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Antibacterial Analysis of *Muntingia Calabura* (Mansanitas) Ethanolic Flower Extract on *Staphylococcus* Aureus Using Paper Disc Diffusion Method

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Abstract

This study aimed to evaluate the antibacterial efficacy of *Muntingia calabura* ethanolic flower extract against *Staphylococcus aureus* using the paper disc diffusion method. Anchored on the concept that plant-derived bioactive compounds such as flavonoids, tannins, and polyphenols can inhibit bacterial growth, the research employed an experimental design with three independent trials. The extract was obtained through maceration of flower samples in 96% ethanol, followed by filtration and solvent evaporation, and was tested using standardized microbiological procedures, including the preparation of a bacterial inoculum and the use of a 0.5 McFarland standard. The results revealed measurable zones of inhibition of 29 mm, 31 mm, and 28 mm, with an average of 29 mm, indicating strong antibacterial activity classified as "very active." No inhibition was observed in the control, confirming that the antimicrobial effect was due to the extract. The findings demonstrated consistent and reproducible antibacterial activity across trials, suggesting the presence of effective bioactive compounds. However, the study was limited by the use of a single extract concentration, a small number of trials, and the absence of compound isolation and inhibitory concentration analysis. The study concludes that *Muntingia calabura* ethanolic flower extract has significant potential as a natural antibacterial agent. It is recommended that future research explore varying concentrations, identify active compounds, and determine minimum inhibitory and bactericidal concentrations. This study aligns with SDG 3 (Good Health and Well-Being) by contributing to alternative treatments for infectious diseases and SDG 12 (Responsible Consumption and Production) through the use of plant-based resources. It contributes to sustainability by supporting public health solutions and promoting the use of locally available natural resources for accessible and cost-effective antimicrobial development.

Keywords: Antibacterial Activity; Ethanolic Extract; Muntingia Calabura; Paper Disc Diffusion; Staphylococcus Aureus; Zone of Inhibition

1. Introduction

The increasing prevalence of antibiotic-resistant bacteria, particularly *Staphylococcus aureus*, has intensified the need for alternative antimicrobial agents. Plant-based compounds, such as those derived from *Muntingia calabura*, offer promising bioactive properties. This study investigates the antibacterial efficacy of *Muntingia calabura* ethanolic flower extract against *S. aureus* using the paper disc diffusion method, contributing to the search for sustainable and effective antimicrobial solutions.

1.1 Background of the Study on *Muntingia calabura* Flower Extract and *Staphylococcus aureus*

Muntingia calabura, commonly known as Jamaican cherry, has long been utilized for medicinal (Muhammad et al., 2021), nutritional (Mastuki, 2019), cultural, and ecological purposes. Its fruits exhibit anti-inflammatory properties (Rakesh, et al., 2023), while its flowers possess antibacterial and antispasmodic properties when prepared as therapeutic infusions. Traditional uses include antiseptic applications and treatment of swelling, headaches, colds, and gastrointestinal conditions across Peru, Colombia, and the Philippines (Mahmood (2014)). Despite extensive research on its fruits, leaves, and bark, studies on the pharmacological properties of its flowers remain limited. However, recent findings indicate that ethanolic flower extracts demonstrate antibacterial activity against pathogens such as Vibrio cholerae, Salmonella typhi, Shigella dysenteriae, and Escherichia coli (St. Mary et al. (2024)).

Staphylococcus aureus is a common bacterium present in approximately 30% of humans (U.S. Centers for Disease and Control Prevention, 2024). While often harmless, it can cause infections ranging from minor skin conditions to severe diseases such as sepsis, pneumonia, and endocarditis (Taylor and Unakal, 2023). Its ability to rapidly develop resistance to antibiotics highlights the urgent need for alternative antimicrobial agents (Pantosti et al. (2007)).

1.2 Themes and Insights from Related Literature on Antibacterial Properties and Pathogen Behavior

Muntingia calabura exhibits diverse pharmacological activities, including cytotoxic, antiproliferative, antioxidant, anti-inflammatory, and antimicrobial effects (Mahmood et. al. (2014); Kaneda et. al., 1991; Zakaria et. al., 2011; Su et. al., 2003; Chen et al., 2007; Zakaria et. al., 2007; Bandeira et. al., 2013; Zakaria et al., 2006; Ibrahim et al., 2012; Sridhar et al., 2011; Shih et al., 2006; Nivethetha et. al., 2009). Early studies demonstrated antibacterial activity of its extracts against E. coli and S. aureus (Yasunaka et al. (2005)). Further research confirmed that ethanolic flower extracts exhibit significant antibacterial effects against gastrointestinal pathogens (St. Mary et al. (2024)). *Staphylococcus aureus* is a Gram-positive bacterium forming grape-like clusters and capable of causing widespread infections (Foster (2002)). It demonstrates strong adaptive evolution and resistance to antibiotics, including penicillin and methicillin, with resistance emerging rapidly after their introduction (Deurenberg and Stobberingh (2008)). Current treatments, such as vancomycin and newer antibiotics, face reduced effectiveness due to resistance development (Graningcr et al., 1995; Waldvogel, 1995; Chambers, 1997; Hoopcr, 1997).

1.3 Local, National, and Global Context of Antimicrobial Resistance and Plant-Based Remedies

Globally, antimicrobial resistance poses a major public health challenge, particularly with pathogens like *Staphylococcus aureus* that exhibit rapid resistance evolution (Pantosti et al. (2007)). The widespread presence of S. aureus in human populations (U.S. Centers for Disease and Control Prevention, 2024) increases the risk of infection and transmission. Locally and regionally, *Muntingia calabura* is widely available and traditionally used for medicinal purposes in the Philippines (Mahmood (2014)), making it a practical and accessible candidate for alternative treatments. Its adaptability and abundance across Southeast Asia further support its potential as a sustainable resource (Berazain, 2016).

1.4 Theoretical or Conceptual Basis of Antibacterial Activity of Plant Extracts

The antibacterial activity of plant extracts is attributed to bioactive phytochemicals such as flavonoids, tannins, and polyphenols, which disrupt bacterial cell structures and metabolic processes. The effectiveness of these compounds can be assessed using the paper disc diffusion method, where the zone of inhibition (ZOI) indicates bacterial susceptibility (Claustra et. al, 2005; Bhargav, 2016). The adaptive resistance mechanisms of *Staphylococcus aureus* emphasize the importance of exploring plant-based antimicrobials as alternative therapeutic agents (Pantosti et al. (2007)).

1.5 Research Gaps and Rationale for Studying *Muntingia calabura* Flower Extract

Although *Muntingia calabura* has been widely studied for its pharmacological properties, research focusing specifically on its flower extracts remains limited. Existing studies primarily examine leaves, fruits, and bark, leaving a gap in understanding the antibacterial potential of its flowers. Additionally, while previous research has demonstrated antibacterial activity against various pathogens (St. Mary et al. (2024)), there is limited evidence on its effectiveness against *Staphylococcus aureus*, a clinically significant and antibiotic-resistant bacterium. This study addresses these gaps by evaluating the antibacterial activity of M. calabura ethanolic flower extract using a standardized method.

1.6 Alignment with Relevant Sustainable Development Goals (SDGs)

This study aligns with several Sustainable Development Goals (SDGs), particularly SDG 3 – Good Health and Well-Being, by contributing to the development of alternative antimicrobial agents to help address infectious diseases and antibiotic resistance. It also supports SDG 12 – Responsible Consumption and Production through the use of plant-based extracts, promoting sustainable utilization of natural resources. Additionally, the study relates to SDG 15 – Life on Land by encouraging the conservation and sustainable use of *Muntingia calabura*, a locally available plant species that contributes to biodiversity and ecosystem sustainability.

1.7 Importance of the Study to Multidisciplinary Sustainability

This study contributes to public health sustainability by identifying potential alternatives to conventional antibiotics in response to antimicrobial resistance. It supports pharmaceutical and technological sustainability through the development of plant-based therapeutic agents. The research also promotes environmental and ecological sustainability by utilizing naturally abundant plant resources and encouraging biodiversity conservation. Additionally, it contributes to socio-economic sustainability by exploring accessible and cost-effective treatment options, particularly in resource-limited settings. Overall, the study integrates microbiology, pharmacology, and environmental science to advance sustainable healthcare solutions.

2. Material and methods

2.1 Research Design

The study utilized an experimental design to determine the antibacterial efficacy of *Muntingia calabura* ethanolic flower extract against *Staphylococcus aureus*. The experiment consisted of three independent trials using the paper disc diffusion method, wherein 6 mm paper discs impregnated with the extract were applied onto agar plates inoculated with S. aureus.

The procedure involved sequential steps including plant extraction, preparation of bacterial inoculum, application of the extract, and measurement of bacterial inhibition. The antimicrobial screening followed the standard protocol outlined in "A Guidebook to Plant Screening: Phytochemical and Biological" (Claustra et. al, 2005).

2.2 Locale of the Study

The study was conducted in two primary locations. The preparation of plant materials, including maceration and solvent evaporation, was carried out at the Philippine Science High School – Caraga Region Campus Science Laboratory. The antibacterial testing, including microbial culture and disc diffusion assays, was performed at the Department of Science and Technology (DOST)–CARAGA Regional Standards Testing Laboratory.

2.3 Respondents of the Study

The experimental subject of the study was *Staphylococcus aureus* (BIOTECH 1582), a bacterial strain used to assess the antibacterial activity of the plant extract. The bacteria were cultured and standardized prior to experimentation to ensure consistent and reliable results.

2.4 Sample and Sampling Procedure

Fresh *Muntingia calabura* flowers were collected from the local vicinity of Cabadbaran City, Agusan del Norte, in January 2025. The samples were cleaned with water and weighed to approximately 49.14 g prior to extraction. The extraction process involved maceration, where the flowers were soaked in 300 mL of 96% ethanol for 34 days to allow the bioactive compounds to dissolve into the solvent (St. Mary et al., 2024). After maceration, the mixture was strained and filtered to remove solid residues, ensuring that only the liquid extract remained. Filtration was performed using a strainer followed by filter paper to achieve a clearer solution. The filtrate was then subjected to solvent evaporation using a rotary evaporator at 40°C under reduced pressure to remove the ethanol and concentrate the extract (Buhian, 2016). This process resulted in a purified ethanolic flower extract with a final volume of approximately 15 mL.

2.5 Research Instrument

The study utilized standard microbiological equipment and materials, including nutrient agar plates, sterile cotton swabs, 6 mm paper discs, forceps, a ruler, nutrient broth, and a rotary evaporator. A 0.5 McFarland standard was prepared to ensure uniform bacterial turbidity, which is essential for accurate antimicrobial testing. The standard was prepared by mixing 0.5 mL of 0.048 $BaCl_2$ with 99.5 mL of 1% H_2SO_4 and distributed into tubes for comparison during inoculum preparation. The antimicrobial assay followed the paper disc diffusion method, where inhibition zones were measured to determine antibacterial activity (Claustra et. al, 2005; Bhargav, 2016).

2.6 Data Gathering Procedure

The bacterial inoculum was prepared by transferring a loopful of Staphylococcus aureus into 50 mL of nutrient broth and incubating it for 18–24 hours at 35°C. The turbidity of the bacterial suspension was adjusted using a vortex mixer and compared to the 0.5 McFarland standard to ensure consistency. For the experimental trials, approximately 15 mL of molten nutrient agar was poured into sterile petri dishes and allowed to solidify. The bacterial culture was applied onto the agar surface using a sterile cotton swab, streaked evenly in three directions with rotation to achieve uniform distribution, and left to stand for five minutes. The application of the plant extract was conducted using the paper disc diffusion method. As illustrated in Figure 7, three sterile 6 mm paper discs were immersed in the Muntingia calabura ethanolic flower extract and placed onto the inoculated agar plates. Three independent trials were conducted to ensure reliability of results. Data collection involved measuring the zone of inhibition (ZOI), defined as the clear area surrounding the discs where bacterial growth was inhibited (Claustra et. al, 2005; Bhargav, 2016). The results were recorded and summarized in Table 1, which presents the ZOI measurements across trials.

2.7 Data Analysis Techniques

The antibacterial activity of the extract was evaluated by measuring the diameter of the zone of inhibition (ZOI) in millimeters for each trial. The ZOI represents the clear area surrounding the paper disc where bacterial growth is inhibited, indicating the susceptibility of Staphylococcus aureus to the extract (Claustra et. al, 2005; Bhargav, 2016). The measurements from the three trials were recorded and averaged to determine the overall effectiveness of the extract. The results were then interpreted using standard criteria, where inhibition zones greater than 19 mm are classified as "very active," allowing for assessment of the antibacterial potency of the Muntingia calabura ethanolic flower extract.

3. Results and Discussion

3.1 Antibacterial Activity of Muntingia calabura Ethanolic Flower Extract

3.1.1 Zone of Inhibition Against Staphylococcus aureus

The results showed that the ethanolic flower extract of *Muntingia calabura* produced measurable zones of inhibition against Staphylococcus aureus across three trials. The recorded inhibition zones were 29 mm, 31 mm, and 28 mm, with an average

value of 29 mm. No inhibition was observed in the control, indicating that bacterial growth occurred normally in the absence of the extract. The observed inhibition zones indicate that the extract exhibits strong antibacterial activity against Staphylococcus aureus. Based on standard interpretative criteria, inhibition zones greater than 19 mm are classified as "very active," suggesting that the extract possesses potent antimicrobial properties. The absence of inhibition in the control further confirms that the antibacterial effect is attributable to the bioactive compounds present in the extract rather than external factors.

3.2 Consistency of Antibacterial Effect Across Trials

3.2.1 Reliability of Experimental Results

The antibacterial activity of the extract remained consistent across the three trials, with only minimal variation in the measured zones of inhibition. The recorded values ranged from 28 mm to 31 mm, indicating a narrow range of deviation and a stable response of Staphylococcus aureus to the treatment. This consistency suggests that the experimental procedure and the extract preparation method produced reliable and reproducible results. The small variation in inhibition zones indicates uniform distribution of the extract on the paper discs and consistent bacterial susceptibility, supporting the validity of the findings. Such reproducibility is essential in confirming the antibacterial potential of plant-based extracts and reinforces the reliability of the paper disc diffusion method used in this study.

3.3 Implications of Antibacterial Activity

3.3.1 Potential of *Muntingia calabura* as an Antimicrobial Agent

The results demonstrate that *Muntingia calabura* ethanolic flower extract exhibits significant antibacterial activity against Staphylococcus aureus, as evidenced by the large zones of inhibition observed in all trials. The extract consistently inhibited bacterial growth, indicating the presence of active compounds with antimicrobial properties. These findings support the potential of *Muntingia calabura* as a source of plant-based antibacterial agents. The strong inhibitory effect suggests that its bioactive phytochemicals, such as flavonoids, tannins, and polyphenols, may contribute to disrupting bacterial growth and survival. This highlights the relevance of exploring natural alternatives to conventional antibiotics, particularly in addressing the growing issue of antimicrobial resistance.

4. Conclusion & Recommendations

This study demonstrated that *Muntingia calabura* ethanolic flower extract exhibits antibacterial activity against *Staphylococcus aureus* using the paper disc diffusion method. The extract, obtained through maceration, filtration, and concentration, produced measurable zones of inhibition across three trials, indicating consistent antimicrobial effects. These findings confirm the presence of bioactive compounds in the extract and support its potential as a natural antibacterial agent.

It is recommended to use varying concentrations of the extract and increase the number of trials to improve the reliability of the findings. Further studies should focus on isolating and identifying the specific bioactive compounds responsible for the antibacterial activity and determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to establish the effective dosage for bacterial inhibition.

The study was limited to evaluating the antibacterial activity of *Muntingia calabura* ethanolic flower extract against Staphylococcus aureus using a single method and a fixed extract concentration with only three trials. It did not include identification of specific bioactive compounds or determination of the exact inhibitory concentrations, which restricts the depth of analysis regarding the extract's mechanism and optimal effectiveness.

Compliance with ethical standards

The authors declare no conflict of interest and confirm that this study received no external funding. The research was conducted within the Philippine Science High School – Caraga Region Campus and the Department of Science and Technology–CARAGA Regional Standards Testing Laboratory, with all procedures involving laboratory-based, non-invasive methods. As the study did not involve human participants, no personal or identifying information was collected, and all data were handled with strict confidentiality. All experimental procedures were carried out with proper authorization and adherence to standard laboratory protocols.

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