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(RESEARCH ARTICLE)

Antibacterial efficacy of Madre de Cacao *(Gliricidia sepium)* and Hagonoy *(Chromolaena odorata)* leaves extract against sewage water bacteria

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Abstract

Herbalism refers to the traditional practice of using plants and plant extracts for medicinal and therapeutic purposes. The practice of using plant extracts for wounds and lesions has been continuously implemented by a number of local families until today. This study aimed to investigate the antibacterial efficacy of common local plant leaves used for poultice: madre de cacao *(Gliricidia sepium)* and hagonoy *(Chromolaena odorata)* leaf extracts against sewage water bacteria. Two extraction methods were utilized to extract the antibacterial properties of the leaves: maceration and hot decoction, in different concentrations of 25%, 15%, and 5%. Results revealed significant antibacterial activity at higher concentrations; specifically, the 25% extract concentration demonstrated the most significant reduction in inhibiting bacteria, suggesting that the effectiveness of the extracts is dose-dependent. Furthermore, maceration proved to be a more efficient method for extracting the antibacterial properties of the leaves compared to hot decoction. While the madre de cacao and hagonoy leaf extracts illustrated antibacterial activity against sewage water bacteria, the difference in colony counts proves that the hagonoy leaves are more potent than the madre de cacao in this investigation.

Keywords: Antibacterial Efficacy; Colony Count; Madre de Cacao; Hagonoy; Sewage Water

1. Introduction

Since the dawn of time, civilizations have maintained a tight relationship with their surroundings. Humans have utilized the commodities the environment has to offer to sustain themselves. As such, medicinal plants and herbs were the primary solutions to many different illnesses in the past. Evidence of the use of plants for medicinal purposes dates as far back as 60,000 years ago across cultures and development levels (Gossell-Williams et al., 2006). Today, their potency is enhanced by pharmaceutical advancements, and their formulation can only be done with the guidance of a professional. However, in countries blessed with a vast diversity of flora, like the Philippines, herbalism is still practiced. Such practices are observable in many ethnic groups where modernization has no hold of them, while most knowledge in regards to the practice of herbalism comes from ancestors and elders and is continuously implemented by a number of local families (Liu et al., 2016; McMillen, 2012; Wangkheirakpam, 2018).

The madre de cacao (*Gliricidia sepium*), which belongs to the genus Gliricidia, is a medium-sized leguminous tree belonging to the family Fabaceae. With origins in the tropical dry forests of Mexico and Central America, it has been distributed to tropical regions around the world due to its versatility. Indigenous knowledge indicates that madre de cacao has a broad spectrum of uses against pests and is an herbal medicine both for humans and animals. In addition to being expectorant, insecticidal, rodenticidal, sedative, and suppurative (Duke and Wain, 1981, as cited by Abulude et al., 2009), it has also been found that the madre de cacao leaves are a good source of coumarins, a toxic substance that can

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kill almost all types of pests and insects (Rabena, 2008). Hagonoy (*Chromolaena odorata*), also known as siam weed and devil weed, among others, belongs to the Asteraceae family. It is an invasive weed species and an herbaceous perennial that is widely used for its wound-healing properties. Various parts of the plant have been used to treat wounds, burns, and skin infections. Furthermore, it has also been shown to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties (Sirinthipaporn, A., & Jiraungkoorskul, W., 2017).

This study aimed to investigate the antibacterial efficacy of madre de cacao and hagonoy leaf extracts against sewage water bacteria. The diverse chemical compositions and traditionally recognized antibacterial properties of these plant extracts emphasize their potential as sustainable and cost-effective agents against bacteria. By delving into the antimicrobial properties of madre de cacao and hagonoy leaves, this study aimed to contribute valuable insights to the field of anti-bacterial management. The findings of this research may not only enhance our understanding of the potential applications of natural extracts in wound treatment but also offer practical implications for developing eco-friendly strategies to combat harmful bacterial growth, thereby promoting public health and environmental sustainability.

2. Materials and Methods

2.1. Research Design

In this study, a comparative experimental research design was employed. This research method involves comparing the effects of two or more treatments or interventions on a dependent variable.

2.2. Subjects of the Study

The primary subjects of this study are the extracts from madre de cacao leaves (*Gliricidia sepium*) and hagonoy leaves (*Chromolaena odorata*). The sewage water serves as a secondary subject, providing the bacterium used to test the effectiveness of the leaf extracts.

2.3. Data Gathering Procedure

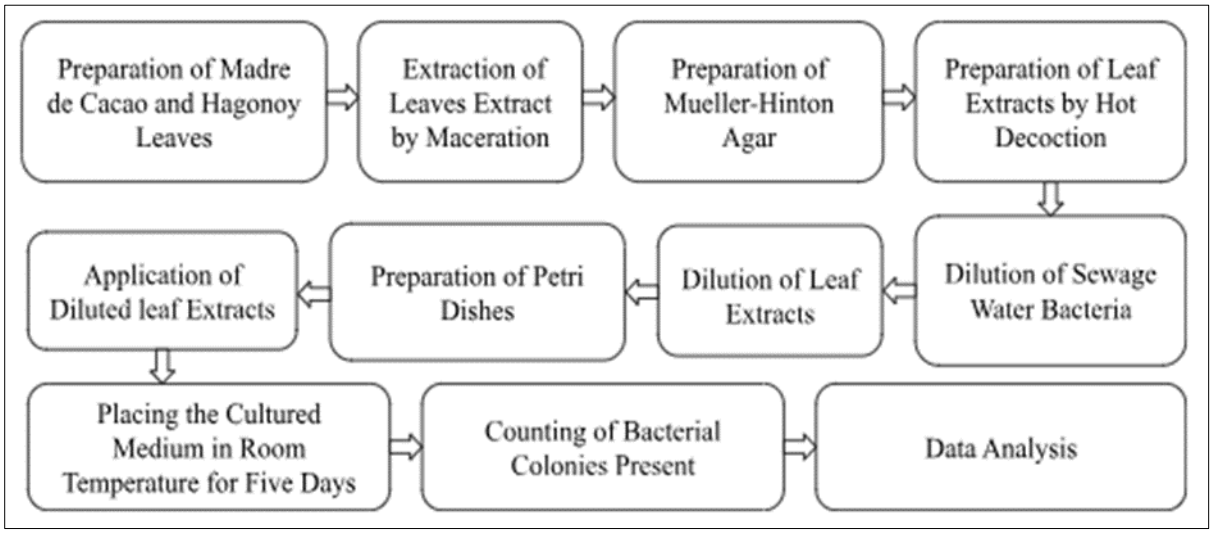

Figure 1 Flowchart of Experimental Procedure

The madre de cacao and hagonoy were washed with distilled water, dried with a cloth, dehydrated at 35°C for three days, and ground into powder using a food processor. For maceration, 50g of powdered leaves were submerged in 500 ml ethanol, sealed with aluminum foil, kept in a cool and dark environment for three days, and filtered through cheesecloth and rotavaped into a paste containing antibacterial compounds. Mueller-Hinton agar was prepared by suspending 19g of agar powder in 500 ml distilled water, heating, and stirring for 15 minutes until dissolved. For hot decoction extraction, 40 ml of boiling distilled water was poured into 20g of powdered madre de cacao leaves, stirred for 10 minutes, and filtered using cheesecloth. Sewage water bacteria were diluted by mixing 2.5 ml sewage water with 22.5 ml distilled water. The resulting leaf extracts were further diluted with pre-diluted sewage water to create final concentrations of 25%, 15%, and 5%. Petri dishes were prepared with 20 ml of Mueller-Hinton agar, covered, and

sterilized using UV light. The diluted leaf extracts and sewage water bacteria were then streaked onto the agar surfaces using sterile cotton swabs. These plates were incubated at room temperature for five days to allow bacterial growth. Finally, bacterial colonies were counted using the "APD Colony Counter App Lite," which facilitated accurate counting by marking each counted colony on the digital images, thereby preventing double-counting.

2.4. Data Analysis

The mean, median, and standard deviation are utilized to find the average number of bacterial colonies among the replicates that formed. Welch's ANOVA was used to compare the effectiveness of the madre de cacao and hagonoy leaf extracts against sewage water bacteria with different methods of extraction and at concentrations of 5%, 15%, and 25%. The analysis identified significant differences in their ability to inhibit bacterial growth and to explore whether the leaf extract concentration influenced their effectiveness. Subsequently, Tukey's Test, a post-hoc analysis, was used to determine which groups of extracts significantly differed from each other in their antibacterial effectiveness.

3. Results and Discussions

The antibacterial activity was assessed through two analyses. A bar graph visualized the colony counts of each extract at various concentrations and extraction methods, enabling a clear comparison of their effectiveness. Additionally, colony counts from the madre de cacao and hagonoy leaf extract treatment groups were compared with the control group, the sewage water treatment without leaf extract. Statistical analysis using ANOVA confirmed significant differences between the madre de cacao and hagonoy leaf extract treatment groups and the control group. To present the antibacterial potential of each extract, a post-hoc comparison table was used to pinpoint their specific efficacy relative to the control group.

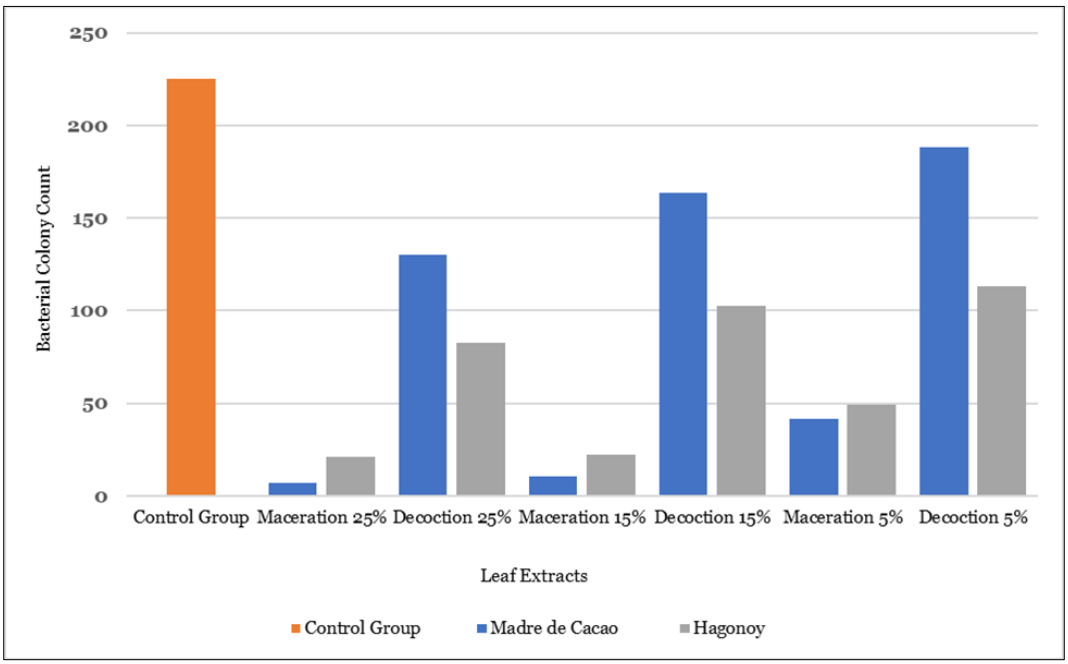

Figure 2 Mean Colony Counts of Treated Leaves

The hagonoy leaves at different concentrations and extracted with different methods exhibited higher antibacterial efficacy compared to madre de cacao. This implies that hagonoy contains more potent antibacterial properties against canal water. This result is supported by Yan Y et al. in 2021, who said different and unique antibacterial compounds could be found in one leaf that may not be present in the other leaf that have potent activity against the tested bacteria.

Welch's ANOVA showed significant differences with a p-value of <.001 in colony counts between the madre de cacao, hagonoy, and control groups. This suggests that the treatment groups impact the colony counts, warranting Tukey's test to pinpoint the most effective leaf extract.

Table 1 Post-hoc Comparison (Tukey's Test) of Leaf Extract Treatment Group vs Control Group

Comparison of Control with	Mean Difference	P-value	Remarks
Macerated Madre de Cacao 25%	254.33	<.001	Significant
Macerated Madre de Cacao 15%	250.7	<.001	Significant
Macerated Madre de Cacao 5%	219.67	<.001	Significant
Hot Decocted Madre de Cacao 25%	130.3	0.010	Significant
Hot Decocted Madre de Cacao 15%	97.3	0.010	Significant
Hot Decocted Madre de Cacao 5%	72.7	0.468	Not Significant
Macerated Hagonoy 25%	240	<.001	Significant
Macerated Hagonoy 15%	239	<.001	Significant
Macerated Hagonoy 5%	211.7	<.001	Significant
Hot Decocted Hagonoy 25%	178.7	<.001	Significant
Hot Decocted Hagonoy 15%	158	<.001	Significant
Hot Decocted Hagonoy 5%	147.7	0.002	Significant

Sig, at <0.05

Statistical analysis using Tukey's post-hoc test revealed significant mean differences in colony counts across all treatment groups. The table suggests a trend where higher mean differences in colony counts compared to the control group indicate stronger antibacterial activity. This is because a larger mean difference signifies a greater reduction in bacterial colonies by the treatment extract compared to the control group. Notably, the macerated madre de cacao leaf extract at a concentration of 25% exhibited the highest mean difference of 254.33 in colony counts compared to the control group. This finding suggests the 25% macerated extract exhibited the strongest antibacterial efficacy, as shown by the greatest reduction in colony counts compared to the control. This observation aligns with previous research by Zorraquino et al. (2021), who proposed that maceration at room temperature may be a more effective method for preserving heat-sensitive antibacterial compounds in plant materials. In contrast, hot decoction methods, which utilize heat, can degrade or inactivate these very compounds, leading to a less potent antibacterial extract. Furthermore, all concentrations of the macerated extracts showed significant antibacterial activity, with the 25% concentration being the most effective in both cases. Conversely, hot decoction exhibited a concentration-dependent effect, with only the higher concentrations (25% and 15%) showing significant antibacterial activity for madre de cacao and all concentrations being effective for hagonoy, though weaker overall. This result is supported by the study of Frenkel et al. in 2021, which stated that the greater the concentrations of an antimicrobial agent, the larger the bacterial concentration it can inhibit because, at higher concentrations, the antimicrobial agent contains more of the antibacterial compounds responsible for killing bacteria.

4. Conclusion

Hagonoy leaves exhibited stronger antibacterial activity compared to madre de cacao leaves based on the mean colony counts throughout all the samples in different concentrations and methods. This indicates that hagonoy leaves possess a higher natural capacity to eliminate bacterial growth. At higher concentrations of 25%, the extracts from both leaves demonstrated stronger antibacterial activity. This is evident in the consistently lower colony counts observed with increasing concentrations in both madre de cacao and hagonoy extracts. This indicates a dose-dependent effect, where higher concentrations exhibit stronger antibacterial activity. Moreover, extraction by maceration yielded considerably lower colony counts compared to hot decoction, according to the post hoc comparison. Thus, maceration was a more effective method for extracting antibacterial compounds from the plant material, as evidenced by the significantly lower bacterial growth observed.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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