J. BIOL. ENVIRON. SCI.,
2025, 19(55), 1-9

Original Research Article

Phytochemistry, Cytotoxicity, and Antibacterial Activity of Almaciga *(Agathis philippinensis)* Aqueous Resin Extract

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Received: 24.09.2023; Accepted: 28.03.2025; Published Online: 01.04.2025

ABSTRACT

This study investigated the phytochemistry, cytotoxicity, and antibacterial activity of almaciga (*Agathis philippinensis*) resin sourced from Governor Generoso, Davao Oriental, using water as the extracting solvent. Phytochemical screening employed various tests, including Borntrager's test for anthraquinones, a coumarin test, the Alkaline Reagent test for flavonoids, the Froth test for saponins, and the Salkowski test for terpenoids. The cytotoxic activity was assessed using the brine shrimp lethality test, while the antibacterial activity was evaluated through the disc diffusion method. The study found the presence of terpenoids and saponins in the aqueous extract of almaciga (*A. philippinensis*) resin. The cytotoxicity results indicated a high mortality rate of 85% at a concentration of 1000 µg/ml, with an LC_{50} value of 401.65 µg/ml, which is classified as toxic according to Meyer's Toxicity Index. Furthermore, the concentrated crude extract demonstrated significant inhibitory effects against *Staphylococcus aureus*, with an average zone of inhibition measuring 21.0 mm. These findings underscore the pharmacological potential of almaciga resin, though further investigation is encouraged to validate the results.

Keywords: *Agathis philippinensis*, almaciga, antibacterial activity, aqueous resin extract, cytotoxicity, *Staphylococcus aureus*

INTRODUCTION

Plants are a promising source of phytochemicals linked to health-promoting effects. Most of these are categorized as secondary metabolites with natural bioactive compounds that give certain physiological effect in the human body. These include alkaloids, glycosides, tannins, steroids, saponins, anthraquinones, coumarins, flavonoids, phenols, and others. These compounds have been described to possess a variety of biological activities such as antioxidant activity (Tayone *et al*. 2021; Naseer *et al*. 2023), anti-allergic and anti-inflammatory (Alavijeh *et al*. 2023; de Vera *et al*. 2022), antimicrobial, and anticancer activity (Haq *et al*. 2023; Devi *et al*. 2022; Zhao *et al*. 2023; Parvez *et al*. 2022).

The Philippines boasts a diverse richness of plant life, much of which is an important resource for multiple purposes and treatments for diseases. Of these, almaciga (*Agathis philippinensis*) is one of the prominent members of the ancient coniferous tree family native to the area (Halos and Principe n.d.). Almaciga resin, also known as 'Manila copal,' is not only locally used to make high-grade glossy varnishes and lacquers but is also used as a fumigant against mosquitoes (Lassak and Brophy 2008).

Past studies have described Davao Oriental and Palawan almaciga resins as varying in quality and market value (Razal *et al*. 2021). Nonetheless, these studies mostly compared solubility, acid value, saponification value, and the determination of functional groups and melting profiles. Further, Lassak and Brophy (2008) analyzed the steam-volatile oils of almaciga resins and identified the occurrence of monoterpenoids. All these notwithstanding, more studies on almaciga from Governor Generoso, Davao Oriental are necessary to further shed light on its distinctive characteristics and possible uses, especially in the health sector and sustainable utilization in local industries.

The objective of this research is to perform an initial screening of the phytochemical and bioactive compounds of Governor Generoso almaciga resin, Davao Oriental, to determine its biological activities. Although

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earlier studies have reported different chemical constituents and properties of almaciga resin, there is a wide gap in knowing its biological potential, especially regarding its cytotoxicity and antibacterial activity (Razal 2018). By determining and characterizing the secondary metabolites of the resin, this work aims to provide useful information about its pharmacological value that could lead to breakthroughs in drug discovery and development.

MATERIALS AND METHODS

Phytochemical screening of almaciga resin aqueous extracts

Sample Collection and Extraction

The collection of almaciga resin samples was done in Eco-Park, Upper Tibanban, Governor Generoso, Davao Oriental, (6° 36' 30" N, 126° 8' 1" E) (Figure 1).

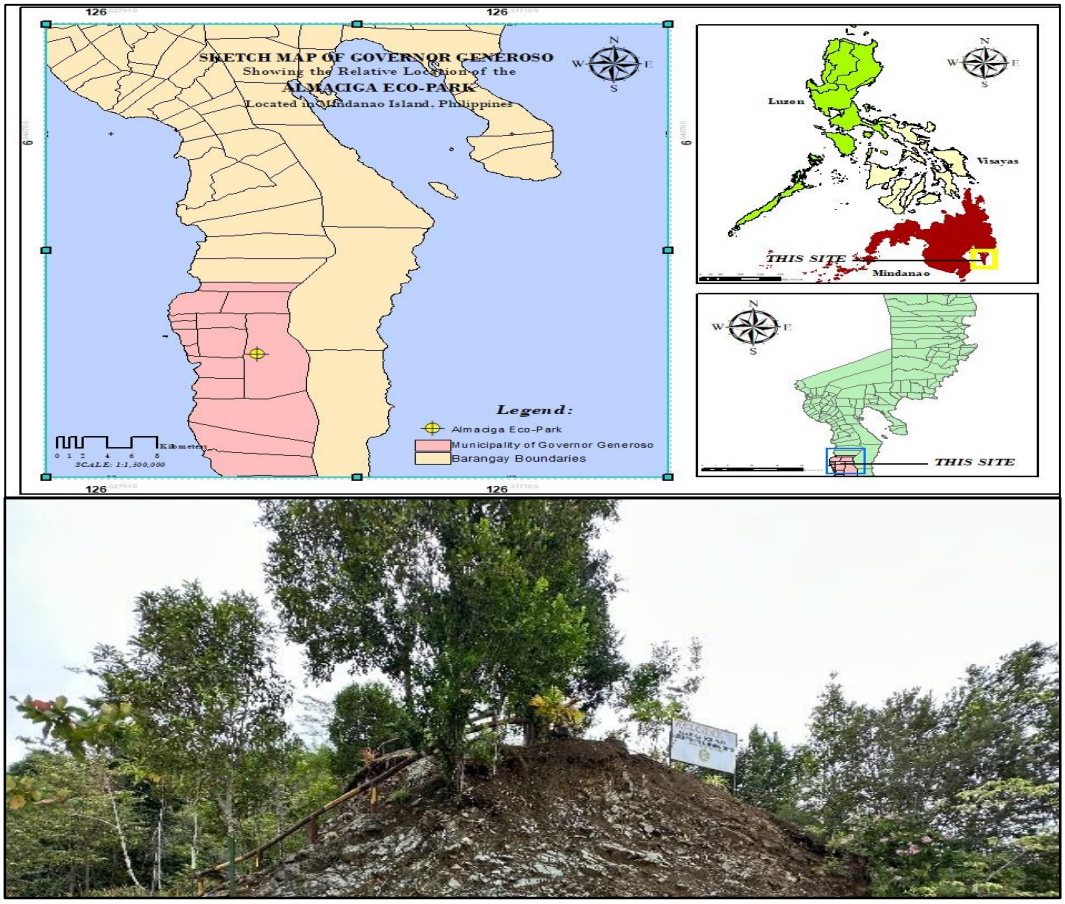

Figure 1. Sampling site for almaciga (*A. philippinensis*) resin.

A representative sample of one (1) kg dried resin (Figure 2) was randomly collected and placed in a sealed container. The resin was grounded manually and pulverized further using a mechanical blender. About 100 g homogenized resin was extracted with 200 mL of distilled water at room temperature for about 48 hours. The extract was filtered using Whatman filter paper no. 1. Afterward, it was concentrated using a water bath below 60°C to slowly evaporate the solvent. The concentrated extract was transferred to a sealed sterilized glass container and stored at 4°C until its analysis.

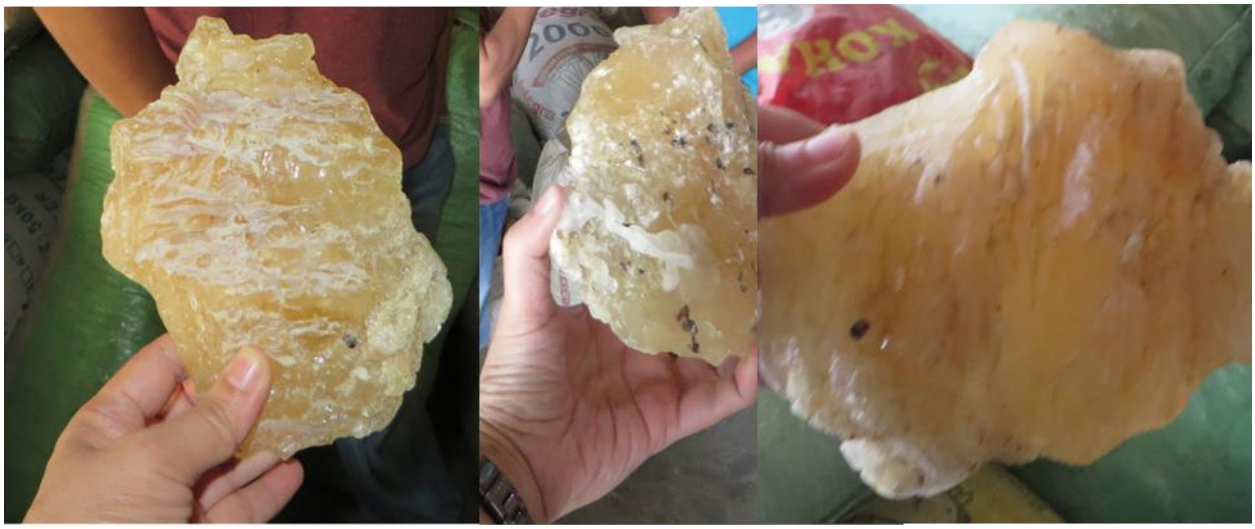

Figure 2. Dried almaciga resin collected from Governor Generoso, Davao Oriental.

Phytochemical Screening
The following tests were conducted with some modifications (Guevarra 2004; Tayone *et al*. 2019).

Test for Anthraquinone (Borntrager's Test)
A dried resin sample was added with 10 mL of distilled water. It was filtered, and the residue was discarded. The aqueous filtrate was extracted twice with benzene. The combined extract was divided into two fractions. One portion was treated with five (5) mL ammonia solution and was shaken. The development of red color in the lower ammoniacal layer implicated the presence of anthraquinone.

Test for Coumarins
One (1) gram of the test residue was moistened with water and placed into a test tube. The mouth of the test tube was covered with paper moistened with a dilute sodium hydroxide solution. The covered test tube was placed in a boiling water bath for 15 minutes. Next, the paper was removed and exposed to ultra-violet light. Yellowish-green fluorescence confirmed the presence of coumarins.

Test for Flavonoids (Alkaline Reagent Test)
A two (2) mL of 2.0% sodium hydroxide mixture was mixed with aqueous plant crude extract. The concentrated yellow color was produced, which became colorless when two drops of the dilute acid mixture were added. This result confirmed the presence of flavonoids.

Test for Saponin (Froth Test)
Three (3) mL of the aqueous solution of the extract was mixed with 10 mL of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 5 min. It was allowed to stand for 30 min and observed for honeycomb froth, indicating saponins' presence.

Test for Terpenoid (Salkowski Test)
A five (5) mL extract was mixed with two (2) mL chloroform and three (3) mL concentrated sulfuric acid to form layers. A reddish-brown coloration confirmed the presence of terpenoids.

Brine Shrimp Lethality Test
Hatching Brine Shrimp Eggs

About 3.8 grams of rock salt was weighed using an analytical balance and dissolved in 100 mL of distilled water. This was poured into a 22 cm x 32 cm rectangular jar. The rectangular jar was composed of two unequal compartments divided by a plastic divider with several 2mm holes. One (1) gram per liter of brine shrimp eggs was sprinkled at the top water level of the larger compartment and was covered to avoid light exposure. On the other hand, the smaller compartment was left open and illuminated with a light source (60-100-Watt bulb) placed a few inches away. After 48 hours, the eggs hatched, and the nauplii were observed in the small illuminated compartment. The food for the nauplii was prepared by dissolving 3 mg of yeast in 3 mL of artificial seawater (Tayone *et al*. 2019).

Toxicity Testing

Using a 10 ml Pasteur pipette, 20 nauplii were transferred into each respective test tube labeled 0 µg/mL, 10 µg/mL, 100 µg/mL, and 1000 µg/mL containing 4.5 mL of brine solution and 0.5 mL of dried crude extract. Methanol and distilled water were used as the positive and negative control, respectively. Furthermore, the number of dead nauplii was counted after 24 hours and was calculated by percentage (Sarah *et al*. 2017; Tayone *et al*. 2019). The mortality of this bioassay was defined as the absence of forward motion during 30 seconds of observation. For each tube, the number of dead and the number of live nauplii were counted, and the % of death was determined (Meyer *et al*. 1982).

Kirby-Bauer Disc Diffusion Assay

Bacterial Isolation of Staphylococcus aureus Culture

Three sterile cotton swabs were used to obtain samples from the skin of three selected human individuals. Since approximately 30% of humans carry *S. aureus* on their skin, this served as the bacterial source (Turner *et al*. 2019). The collected swabs were immediately placed into separate sterile test tubes containing 5 mL of nutrient broth. The tubes were then incubated at 37°C for 24 hours to promote bacterial growth. Following incubation, the petri dishes containing sterile Mannitol Salt Agar (MSA) were inoculated by dipping the inoculation loop in the bacterial source and incubated at 37°C for 24-48 hours (Aryal 2017).

Culture Media Preparation & Inoculation and Disc Diffusion Method

The Mueller-Hinton Agar (MHA, Himedia Laboratories) solution was 121°C autoclaved for 15 minutes for sterilization. The medium was cooled to around 45-50°C after autoclaving and poured into petri dishes in a sterile environment. The plates were stored at 2-8°C after solidification in sealed plastic bags to avoid moisture loss (CLSI 2020).

For inoculation, bacterial colony on MSA was lightly touched with sterilized inoculating loop and inoculated to a Mueller-Hinton Agar (MHA) plate by streaking it in three directions across the surface of the plate, with the plate rotated about 60° between streaks for uniform distribution of the inoculum. The inoculated plates were dried for 3-5 minutes at room temperature with the lids closed before applying the test disks (CLSI 2020; Hudzicki 2009).

A 6 mm diameter filter paper disks were sterilized through dry heat or autoclaving. The disks were soaked in the aqueous crude almaciga (*A. philippinensis*) resin extract to achieve maximum saturation. The extract-soaked disks were placed on the surface of the inoculated MHA plates using sterile forceps with even spacing and tight contact with the agar. A 250 mg ciprofloxacin disk was used as positive control, and a distilled water-soaked disk used as a negative control. The plates were inverted and incubated at 35±2°C for 16-18 hours. The diameters of the zones of inhibition that formed around each disk after incubation were measured in millimeters using a ruler or caliper. Tests were performed in triplicate to assess reproducibility (CLSI 2020; Hudzicki 2009). The bacterial culture was preserved on nutrient agar plates and kept at 4°C. Following experiment, the culture and contaminated items were discarded by autoclaving. Agar plates, broth cultures, and any pipettes or loops used were put in an autoclavable biohazard bag and sterilized at 121°C, 15 psi for 15-20 minutes. The autoclaved materials were then disposed of safely as normal waste.

Statistical Analysis

All experimental measurements were carried out in triplicate and were expressed as the mean ± standard deviation. LC_{50} value was determined through linear regression curve analysis. Toxicity criteria were defined using Meyers Toxicity Index. A One-Way Analysis of Variance (ANOVA) for antibacterial activity was used to determine the significant difference between crude extract and positive control. P value < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening

The investigation of the secondary metabolites present within the aqueous extract of almaciga (*A. philippinensis*) resin as presented in Table 1 reveals valuable insights into the plant's phytochemical profile and its potential applications in medicine and pharmacology.

Table 1. Secondary metabolites screening of almaciga aqueous resin extract

Secondary Metabolites	Test	Result	Remarks
Anthraquinone	Borntrager's Test	No color change	-
Coumarins	Coumarins' Test	No color change	-
Flavonoids	Alkaline Reagent Test	No color change	-
Saponin	Froth Test	Honeycomb formation	+
Terpenoids	Salkowski Test	Reddish-brown formation	+

Legend: absence (-); presence (+)
Each test was done in three replicates.

The Froth Test established the presence of saponins, indicated by honeycombing. Saponins are widely recognized glycosides showing a diverse array of biological activities such as antimicrobial, antifungal, and anticancer activities (Okwu and Ndu 2021). Their surfactant properties increase membrane permeability, enabling increased absorption of other therapeutic agents. The occurrence of saponins in almaciga resin may suggest that it is used as a traditional medicine, where it can induce health benefits through these bioactivities (Sharma *et al*. 2021).

The Salkowski Test positive result is a sign of the presence of terpenoids, which are a group of natural organic compounds with a strong odor and biological activity. Monoterpenoids like limonene and terpineol, common in almaciga (*A. philippinensis*) resin, exhibit antibacterial, antifungal, and antiviral activities (Ben Miri 2025). Terpenoids are also significant in traditional medicine uses, as they possess anti-inflammatory and analgesic activities (Duke 2018). The results were consistent with earlier findings that terpenoids are the key components responsible for the pharmacological activity of resinous plant extracts (Lassak and Brophy 2008).

On the other hand, the screening indicated the lack of anthraquinones, coumarins, and flavonoids, a significant finding, particularly when compared to other related species. For example, in the case of the *Araucaria columnaris*, flavonoids and coumarins were detected, which might point to a comparative absence of some bioactive compounds in almaciga (*A. philippinensis*) (Jadav and Gowda 2017). Flavonoids and coumarins have antioxidant, anti-inflammatory, and antimicrobial activities, so their absence might point to the fact that almaciga (*A. philippinensis*) might lack some of the same health benefits of some of its family members. Yet it is important to mention that this does not dismiss the possible effectiveness of almaciga (*A. philippinensis*) resin when viewed in terms of its saponin and terpenoid content.

The discovery of the existence of terpenoids in almaciga (*A. philippinensis*) resin rings true with *Agathis robusta* studies wherein a dominance of oxygenated monoterpenoids was also found (Verma *et al*. 2016). Further, the findings from Tayone *et al*. (2020) to clarify the role of terpenoids in other plant extracts indicate that plant parts such as leaves, bark, and resin may provide different profiles of bioactive compounds. The diversity highlights the importance of detailed phytochemical studies in multiple parts and plants to identify useful

therapeutic agents. Previous studies have shown that terpenoids and saponins not only exhibit inherent biological activities but can also increase the bioavailability and activity of other compounds when employed in formulations or combined therapy (Das *et al*. 2020).

Cytotoxic Activity

Table 2 shows the mortality rate of nauplii with different concentrations. The highest mortality rate of 85% was achieved at 1000 µg/ml and the lowest (25%) at 10 µg/ml of Almaciga (*A. philippinensis*) aqueous resin crude extract, while the positive control, methanol showed a 100% mortality rate. Using linear regression analysis (y = 0.0604x + 25.74, r = 0.946), the calculated LC_{50} was 401.65 µg/ml. If the LC_{50} value is below 1000 µg/ml, the substance is considered toxic, whereas a value above 1000 µg/ml indicates it is not toxic (Meyer *et al*. 1982). In this instance, the LC_{50} of 401.65 µg/ml was sufficient to result in the death of fifty percent (50%) of the nauplii.

Table 2. The nauplii mortality rate at different almaciga (*A. philippinensis*) resin aqueous extracts concentrations

Crude Extract Concentration, µg/ml	% Mortality
0	16.7
10	25
100	43.3
1000	85
Positive Control	100

Each test was done in three replicates.

Brine shrimp lethality bioassay is a well-known initial screening tool employed to evaluate the toxicity of different extracts and compounds. This bioassay is best applied in early research for the determination of a compound's possible toxicity and safety profile (Sukkum *et al*. 2017). The very high LC_{50} value of 401.65 µg/mL reveals that although the almaciga (*A. philippinensis*) resin exhibits significant cytotoxicity, more work should be carried out to define the exact compounds accountable for this action.

The earlier work has reported the occurrence of diverse phytochemicals in plant resins, including saponins and terpenoids, that have been reported to possess cytotoxic activity (Bohm *et al*. 2018; Goh *et al*. 2020). Saponins, for example, are known to interfere with cell membranes, causing cell lysis and death, whereas terpenoids can modulate cell signaling pathways that regulate cell survival and apoptosis (Santos *et al*. 2024). The cytotoxicity seen in almaciga (*A. philippinensis*) resin is similar to that reported in other plant extracts with medicinal value. For instance, extracts of plants such as *Euphorbia hirta* and *Cinnamomum verum* have also shown cytotoxicity in parallel assays, highlighting the promise of plant compounds as anticancer drug leads (Tayone *et al*. 2020; El-Hallouty *et al*. 2020). These studies underscore the significance of investigating and describing the bioactive constituents in almaciga (*A. philippinensis*) resin, which may result in the discovery of new drugs.

Although the present results show that almaciga (*A. philippinensis*) resin possesses significant cytotoxic activity, it is imperative that future research continues to explore the mechanisms of action. The isolation of active compounds and the assessment of the individual cytotoxicities of such compounds may shed light on their applicability in terms of therapeutic potential. Further, research needs to be conducted to explore the possible synergistic activities resulting from combining almaciga (*A. philippinensis*) resin extracts with other anticancer or antimicrobial drugs. This may increase the overall effectiveness and decrease the dose required for therapeutic outcomes, possibly limiting side effects (Wang *et al*. 2020).

Antibacterial Activity

The findings from the Kirby-Bauer disc diffusion test reveal that the aqueous extract of almaciga (*A. philippinensis*) resin exhibits an average inhibition zone of 21.0 ± 1.41 mm, indicating a noteworthy level of antibacterial activity. In comparison, the control antibiotic Ciprofloxacin shows a larger inhibition zone of 40.0 mm ± 1.63 (p = 0.016), suggesting that while almaciga resin demonstrates a weaker antibacterial effect, it could still serve as a viable natural alternative to synthetic antibiotics. This antibacterial property of the resin points to

its potential application in conventional medicine and the prospect of discovering new antimicrobial agents (Kumar *et al*. 2020).

The antibacterial efficacy of almaciga (*A. philippinensis*) resin is likely linked to its phytochemical composition, particularly limonene. Limonene is known for its ability to disrupt bacterial cell membranes and inhibit metabolic functions (Han *et al*. 2021). Recent studies have also identified other compounds, such as terpenes and flavonoids, commonly found in tree resins, which may exhibit synergistic effects against various pathogens and enhance overall antimicrobial activity (Rasul *et al*. 2020).

Given the rising issue of antibiotic resistance, there is increasing interest in exploring plant-derived antimicrobial agents. The World Health Organization (WHO) has emphasized the importance of finding alternative treatments for resistant bacterial strains (WHO 2021). Evidence from research on plant extracts, including those from *Agathis dammara*, supports the presence of similar active components that could be effective against resistant strains, highlighting their potential for medicinal applications (Sadiq *et al*. 2022). The demonstrated susceptibility of *S. aureus* to other natural products suggests that further exploration of almaciga (*A. philippinensis*) resin could lead to the development of effective therapeutic options or new formulations for antibiotic development (Wang *et al*. 2020; Lassak and Brophy 2008).

While the research underscores the promising antibacterial properties of almaciga (*A. philippinensis*) resin, there remains a need for further investigation. A detailed exploration of the structure-activity relationship of each phytochemical in the resin could provide insights into their distinct contributions to antimicrobial activity. The initial findings suggest that almaciga (*A. philippinensis*) resin is a strong candidate for further research into natural treatments for bacterial infections, including those that are resistant to conventional therapies. As the challenge of antibiotic resistance escalates globally, harnessing natural resources like almaciga resin could enhance therapeutic strategies and promote sustainable healthcare practices.

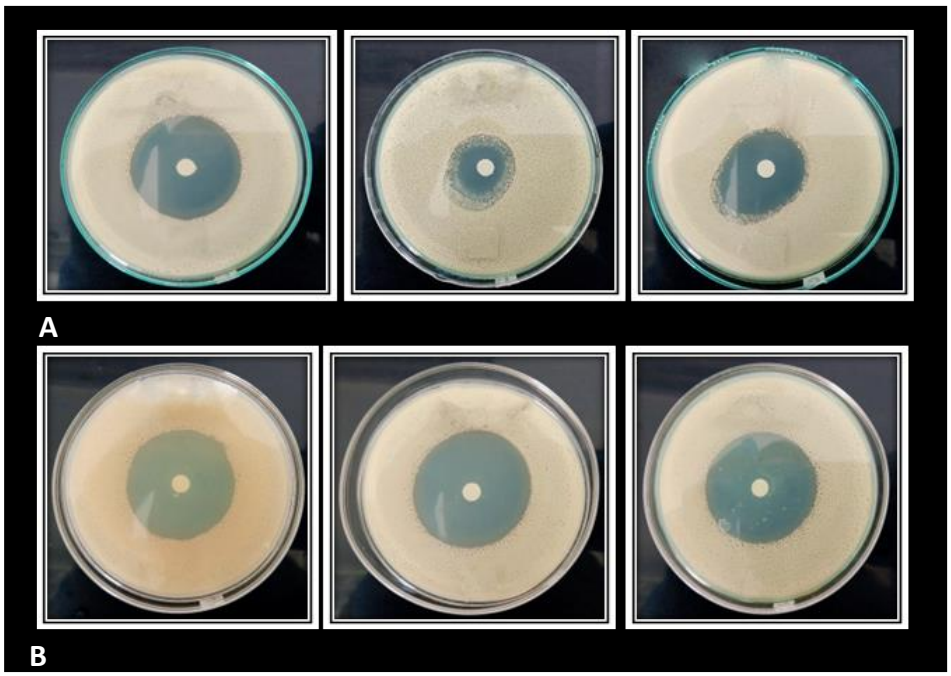

Figure 3. Sample plates for the zone of inhibition of (A) crude concentrated almaciga resin aqueous extract and (B) Ciprofloxacin as the positive control. Each test was done in three replicates.

CONCLUSIONS

The research identified the occurrence of terpenoids and saponins in almaciga (*A. philippinensis*) resin crude aqueous extract. The cytotoxicity evaluated using the brine shrimp lethality bioassay revealed a high percentage of mortality at 85%, with an LC_{50} value of 401.65 µg/mL. These data imply that almaciga (*A. philippinensis*) resin

has a significant amount of cytotoxic compounds that could be well-extracted with distilled water. Moreover, the crude concentrated extract also exhibited antibacterial activity against *S. aureus* with an inhibition zone of 21 mm. Therefore, extracts from almaciga (*A. philippinensis*) resin show potential as a natural source of antibacterial agents effective against *S. aureus*. Future studies must be extended to screen other pathogenic bacteria, employing different parts of almaciga (*A. philippinensis*) and different extraction solvents in order to realize its full antimicrobial potential.

ACKNOWLEDGEMENTS

The authors are grateful to the Research, Innovation, and Extension (RIE) division of DOrSU for the source of funds.

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