

Antimicrobial and Cytotoxic Potential of *Barringtonia asiatica* Seed Extracts: A Multi-Assay Approach

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Abstract. This study aimed to evaluate the phytochemical constituents, antimicrobial activities, and cytotoxicity of *Barringtonia asiatica* seed extract. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, and flavonoids in both aqueous and ethanol extracts, with terpenoids and steroids present only in the ethanol extract. Antibacterial assays showed that both extracts exhibited resistance against *Escherichia coli* and *Staphylococcus aureus*, with streptomycin being the most effective treatment. Antifungal assays demonstrated that commercial fungicides were the most effective against *Fusarium verticillioides* and *Aspergillus niger*, followed by *B. asiatica* ethanol and aqueous extracts. Cytotoxicity tests using brine shrimp lethality assay revealed that *B. asiatica* ethanol and aqueous extracts were highly toxic, with LC₅₀ values of 24.55 ppm and 181.97 ppm, respectively. The presence of terpenoids, cardiac glycosides, and saponins may contribute to their cytotoxic effects. These findings suggest that *B. asiatica* seed extracts contain phytochemicals with potential antimicrobial and cytotoxic properties, warranting further drug discovery and development research.

Keywords: Antimicrobial activity; *Barringtonia asiatica*; Cytotoxicity; Phytochemical analysis; Traditional medicine.

1.0 Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components with therapeutic value (Nostro *et al.*, 2000). According to the World Health Organization (WHO), in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs. In the Philippines, the importance of the country's diverse medicinal plants lies in their chemotherapeutic value in traditional healthcare and their potential as sources of new chemical entities for drug discovery. Although the country boasts of its remarkable biodiversity and rich cultural traditions of plant use, scientific understanding of medicinal plants remains largely unexplored, and pharmacological investigation of Philippine flora has recently gained momentum (Basco *et al.*, 2016).

The problem of microbial resistance is growing, and prospects for using antimicrobial drugs in the future are still uncertain. Immediate action must be taken to reduce this problem, aiming for efficient antimicrobial drugs. Using plant extracts and phytochemicals with known antimicrobial properties is of great significance in treatments. Several studies have been conducted in different countries to prove their efficiency in recent years. Many plants have been used because of their antimicrobial properties due to compounds synthesized in the secondary

metabolism of the plant known by their active substances, such as phenolic compounds, which are part of essential oils and tannins.

Currently, data on the antimicrobial properties of several plants have been measured empirically concurrently with the increasing number of reports on the antimicrobial resistance of pathogenic microorganisms. Products derived from plants may potentially control microbial growth in various situations and cases of disease treatment. Numerous studies have aimed to describe the chemical composition of plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or in combination with conventional antimicrobials (Jacob et al., 2016). *Barringtonia asiatica*, commonly known as the fish poisoning tree, is a medium-sized tree found in coastal areas, particularly on Car Nicobar Island (Ravikumar et al., 2015). It is known for its ichthyotoxic properties and its seeds are traditionally used for fishing in shallow waters during calm seasons (Murdiansyah et al., 2019; Ravikumar et al., 2015). This tree has multiple traditional uses beyond fishing, including medicinal applications. Various parts of *B. asiatica* have been utilized in herbal medicine. The leaves are used for therapeutic purposes such as treating fractures and wounds, de-worming, and pain relief (Ravikumar et al., 2015). Antimicrobial resistance (AMR) is a critical global health challenge threatening modern medicine and potentially cause millions of deaths annually by 2050 (Charlebois et al., 2020). The emergence and spread of AMR are driven by various factors, including inappropriate antimicrobial prescription in healthcare, misuse of antimicrobials in livestock, and propagation of antibiotic-resistance genes in the environment (Ogawa, 2017).

Recent studies have revealed that microbial communication systems, such as quorum sensing and pathogen-host communication mechanisms, play a significant role in the formation and regulation of AMR (Huang et al., 2020). Additionally, non-genetic heterogeneity resulting from fluctuations in gene expression can lead to the fractional killing of microbial populations, causing drug therapies to fail and enhancing the probability of acquiring genetic drug resistance mutations (Charlebois et al., 2020). To effectively combat AMR, a One Health approach is crucial, integrating knowledge of antimicrobial resistance genes (ARGs) across different microbial reservoirs in human, animal, and environmental health (Despotovic et al., 2023). The priorities for action include improving surveillance, enhancing stewardship and infection prevention, promoting basic and applied research, and coordinating global policies (Ogawa et al., 2018). Furthermore, understanding the molecular genetics and evolutionary mechanisms of drug-resistant bacteria is essential to develop fundamental solutions to overcome AMR (He, 2022). Innovative strategies, such as harnessing heat shock protein 90 (Hsp90) and exploring the potential of microbial extracellular vesicles, offer promising avenues for treating drug-resistant infections and developing novel antimicrobial approaches (Cowen, 2008; Jiang et al., 2024).

Existing research on *Bacopa asiatica* has revealed several gaps in our understanding of its phytochemical composition and antimicrobial and cytotoxic properties. While some studies have identified alkaloids, saponins, glycosides, and tannins in *B. asiatica* (Aparna et al., 2015), there is a lack of comprehensive phytochemical profiling using advanced analytical techniques, such as LC-MS and GC-MS. These methods have been successfully employed for other plants, such as *Centella asiatica* (Ondeko et al., 2020), and could provide a more detailed understanding of the chemical constituents of *B. asiatica*. However, the antimicrobial properties of *B. asiatica* have not been thoroughly investigated. Unlike studies on other plants, such as *Senecio asirensis* (Kamaly et al., 2024) and *Toddalia asiatica* (Hu et al., 2013), which have evaluated antimicrobial activity against a range of microorganisms, similar comprehensive studies on *B. asiatica* are lacking. There is a need for research that assesses its efficacy against Gram-positive and Gram-negative bacteria as well as against fungi. However, the cytotoxic properties of *B. asiatica* extracts have not been extensively studied. Research on other plants, such as *Senecio asirensis* (Kamaly et al., 2024) and *Toddalia asiatica* (Hu et al., 2013), has evaluated their cytotoxic activities against multiple cancer cell lines. Further studies are needed to explore the potential anticancer properties of *B. asiatica*. To address these gaps, a multiassay approach is required. This should include comprehensive phytochemical analysis using advanced techniques, antimicrobial testing against various pathogens, and cytotoxicity assays in various cancer cell lines. Additionally, as performed for *Senecio asirensis* (Kamaly et al., 2024), molecular docking studies could provide insights into the mechanisms of action of the bioactive compounds in *B. asiatica*.

The use of medicinal plants for primary health care is of substantial help to developing countries such as the Philippines in meeting their drug requirements. However, medicinal plant use has been based primarily on empirical grounds. There is a need for the scientific validation of such empirical knowledge (Philippine Council

for Health Research and Development, 1991). Plant extracts provide unlimited opportunities for new drug development due to the unmatched availability of chemical diversity, which plays an imperative role in curbing new and re-emerging infectious diseases (Vaghasiya *et al.*, 2009). Therefore, this study evaluated the biofunctional activities of *B. asiatica* seed extract. The utilization of this plant provides an alternative source of antibiotics and cytotoxic drug agents that can promote less expensive, more effective, and novel ways of obtaining bioactive substances. Research on antimicrobial agents has potential applications in pharmaceuticals, agriculture, and food safety. In pharmaceuticals, these agents could lead to developing more effective antibiotics to combat drug-resistant pathogens, thereby addressing a critical global health concern. In agriculture, antimicrobial agents can improve crop protection products, reduce crop losses due to bacterial and fungal infections, and potentially increase food production. For food safety, these agents can be incorporated into food packaging materials or used as sanitizers to prevent foodborne illnesses, extend the shelf life of perishable goods, and ensure safer food supply chains. Developing novel antimicrobial agents could also lead to more environmentally friendly and sustainable practices in all three sectors, reducing reliance on traditional chemical-based solutions and minimizing potential negative impacts on human health and ecosystems.

2.0 Methodology

2.1 Collection and Preparation of *B. asiatica*

The fruit of *B. asiatica* was obtained from the municipal beach of Pagudpod, Ilocos Norte. The collected samples were air-dried at room temperature for about seven (7) to ten (10) days prior to the separation of seeds and pulp. Dried seeds were sliced thinly, pulverized using a grinder after drying, and kept in a sealed container with proper labels.

Ethanol Extraction of B. asiatica

The seed samples were extracted using 95% lab-grade ethanol. Pulverized samples of *B. asiatica* (500 g) were dispensed in a clean, sterile bottle containing 1000 ml of 95% laboratory-grade ethanol and kept for 48 h before filtration. The extracts were filtered using Whatman filter paper no.1 to separate the filtrate. The filtrates were refluxed for four (4) hours at 70-80°C until a sticky residue was obtained, which was stored for phytochemical, antimicrobial, and cytotoxicity analyses.

Aqueous Extraction of B. asiatica

Pulverized samples of *B. asiatica* (50 g) were dispensed in a clean, sterile bottle containing 1000 ml of sterile distilled water. The suspension was pasteurized for two hours before filtration using Whatman filter paper no.1. It was stored in a clean, sterile bottle and refrigerated before use.

2.2 Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was performed using the standard procedures of Sofowara (1993), as cited and modified by Jacob and David (2016). Results were determined based on reaction and color intensity and interpreted as + if the chemical is present in traceable amounts, ++ if the chemical is present in appreciable amounts, and – if the chemical is absent. The phytochemicals tested were as follows:

Test for Alkaloids

The extracts were dissolved in dilute hydrochloric acid and filtered. The filtrate was then treated with Wagner's reagent. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

Test for Saponins

Ten (10) ml of the filtered ethanolic extract was mixed with five (5) ml of distilled water in a test tube and shaken vigorously to obtain a stable, persistent froth. Three drops of olive oil were added to the test tube to form the emulsion, indicating the presence of saponins.

Test for Flavonoids

Two (2) to three (3) drops of 1% NH₃ solution were added to two (2) ml of the extracts in a tube. Yellow indicates the presence of flavonoids.

Test for Cardiac Glycosides

Approximately one (1) ml of concentrated H_2SO_4 was prepared in a test tube. Then, five (5) ml of the ethanolic extract of the samples was mixed with two (2) ml of glacial acetic acid ($\text{CH}_3\text{CO}_2\text{H}$) containing one (1) drop of FeCl_3 . The mixture was carefully added with one (1) ml of concentrated hydrogen sulfate prepared so that the H_2SO_4 would be underneath the mixture. The test tube was observed to contain a brown ring, which indicates the presence of cardiac glycosides.

Test for Terpenoids

Using Salkowski's test, 0.2 ml of the extracts was dissolved in 2) ml of chloroform. Concentrated H_2SO_4 was then carefully added to form the lower layer. The reddish-brown color at the interphase indicates the deoxy sugar characteristics of cardenolides.

Test for Tannins

0.5 ml of the extract was boiled in 20 ml of distilled water in a test tube and filtered. 0.1% FeCl_3 was added to the filtrate samples, and brownish-to-green or blue-to-black coloration was observed, indicating the presence of tannins. Green coloration indicates the presence of gallotannins, whereas brown coloration indicates the presence of pseudo-tannins.

Test for Steroids

0.5 ml of the extract was mixed with two (2) ml of acetic anhydride, followed by two (2) ml of sulfuric acid. The samples' change in color from violet to blue or green indicated the presence of steroids.

2.3 Antibacterial Assay

The antibacterial properties of ethanol and aqueous extracts of *B. asiatica* were assessed using the procedure described by Bauer *et al.* (1996). Following the aseptic technique, the assay was performed inside a laminar flow chamber containing a high-efficiency particulate air (HEPA) filter to avoid contaminants that may affect the process.

2.4 Preparation of Mueller Hinton Agar (MHA)

Approximately 38 g of Mueller Hinton agar (MHA) was dispensed in a clean, sterile Erlenmeyer flask with one (1) liter of distilled water. The mixture was heated until homogenized. It was then sterilized in an autoclave for 15 minutes at $121^\circ\text{C}/15$ psi. After sterilization, the samples were allowed to cool and plated on sterilized Petri dishes.

2.5 Disc Diffusion Assay

The antibacterial assay was performed using the disc diffusion method of Bauer *et al.* (1996). This involved the use of filter paper discs as carriers of antimicrobial agents. Whatman filter paper no.1 was cut into discs with six (6) mm diameter and impregnated with distilled water, streptomycin, 95% Ethanol, *B. asiatica* ethanol extracts, and *B. asiatica* aqueous extracts. *E. coli* and *S. aureus* cultures were then spread thoroughly onto MHA plates using sterile cotton swabs. The impregnated discs were placed equidistant on the surface of the medium. The plates were incubated at 37°C and turned upside down to prevent contamination. The zone of inhibition of each paper disc was observed and recorded every eight (8) hours within a 24-hour incubation period. The zone of inhibition was measured using a calibrated digital Vernier caliper.

2.6 Antifungal Assay

Preparation and Revival of the Test Organism

Approximately 250 g of potato was boiled in ((1) liter of distilled water until it became tender. The resulting mixture was strained and reconstituted in water until a volume (1) liter was obtained. Ten (10) grams of sugar were added with 20 g agar until a homogenized mixture was obtained. The resulting mixture was dispensed into a clean Erlenmeyer flask, plugged with cotton, and covered with clean paper to absorb moisture. It was then sterilized using an autoclave at $121^\circ\text{C}/15$ psi for 15 min. After sterilization, they were allowed to cool and plated on Petri dishes, which were used to revive the pure culture.

Cultures of *F. verticillioides* and *A. niger* were obtained from the fungal collection at the Microbiology and Bio-Industry Laboratory of Biological Sciences, Isabela State, University, Echague, Isabela. Cultured cells were

aseptically transferred onto potato dextrose agar (PDA) supplemented with 1 g/ml streptomycin to prevent bacterial interactions. These were allowed to grow for five–seven days until fully ramified plates were obtained.

Treatment Preparation and Inoculation of Test Organism

Two (2) ml of the prepared *B. asiatica* ethanol extract were poured onto sterile Petri plates. Approximately 15-20 ml of sterilized PDA was added and swirled clockwise to cool and solidify. Each plate containing the mixtures was inoculated with 10 mm of the fungal disc for the revival of pure cultures of *F. verticillioides* and *A. niger*. These were incubated at room temperature (28-32°C), and growth was measured using calibrated Vernier calipers every 24 h for seven (7) days. Aseptic techniques were used to prevent contamination by pathogens.

2.7 Cytotoxicity Test using Brine Shrimp Lethality Assay

Brine shrimp eggs were obtained from the Bureau of Fisheries and Aquatic Resources, San Mateo, Isabela, the Philippines. They were hatched in prepared artificial seawater as McLaughlin and Rogers (1998) described. Brine shrimp eggs were added to artificial seawater, where 30 g of salt was diluted per liter of water in a glass chamber and kept under constant aeration and illumination. After 48 h of incubation, brine shrimp nauplii were attracted to one side of the vessel using a light source and collected using a pipette.

2.8 Cytotoxic Lethality Assay

The cytotoxic properties of the *B. asiatica* extract were monitored using the brine shrimp lethality test described by McLaughlin and Roger (1998) with modifications. Nine newly hatched nauplii were placed in an ELISA well with three (3) nauplii per well. Triplicates were performed for each treatment concentration. The setup containing extracts of the seed extract at different concentrations was left uncovered under a lamp. The number of dead nauplii was monitored 6th, 12th, 18th, and 24th hours. Observations of live and dead nauplii were compared to the standard. Live brine shrimps were observed to be actively squirming and constantly moving, whereas dead nauplii were observed to be non-motile and floating. The number of dead nauplii was counted using a stereomicroscope. Percent mortality was documented, and the LC₅₀ was determined through Probit Analysis.

2.9 Experimental Design and Layout

Antibacterial, antifungal, and cytotoxic assays were performed separately. Each test was carried out using a Completely Randomized Design (CRD) with three (3) replicates. The treatments used in the antibacterial assay (Figure 1) were as follows:

- Treatment 1 – Distilled water (+ control)
- Treatment 2 – Streptomycin (- control)
- Treatment 3 – 95% Ethanol
- Treatment 4 – *B. asiatica* ethanol extracts
- Treatment 5 – *B. asiatica* aqueous extracts

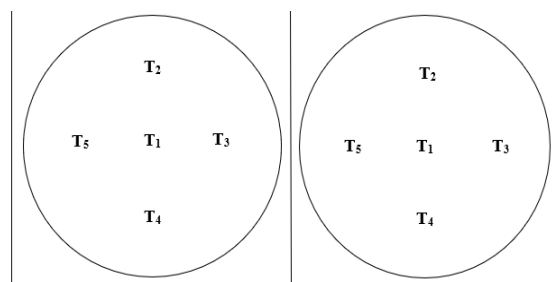


Figure 1. Experimental design for antibacterial assay

These treatments were used for both *E. coli* and *S. aureus*.

For the antifungal assay, the ethanol and aqueous extracts of *B. asiatica* were treated as follows:

- Treatment 1 – Distilled water (- control)
- Treatment 2 – 95% lab-grade Ethanol
- Treatment 3 – Commercial Fungicide (+ control)
- Treatment 4 – *B. asiatica* ethanol extract
- Treatment 5 – *B. asiatica* aqueous extract

T ₁ R ₁	T ₂ R ₁	T ₃ R ₁	T ₄ R ₁	T ₅ R ₁
T ₁ R ₂	T ₂ R ₂	T ₃ R ₂	T ₄ R ₂	T ₅ R ₂
T ₁ R ₃	T ₂ R ₃	T ₃ R ₃	T ₄ R ₃	T ₅ R ₃

Figure 2. Experimental design for antifungal assay

The same treatments were used for *F. verticillioides* and *A. niger*.

For the cytotoxicity (Figure 3), the treatment used for the ethanol and aqueous extracts of *B. asiatica* was:

- Treatment 1 – 1000 ppm concentration
- Treatment 2 – 500 ppm concentration
- Treatment 3 – 250 ppm concentration;
- Treatment 4 – 125 ppm concentration;
- Treatment 5 – Saltwater (- control)
- Treatment 6 – Ethanol (+ control)

T ₁ R ₁	T ₂ R ₁	T ₃ R ₁	T ₄ R ₁	T ₅ R ₁	T ₆ R ₁
T ₁ R ₂	T ₂ R ₂	T ₃ R ₂	T ₄ R ₂	T ₅ R ₂	T ₆ R ₂
T ₁ R ₃	T ₂ R ₃	T ₃ R ₃	T ₄ R ₃	T ₅ R ₃	T ₆ R ₃

Figure 3. Experimental design for cytotoxicity assay

All recorded data were treated statistically using one-way analysis of variance (ANOVA), and means were compared using LSD at $p < 0.05$.

2.10 Data Gathered

The following data were gathered during the study period:

- a. Phytochemical Properties of *B. asiatica*
- b. Zones of inhibition of *B. asiatica* ethanol and aqueous extracts against *E. coli* and *S. aureus*;

- c. Mycelial diameter of *F. verticillioides* and *A. niger*; and
- d. Mortality rate and LC₅₀ of the fungal isolates in brine shrimp.

2.11 Ethical Considerations

The ethical considerations of this study, conducted at Isabela State University, were rigorously upheld to ensure responsible research practices. The study adhered to the ethical guidelines set by the university's Institutional Review Board, focusing on the welfare of all participants and environmental stewardship. Special attention was given to sustainable sourcing practices for *Barringtonia asiatica* seeds to avoid negative ecological impacts and to ensure compliance with local biodiversity conservation laws. Additionally, the study was designed to minimize harm and prioritize safety throughout the laboratory procedures, ensuring that all experiments involving cytotoxic and antimicrobial assays were conducted following stringent safety protocols. Informed consent was obtained for any human cell line usage while maintaining strict data confidentiality and adequately disposing of biohazardous materials to prevent environmental contamination. These ethical practices underline the commitment of Isabela State University to uphold its integrity and responsibility in scientific research.

3.0 Results and Discussion

3.1 Phytochemical Constituent of the *B. asiatica*

Table 1 presents the phytochemical constituents of ethanol and aqueous extracts of *B. asiatica*. Seven (7) phytochemical constituents were screened: alkaloids, saponins, tannins, cardiac glycosides, flavonoids, steroids, and terpenoids.

Table 1. Phytochemical composition of *B. asiatica* using aqueous and ethanol extract

Phytochemicals	Aqueous extract	Ethanol extract
Alkaloids	+	+
Saponins	++	++
Tannins	+	++
Cardiac Glycosides	+	+
Flavonoids	+	+
Steroids	-	+
Terpenoids	-	+

Legend: (+) present in traceable amount, (++) present in appreciable amount, (-) absent

Of the seven (7) phytochemical constituents screened, the *B. asiatica* aqueous extract exhibited five (5) phytochemicals: alkaloids, saponins, tannins, cardiac glycosides, and flavonoids. In contrast, the ethanol extract of *B. asiatica* exuded seven (7) phytochemicals, namely: alkaloids, saponins, tannins, glycosides, flavonoids, and terpenoids. However, terpenoids and steroids were absent in the aqueous extracts. Alkaloids, cardiac glycosides, and flavonoids were observed in traceable amounts, whereas saponins were present in appreciable quantities in both extracts. However, phytochemical constituents, such as terpenoids and steroids, were present in the ethanol extract but absent in aqueous extracts. Tannins, however, were found to be appreciable in the ethanolic extract but in a significant amount in the aqueous extracts.

The results showed that The ethanol extracts contained more phytochemicals than the aqueous extracts. This may be because of the higher solubility of the active components in organic solvents (de Boer et al., 2005). Phytochemicals are naturally occurring constituents of plants. Although the knowledge of how these phytochemicals provide medicinal value to humans reveals a recent scientific understanding, the use of plants and plant extracts to heal, relieve pain, and promote good health dates back to the beginning of medical science. (Basco et al., 2016; Jacob and David, 2016). In this study, alkaloids in perceptible amounts have been shown to act as a pain reliever and a contemporary anesthetic in ophthalmology with stimulating results and antipyretic effects (Yadav and Agarwala, 2011).

As cited by Jacob and David (2016), the presence of saponins includes significant biological effects such as erythrocyte hemolysis, enzyme inhibition, cholesterol and bile acid metabolism, antifungal activity, anti-carcinogenic, and impact on reproduction. This compound can also delay the growth of cancer cells, boost the immune system and energy, lower cholesterol, act as a natural anti-inflammatory, antibiotic, and anti-oxidant, and can reduce the uptake of certain nutrients, including glucose and cholesterol, in the gut through intraluminal physicochemical interactions (De Silva et al., 2013; Aberoumand, 2012).

Tannins are a broad class of compounds that fight cavities and diarrhea; some even protect against heart diseases and cancer. Tannins are well known for their astringent properties; thus, they have been used as bases for several herbal treatments. They act by iron deprivation, hydrogen bonding, or specific interactions with proteins, such as enzymes, cell envelopes, and complex formation with polysaccharides (Dharmananda, 2003; Hisanori *et al.*, 2001). Herbs containing tannins as their component are used to treat intestinal disorders such as diarrhea and dysentery (Just *et al.*, 1998). However, in the study of Jacob and David (2016), it was stated that the presence of tannins in plants can also cause adverse effects on productivity, reduced nutrient availability, reduced digestibility, impaired digestive physiology, and maybe mucosal perturbations for those who will intake such plants. Plants that are naturally poisonous contain cardiac glycosides. Cardiac glycosides commonly comprise one or more sugars combined with an alcohol, phenol, or another complex molecule (Hollman, 1985). Cardiac glycosides are the common agents of choice in treating congestive cardiac failure (CCF), and these compounds are said to have a narrow therapeutic index. They hence can often cause intoxication (Rashmika & Manish, 2012). Pongrakhananon (2013) indicated that these constituents are primarily involved in treating cardiac failure since they increase cardiac output and are used to treat congestive heart failure and cardiac arrhythmia.

Flavonoids are secondary metabolites of polyphenolic plants (Janićijević *et al.*, 2007). According to a study by Janićijević *et al.* (2007), flavonoids play a vital role in the protection of plants from insects and mammalian herbivores (Harbone *et al.*, 2000) and have the ability to modify enzymatic and chemical reactions that have either positive or negative impacts on human health (Beecher, 2003). Flavonoids are also considered angiotensin-converting enzyme inhibitors in regulating hypertension (Balasuriya & Rupasinghe, 2011). They are also involved in stress phenomena, such as antibiotics and modulating molecules (Petrussa *et al.*, 2011). According to a study by Middleton *et al.* (2000), plant flavonoids can act as therapeutic agents in mammalian cells and can be implicated in inflammation, heart disease, and even cancer. Steroids are vital for the average growth and development of plants. Based on the study by Ylstra *et al.* (1995), steroid hormones can stimulate germination and pollen tube growth. Steroids possess physiological roles similar to the plant, allelochemical substances related to animal hormones, and plant-specific allelochemical substances, which often display protective actions against phytophagous animals or parasitic fungi. According to Zhang *et al.*'s (2014) report, terpenoids or terpenes have varied biological activity profiles, including cytotoxicity (i.e., sesquiterpene lactones), antimalarial drugs (i.e., sesquiterpene lactone endoperoxide), growth hormones, tumor promoters, and anticancer agents against paclitaxel (i.e., diterpenes).

3.2 Antibacterial Assay

The extracts of *B. asiatica* were examined for their antibacterial activity against the test bacteria *E. coli* and *S. aureus* using a disc diffusion assay. The data presented in Tables 2 and 3 show the results of the antibacterial assay against *E. coli* and *S. aureus*, respectively.

Table 2. Antibacterial activity of *B. asiatica* against *E. coli*

Treatments	Zone of inhibition (mm)		
	8 hrs	16 hrs	24 hrs
Distilled water	6.00 ^b	6.00 ^c	6.00 ^b
Streptomycin	27.96 ^a	32.74 ^a	32.99 ^a
95% Lab grade ethanol	10.28 ^b	10.33 ^b	10.96 ^b
<i>B. asiatica</i> ethanol extract	7.12 ^b	7.49 ^c	8.13 ^b
<i>B. asiatica</i> aqueous extract	6.59 ^b	6.86 ^c	7.47 ^b
ANOVA	*	*	*

Legend: *significant. Note: Results are presented as the mean $\pm$ standard deviation. Results with the same superscript letter are not significantly different at the 5% significance level, using the least significant difference.

At 8 h, the Analysis of Variance (ANOVA) showed a significant result wherein streptomycin obtained the highest value in terms of zone of inhibition with a mean value of 27.96 mm followed by 95% Lab grade ethanol, *B. asiatica* ethanol extract, *B. asiatica* aqueous extract, and distilled water. There was a significant difference between streptomycin and 95 % lab-grade ethanol at 16 h. At 24 h, streptomycin maintained the highest zone of inhibition (32.99 mm) and was significant in 95% Lab grade ethanol, *B. asiatica* ethanol extract, *B. asiatica* aqueous extract, and distilled water. The results showed no statistically significant difference between the inhibition zones in 95% Lab grade ethanol, *B. asiatica* ethanol extract, *B. asiatica* aqueous extract, and distilled water. According to standard

zone sizes (Bauer *et al.*, 1996), streptomycin with a size of 15 mm or more is sensitive. Since the zones of inhibition exhibited by all the other treatments were less than 11 mm, *E. coli* was resistant to the treatments.

Table 3. Antibacterial activity of *B. asiatica* against *S. aureus*

Treatments	Zone of inhibition (mm)		
	8 hrs	16 hrs	24 hrs
Distilled water	6.00 ^b	6.00 ^c	6.00 ^c
Streptomycin	33.79 ^a	34.38 ^a	34.70 ^a
95% Lab grade ethanol	10.36 ^b	11.35 ^b	11.57 ^b
<i>B. asiatica</i> ethanol extract	7.48 ^b	7.75 ^c	7.80 ^c
<i>B. asiatica</i> aqueous extract	6.98 ^b	6.99 ^c	7.28 ^c
ANOVA	*	*	*

Legend: *significant. Note: Results are presented as the mean± standard deviation. Results with the same superscript letter are not significantly different at the 5% significance level, using the least significant difference.

ANOVA at 8 h showed significant differences between streptomycin and 95% Lab grade ethanol, *B. asiatica* ethanol extract, *B. asiatica* aqueous extract, and distilled water. Significant results were also obtained after 24 h, with streptomycin having the highest zones of inhibition at 34.70 mm. According to standard zone sizes (Bauer *et al.*, 1996), *S. aureus* is resistant to 20 mm or less. According to Ragasa *et al.* (2011), compounds from *B. asiatica* seed extracts were slightly active against *S. aureus*. They were more active against *C. albicans* due to the presence of different triterpenes in the extracts.

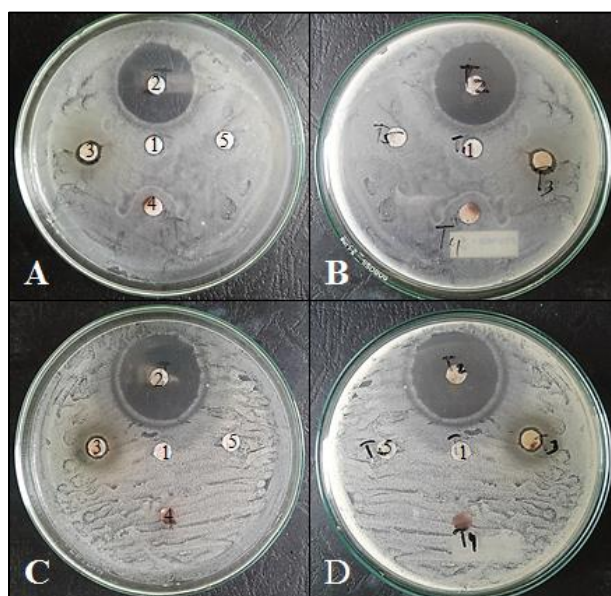


Figure 4. Zones of inhibition of (A, B) *S. aureus* and (C, D) *E. coli* on Mueller-Hinton agar (MHA) after 24 h of incubation. Treatments (1) distilled water (2) streptomycin (3) 95% lab grade ethanol (4) *B. asiatica* Ethanol extracts (5) *B. asiatica* aqueous extracts

One of the biggest threats to human existence is microbial resistance to synthetic medicines or drugs. The use of conventional synthetic drugs in offering remedies to existing human, animal, and plant disease therapies has proven to be unsuccessful. According to the study by Orhana *et al.* (2010), flavonoids have strong antibacterial activity, and the extract would also display antimicrobial activity against some of the bacteria. Lamb and Tim Cushnie (2005) explained the antibacterial mechanisms of action of selected flavonoids. Quercetin activity was at least partially attributed to the inhibition of DNA gyrase activity. It has also been proposed that sophoraflavone G and (-)-epigallocatechin gallate inhibit cytoplasmic membrane function and that licochalcones A and C inhibit energy metabolism. Other flavonoids whose mechanisms of action have been investigated include robinet, myricetin, apigenin, rutin, galanin, 2,4,2 -trihydroxy-5 -methyl chalcone, and lonchocarpol A.

The presence of alkaloids in the phytochemical analysis indicates their antibacterial properties because alkaloids are a large and structurally diverse group of compounds that serve as scaffolds for essential antibacterial drugs such as metronidazole and quinolones. The antibacterial mechanism of action has been investigated for alkaloids

in indolizidine, isoquinoline, quinolone, and polyamine classes (Tim Cushnie *et al.*, 2014). In the indolizidine class, alkaloids such as pergularinine and tylophorinidine act by inhibiting nucleic acid synthesis, as they inhibit the enzyme dihydrofolate reductase in cell-free assays (Rao & Venkatachalam, 2000). Studies on benzophenanthridine and protoberberine isoquinolines in the isoquinoline class suggest that these compounds perturb the Z-ring and inhibit cell division. Researchers working with phenanthridine isoquinoline ungeremine suggest that this alkaloid acts by inhibiting nucleic acid synthesis after observing inhibition of type I topoisomerases in cell-free assays (Tim Cushnie *et al.*, 2014). Research on alkyl methyl quinolones suggests that these are respiratory inhibitors, as they reduce O₂ consumption in treated bacteria but do not affect 3H uptake (Tominaga *et al.*, 2002). Lastly, studies on the polyamine alkaloid squalamine suggest that it acts by compromising the outer membrane and cytoplasmic membrane integrity (Tim Cushnie *et al.*, 2014). The flavonoids and alkaloids in both extracts indicated their antibacterial effect on the test bacteria.

3.3 Antifungal Assay

The antifungal activity of the ethanol and aqueous extracts of *B. asiatica* against the test bacteria *F. verticilloides* and *A. niger* was determined using an antifungal bioassay. The data presented in Table 4a show the results of the antifungal assay of *B. asiatica* against *F. verticilloides* and in Table 4b for *A. niger*. The results of the antifungal assay of *B. asiatica* against *F. verticilloides* showed a significant difference on day 1, where the fungicide obtained the lowest mycelial growth and was comparable to the ethanol extract with a mean value of 11.30 mm, followed by the aqueous extract at 13.40 mm, ethanol at 13.75, and the highest mean exhibited by distilled water at 17.63 mm. From day 2 to day 7, fungicide showed a significant difference in both aqueous and ethanol extracts, as well as ethanol and distilled water. The highest mycelial growth and the least effective were exhibited by distilled water, as fungi can grow here. The most effective antifungal agent is fungicide, followed by *B. asiatica* ethanol, aqueous extracts, and ethanol.

For the results of the antifungal assay of *B. asiatica* against *A. niger* in Table 4b, fungicide has the lowest mycelial growth at 10 mm. It differs significantly from the aqueous extract at 14.38 mm, ethanol at 13.75 mm, and distilled water at 16.63 mm during Day 1. Fungicide was still significantly different in all treatments from days 1 to 7. Distilled water had the highest mycelial growth at 45.55 mm, followed by ethanol at 36.18, aqueous extract at 31.24, and ethanol extract at 28.74. The most effective antifungal agents are fungicide, *B. asiatica* ethanol and aqueous extracts, and ethanol with distilled water as the least effective treatment.

Statistically, the results of this experiment suggest that all treatments were significantly different from each other. Various medicinal plant extracts have shown inhibitory effects against phytopathogenic fungi in vitro (Shalini & Srivastava, 2009). The antifungal activity results were supported by previous studies that showed that the crude methanolic extract of *B. asiatica*, including leaves, fruits, seeds, stems, and root bark, exhibited an excellent level of broad-spectrum antifungal activity (Khan & Omoloso, 2002). In addition, the methanolic extract of *B. asiatica* flower also exhibited the growth of *Microsporum canis* and *Trichophyton rubrum* at 1000 µg/ml, and *Epidermophyton floccosum* at 125 µg/ml (Locher *et al.*, 1995).

Mycelial inhibition of *B. asiatica* may be associated with the presence of phytochemical constituents. A distinguishing characteristic of tannins is their binding to proteins, essential compounds, and large molecules (Okuda & Ito 2011). The presence of tannins in the seeds may have affected the production of chitin, a protein structure of the cell wall. Chitin deficiency may result in a flaccid cell wall that can expose the cells to the outside environment. In addition, the presence of terpenoids in plants causes cytotoxic effects, growth hormones, and tumor promoters (Zhang *et al.*, 2014). Once tannins act on the cell wall, the toxin present in terpenoids can act on the organelles inside the cell, causing damage to the cell organelles. Wooley (2001) suggested that alkaloids (*i.e.*, pyrrolizidine and indolizidine) cause serious toxicity and even death for horses, cattle, and sheep. Alkaloids play a role in plant defense (Evans *et al.*, 2006), which explains the inhibition performance of *B. asiatica*. The presence of alkaloids in the plant might have destroyed the mycelia of the fungi, resulting in mycelial inhibition. Alkaloids and steroids contradict each other because steroids are growth-promoting phytohormones in aquatic filamentous fungi (Dinnan *et al.*, 2011). Poisonous plants are known to contain cardiac glycoside (Hollman *et al.*, 1985). Cardiac glycosides are the common agents of choice in treating congestive cardiac failure (CCF), and these compounds are said to have a narrow therapeutic index.

They hence can often cause intoxication (Rashmika & Manish, 2012). The cell wall of a fungus is affected by the antifungal agent acting on the fungi. Cardiac glycosides are believed to act on the cells of the heart. They may have acted on the cells of the cell wall, decomposing it and causing growth retardation. Flavonoids can modify enzymatic and chemical reactions, which have positive or negative effects (Beecher *et al.*, 2003). In a study by Middleton *et al.* (2000), plant flavonoids can act as therapeutic agents in mammalian cells and have been implicated in inflammation, heart disease, and cancer. The presence of flavonoids in *B. asiatica* may have caused inflammation in the fungi cells, causing them to die, and could be one of the reasons for mycelial growth inhibition. According to Geyter *et al.* (2007), saponins have the potential to be utilized as natural insecticides, where they can exert robust insecticidal activity in a wide range of insect types and stages and can also be used to prevent transmission of insect-mediated disease. The difference in antimicrobial properties of a plant extract might be attributed to the age of the seeds used, physical factors (temperature, light water), contamination by field microbes, and incorrect preparation and dosage (Calixto, 2000; Okigbo & Igwe, 2007).

3.4 Cytotoxicity Test Using Brine Shrimp Lethality Assay

The brine shrimp lethality assay (BSLA) has been used routinely in the primary screening of crude extracts and isolated compounds to assess toxicity towards brine shrimp. This could also provide an indication of possible cytotoxic properties of the test materials (McLaughlin *et al.*, 1991). Figures 5 and 6 show the mortality rate of nauplii exposed to different concentrations of ethanol and aqueous extracts of *B. asiatica*.

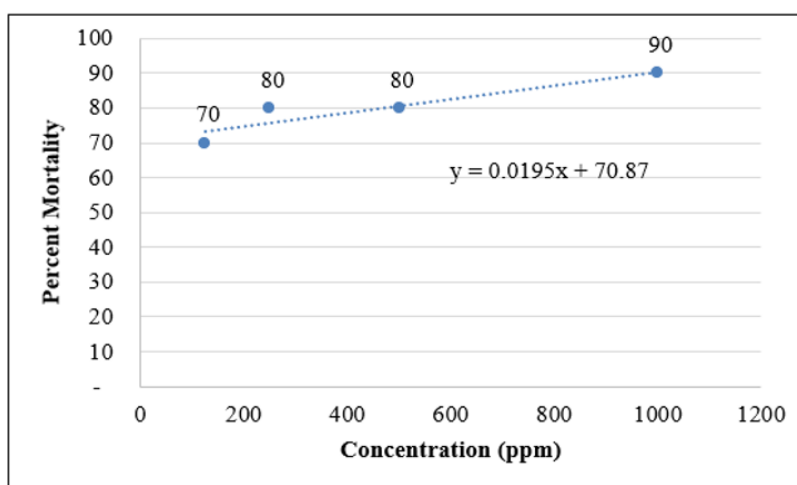


Figure 5. Cytotoxic lethality assay of *B. Asiatica* ethanol extract to *A. Salina* after 24 hours

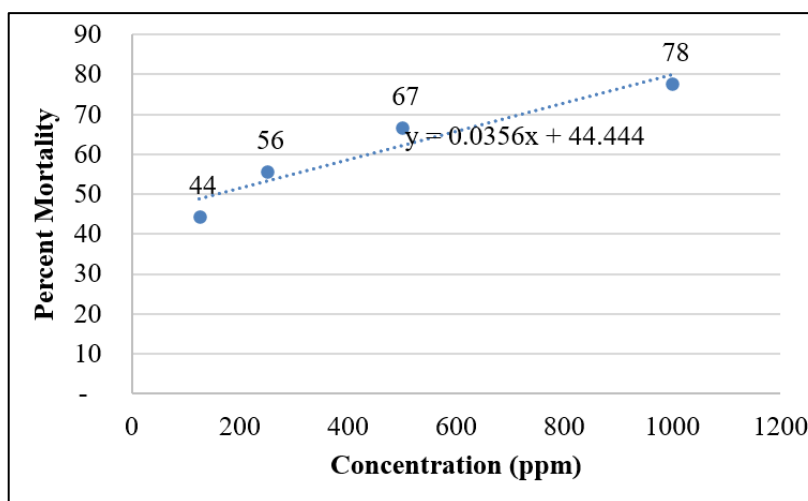


Figure 6. Cytotoxic lethality assay of *B. asiatica* aqueous extract to *A. salina* after 24 hours

Concentrations of *B. asiatica* extract used ranged from 125 to 1000 ppm, with pure artificial seawater as a control. It was observed that the highest mortality rate in ethanol extracts was exhibited at 1000 ppm, which makes it lethal to 90% of the test organisms after 24 h of observation. In contrast, 250 ppm exuded 80% lethality, whereas 125 ppm showed 70% lethality. In addition, the highest mortality rate of aqueous extracts was observed at 1000 ppm, which was lethal to 78% of the same test organism. Moreover, 125 ppm displayed 44% lethality, and 250 ppm showed 56% lethality. The graphs show that the higher the concentration, the higher the mortality rate. The lethal concentration (LC₅₀) of *B. sciatica* ethanol extracts was observed at 24.55 ppm, while in aqueous extracts, 181.97 ppm concentration. McLaughlin and Rogers (1998) state that LC₅₀ of ≤ 249 ppm is highly toxic; LC₅₀ of 250 - 499 ppm is moderately toxic; LC₅₀ of 500-1000 ppm is mildly poisonous, and values above 1000 ppm are non-toxic. Therefore, the ethanol and aqueous extracts of *B. asiatica* are considered highly toxic.

The results of the present study are similar to those of Mojica and Micor (2007). Their study showed that the aqueous extract of *B. asiatica* seeds is highly toxic against *A. salina*. Their results showed that the LC₅₀ was lethal at 2.25 ppm after 48 h of exposure. In comparison to the result of the present study, the LC₅₀ was found deadly at 181.97 ppm, which is also considered highly toxic, according to McLaughlin and Rogers (1998). Another survey by Umuri *et al.* (2018) demonstrated the cytotoxic activities of *B. asiatica* essential oil from different plant parts. The results of their research are congruent with the results of the present study, wherein the ethanol extract of *B. asiatica* was found to be toxic at 24.55 ppm after 24 h of exposure. In association with the result of their study, the 50% lethality concentration of the leaves and the stem-bark essential oil were observed at 56.87µg/mL and 457.63µg/mL, respectively. Other species of *Barringtonia*, like *B. racemose* and *B. acutangula* (L.) Gaertn. also exhibit cytotoxic activity against different types of test organisms (Osman *et al.*, 2015; Mohanty *et al.*, 2016).

The presence of terpenoids, cardiac glycosides, and saponins in the ethanol and aqueous extracts of *B. asiatica* had toxic effects on the test organism. According to Zhang *et al.* (2014) and Rashmika and Manish (2012), the presence of terpenoids varies biological activity, which includes cytotoxicity, antimalarial drugs, growth hormones, and anticancer against paclitaxel. However, cardiac glycoside compounds are also said to have a narrow therapeutic index and, hence, can cause intoxication. Currently, the *A. salina* (brine shrimp, fairy shrimp, or sea monkeys) lethality assay is commonly used to evaluate the cytotoxic effects of bioactive chemicals (Quazi *et al.*, 2017). Brine shrimp nauplii have been previously utilized in various bioassay systems. Several novel antitumor and pesticidal natural products have been isolated using this bioassay (Meyer *et al.*, 1982; Sam TW, 1993). According to the National Cancer Institute (NCI, USA), this bioassay is used as a pre-screening tool for antitumor drug development because of the significant correlation between the brine shrimp assay and the *in vitro* growth inhibition of human solid tumor cell lines (Basco *et al.*, 2016).

Environmental pollution poses a significant risk to human health and ecosystems, highlighting the urgent need for practical and sustainable bioremediation and antimicrobial solutions. While research on *Barringtonia asiatica* seed extracts has shown promising antimicrobial and cytotoxic effects, supporting their potential in combating pathogens, recent findings on the phytoremediation capacity of *Ipomoea reptans* (Kangkong) offer insights into another approach for mitigating toxic contaminants. Ingente and Anselmo (2024) demonstrated that *I. reptans* effectively reduces Pb levels in a hydroponic system, highlighting the potential of plants for heavy metal remediation in contaminated water sources. This intersection of phytoremediation and antimicrobial research underscores the need for a multifaceted approach to address pollution and infection risks, with natural extracts and plant-based systems playing vital roles in health and environmental remediation efforts.

4.0 Conclusion

The ethanol extract of *B. asiatica* contains phytochemical constituents, such as alkaloids, saponins, tannins, glycosides, steroids, flavonoids, and terpenoids. Moreover, five phytochemical constituents were present in the aqueous extract: alkaloids, saponins, tannins, glycosides, flavonoids, steroids, and terpenoids. Based on the results of the antibacterial and antifungal activities, it was found that *B. asiatica* showed potential as a medicinal plant used by traditional herbal medical practitioners as a natural antimicrobial agent; thus, it can be further used to determine the bioactive products that may lead to the development of new drugs. The presence of flavonoids and alkaloids in both extracts indicated its antibacterial effect on the test bacteria. At the same time, the mycelia inhibition of *B. asiatica* can be associated with the presence of phytochemical constituents such as tannins and

terpenoids. The selected ten medicinal plants are the source of the secondary metabolites, i.e., alkaloids, flavonoids, and terpenoids; Brine shrimp lethality assay was used for the preliminary cytotoxicity assay of plant extract and others based on the ability to kill a laboratory cultured larva (nauplii). Nauplii were exposed to different concentrations of the plant extract for 24 h. The number of motile nauplii was calculated to determine the extract's efficacy. It is simple, cost-effective, and requires only a small amount of test material. The lethal concentration (LC₅₀) of *B. asiatica* ethanol extracts was observed at 24.55 ppm. In aqueous extracts, 181.97 ppm of concentration. According to McLaughlin and Rogers (1998), an LC₅₀ of ≤ 249 ppm is considered highly toxic; an LC₅₀ of 250 - 499 ppm is moderately toxic; an LC₅₀ of 500-1000 ppm is mildly toxic, and values above 1000 ppm are non-toxic. Therefore, the ethanol and aqueous extracts of *B. asiatica* are considered highly toxic.

5.0 Contributions of Authors

In this study, author 1 conceptualized and designed the research, including formulating the research questions and hypotheses, contributing significantly to data collection and analysis and ensuring the accuracy and consistency of the data gathered. Played a crucial role in reviewing the literature and drafting the introduction, providing insights into relevant theories and prior research findings, instrumental in interpreting the results, and assisted with statistical analyses, bringing clarity to the findings, focusing on the discussion and implications, linking the findings to practical applications, and suggesting avenues for future research. Author 2. Contributed to the final manuscript by reviewing and refining it for coherence, accuracy, and clarity, and approved the final version for submission, and lastly oversaw the entire project

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7.0 Conflict of Interests

no conflict of interest.

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