to the plant's rich phytochemical composition, which includes flavonoids, tannins, alkaloids, and essential oils—compounds known for their antibacterial properties. Likewise, Zahara (2019) highlighted the traditional medicinal use of C. odorata, particularly in wound healing and infection treatment, further supporting its role as a natural antimicrobial agent.

Moreover, previous studies have shown that solvent-based extractions yield significantly higher concentrations of bioactive compounds compared to fresh or aqueous extracts. Jiboom et al. (2021) reported that ethanolic and methanolic extracts of C. odorata exhibited strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, with inhibition zones ranging from 12 mm to 18 mm. Similarly, Ukwubile et al. (2020) observed that hexane and chloroform extracts of C. odorata displayed notable antibacterial effects due to the enhanced solubility of non-polar phytochemicals. The disparity between these studies and the current findings suggests that the method of extraction plays a pivotal role in determining antimicrobial efficacy.

The extraction method plays a crucial role in the bioactivity of plant extracts. Previous research has highlighted those crude extracts obtained through solvent-based extraction, such as ethanol or methanol, yield higher concentrations of bioactive compounds compared to aqueous or fresh extracts (Adeshina et al., 2019). In this study, only a fresh extract was used, which may not have effectively extracted the plant's active compounds responsible for antibacterial properties. This suggests that future research should explore alternative extraction techniques to determine whether ethanol or methanol extraction enhances the antimicrobial potency of *C. odorata.*

Furthermore, the results suggest that while C. odorata may contain bioactive compounds, its antibacterial efficacy depends on concentration and extraction efficiency. The findings align with those of Irobi et al. (1994), who noted that antibacterial activity in medicinal plants is significantly influenced by the method of preparation. The absence of inhibition

zones in this study may indicate that fresh extracts do not release sufficient bioactive compounds to exert antibacterial effects. Phytochemical analysis of the fresh extract is recommended to assess the presence and concentration of antimicrobial compounds, as suggested by Ahmed et al. (2022), who emphasized that variations in phytochemical composition significantly affect antimicrobial efficacy.

Overall, the findings suggest that fresh Chromolaena odorata extract does not effectively inhibit the growth of S. aureus under the conditions tested. The lack of antibacterial activity highlights the need for optimized extraction methods to enhance the release of bioactive compounds. Future studies should focus on solvent-based extraction techniques, comparative antimicrobial assays using different bacterial strains, and the quantification of key phytochemicals responsible for antimicrobial properties. Additionally, advanced analytical techniques, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), should be employed to identify and quantify the specific bioactive compounds contributing to antimicrobial activity (Ekwunife et al., 2021).

Conclusions

This study investigated the antibacterial efficacy of fresh *Chromolaena odorata* leaf extract against *Staphylococcus aureus* using the agar well diffusion method. The results demonstrated that the fresh extract exhibited no inhibitory activity, as evidenced by the absence of zones of inhibition across all concentrations (100%, 50%, and 25%) and replicates. These findings were comparable to the negative control (distilled water), while the positive control, Amoxicillin Trihydrate, produced a mean inhibition zone of 21.9 mm, confirming the reliability of the experimental procedure. The results indicate that fresh *C. odorata* extract lacks significant antibacterial properties under the conditions applied in this study.

The absence of antibacterial activity in the fresh extract contrasts with previous research, which has reported the antimicrobial potential of *C. odorata* in crude or solvent-based extracts. This discrepancy suggests that the extraction method significantly influences the bioavailability and potency of the plant's active compounds. The inability of the fresh extract to inhibit *S. aureus* growth implies that its bioactive constituents may not be present in sufficient concentrations or may require further processing to enhance their antibacterial effects**.**

Recommendations

Based on the findings of this study, several recommendations are proposed to further explore the antibacterial potential of *Chromolaena odorata*, particularly in relation to its traditional medicinal applications. Since the fresh extract exhibited no antibacterial activity against *Staphylococcus aureus*, future research should investigate alternative extraction methods, such as ethanol or methanol extraction, which may enhance the concentration of bioactive compounds responsible for antimicrobial effects. Traditional healing practices often involve decoctions, poultices, or prolonged extraction processes that may release

more potent phytochemicals. Therefore, replicating these methods in a controlled laboratory setting could provide valuable insights into how traditional preparations achieve their therapeutic effects.

Additionally, conducting a phytochemical analysis of both fresh and processed extracts would help identify and quantify active compounds such as flavonoids, tannins, alkaloids, and essential oils, which have been linked to antimicrobial activity in previous studies. Expanding the study to include a wider range of bacterial species, both Gram-positive and Gram-negative, could determine whether *C. odorata* exhibits selective antibacterial activity, which may explain its historical use in treating certain infections. Furthermore, increasing the sample size and number of replicates in future experiments would improve the reliability and statistical significance of the results.

Exploring different plant parts, such as stems, roots, or flowers, and preparing the extract in alternative formulations like essential oils or nanoparticle-based solutions may enhance its antibacterial efficacy. Given that fresh extracts may degrade quickly, examining their stability under different storage conditions could provide further insights into their effectiveness over time. Since *C. odorata* has long been used in traditional medicine for wound healing, burns, and skin infections, future studies should consider in vivo testing to assess its wound-healing properties in conjunction with antimicrobial effects. Additionally, investigating potential synergistic applications with existing antibiotics could determine whether *C. odorata* can serve as a complementary treatment, preserving its relevance in modern medicine while honoring its traditional uses.

Compliance with Ethical Standards

This study was conducted in full compliance with ethical research standards to ensure the integrity of the process and the protection of all participants. The research involved the in vitro testing of the antibacterial activity of *Chromolaena odorata* extract against *Staphylococcus aureus* and did not involve human or animal subjects. All procedures were performed following biosafety protocols to prevent contamination and ensure laboratory safety. No conflicts of interest were present in the conduct of this study. Plagiarism was strictly avoided, with proper citation of all referenced works to maintain academic integrity. The interpretation of findings was conducted objectively, without bias, ensuring that results were analyzed and reported with accuracy and transparency. The data obtained were used solely for research purposes, contributing to the scientific understanding of *C. odorata*'s antibacterial properties and its potential applications in medicine.

Acknowledgments

The researcher extends sincere appreciation to the instructor, Sir Hansen Villanueva, for his invaluable guidance and for providing the structured procedures essential to this study. Gratitude is also expressed to Ma’am Jaryll, the laboratory assistant, for her support in preparing the necessary materials and equipment, ensuring the smooth execution of the experiment. Furthermore, the researcher acknowledges the collaborative

efforts and assistance of fellow MAT Biology 1 students, whose support contributed to the successful completion of this study. Lastly, appreciation is extended to Ma'am Vicky for accommodating the laboratory activities by allowing the use of the back part of the classroom during her scheduled class.

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APA Citation:

Attam, R. L. (2025). ANTIBACTERIAL SUSCEPTIBILITY ASSESSMENT OF FRESH CHROMOLAENA ODORATA LEAF EXTRACT. Ignatian International Journal for Multidisciplinary Research, 3(3), 418–427. https://doi.org/10.5281/zenodo.15034789

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