

Original Article

Fresh Leaf Extracts of Selected Philippine Medicinal Plants: Phytochemical Composition and Antibacterial Activity Against *Escherichia coli*

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Abstract

Urinary tract infections (UTIs) caused by *Escherichia coli* have become an increasing public health concern due to antibiotic resistance. This study focused on the antibacterial properties of fresh leaf extracts of five Philippine medicinal plants, with special focus on aratilis (*Muntingia calabura*), and aimed to evaluate and compare the phytochemical constituents and the antibacterial effects of crude leaf extracts against *Escherichia coli*. Using the manual maceration method, fresh leaf extracts of guyabano (*Annona muricata*), alugbati (*Basella alba*), aratilis (*Muntingia calabura*), bignay (*Antidesma bunius*), and banaba (*Lagerstroemia speciosa*) were prepared. Phytochemical screening was performed using qualitative assays, and antibacterial activity against *Escherichia coli* was assessed using the Kirby-Bauer disc diffusion method. Data were analyzed and compared inferentially to assess the effectiveness of the two treatments in terms of diameter. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids. Among the plant extracts, only aratilis exhibited antibacterial activity, with a mean inhibition zone of 7.49 ± 0.30 mm, while cefuroxime produced the largest observed mean zone of inhibition of 13.59 ± 1.14 mm. The independent samples t-test revealed a statistically significant difference between the treatments, $t(4) = 8.970$, $p < .001$. Cefuroxime produced larger inhibition zones than aratilis, though it demonstrated measurable antibacterial activity against *E. coli*. The presence of tannins and flavonoids in aratilis was shown to

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confer modest antibacterial activity against *Escherichia coli* compared to cefuroxime. The findings strengthen the potential and viability of aratilis as a candidate for further phytochemical and pharmacological research.

Keywords: antibacterial activity, antimicrobial resistance, flavonoids, *Muntingia calabura*, phytochemical constituents, tannins

1. Introduction

Herbal medicine contributes to existing knowledge of the sources of materials for traditional medicine and modern pharmaceuticals (Rodrigues, 2024). The value of natural products is evident in the context of modern medicine. In the studies by Newman & Cragg (2020) and Tache et al. (2022), approximately 40% of the drugs approved by the U.S. Food and Drug Administration (FDA) were of natural origin. In addition, approximately 50% of all new anti-cancer drugs approved since the 1940s were either natural products or derivatives of natural products.

There is an ample evidence of natural metabolites exhibiting antimicrobial, anticancer, and anti-inflammatory activities, thereby underscoring the potential for these products to be developed for use in the pharmaceutical industry. The Philippines has a vast diversity of flora, with over 13,500 plant species, of which only 120 have been scientifically assessed for safety and efficacy (Philippine Sustainable Development Network Foundation, Inc., 2020). The Philippine government has incorporated herbal medicine into the traditional and alternative health care programs. Medicinal plants offer an economical alternative to expensive synthetic drugs (Meñiza et al., 2024).

Since a significant percentage of modern medicines are derived from plant sources, studies on plant-based therapies are likely to increase public awareness of the sources of potential leads for pharmacological assays and ultimately broaden the prospects for the discovery of innovative drugs aimed at treating common infectious diseases (Atanasov et al., 2021; Chaachouay & Zidane, 2024; Igoli et al., 2025).

Urinary tract infections (UTIs) constitute a major global healthcare problem, with roughly 150 million cases occurring annually, making them the most prevalent bacterial infections worldwide (Hassan et al., 2025). *Escherichia coli* has the highest prevalence among bacterial causes of UTI, accounting for 80–95% of all UTI cases (Devnikar et al., 2024; Tache et al., 2022). Due to its virulence factors, it can colonize the periurethral region and ascend the urinary tract. There are major clinical concerns regarding the lack of prompt and adequate antimicrobial therapy for ascending infections with *Escherichia coli*, as they have the potential to cause pyelonephritis and, in severe cases, result in bloodstream infections and urosepsis (Devnikar et al., 2024).

Sireesha & Swamy (2025) reported that urinary tract infection (UTI) is considered one of the most common bacterial infections worldwide, especially among women. *Escherichia coli*, also known as *E. coli*, is the most common bacterium that causes infection because of its ability to attach to and cling to the urinary tract and bladder using its hair-like structures called fimbriae, and to form biofilms, which allow it to hide and protect itself from the immune system.

The emergence, continuous growth, and mutations of antibiotic-resistant bacteria, such as strains of uropathogenic *E. coli* (UPEC) that are resistant to common antibiotics like beta-lactams, fluoroquinolones, and trimethoprim-sulfamethoxazole, have a significant global effect. This has been associated with the increase in extended-spectrum beta-lactamase (ESBL)-producing organisms, which result in limited choices in antibacterial treatments, creating the need for continuous development of new antimicrobial remedies (Biswas et al., 2025).

Other pathogens, such as *Staphylococcus* species, account for 10% to 15%; *Klebsiella*, *Pseudomonas*, *Proteus*, and *Enterococcus* play minor roles in causing the infection (Vlad et al., 2024). Women of childbearing age are at an even greater risk and may experience recurrent infections in as many as 30% of cases (Zhou et al., 2023; Baimakhanova et al., 2025). UTIs are the most prevalent causes of morbidity in the Philippines and accounted for over 250,000 reported cases in 2014 (Cano et al., 2020; Magtalas et al., 2024; Advani et al., 2025). The preliminary surveys, including that of Aritao National High School, demonstrated that many students with UTI could not afford to purchase synthetic drugs and were therefore using home remedies.

E. coli is an important test organism because of the potential impact of recurrent infections, its rapid development of antibiotic resistance. Its dominance in the etiology of UTIs makes it an excellent test organism to evaluate the potential of affordable, accessible, and effective traditional remedies for UTIs. This has promoted the exploration and initial use of the antimicrobial activity of phytochemicals to develop new plant-derived compounds as potential multi-drug-resistant (MDR) agents against gram-negative *E. coli*.

Philippine medicinal plants play a significant role in ethnomedicine and exhibit the potential to warrant systematic study due to their antimicrobial activity. Medicinal plants like guyabano (*Annona muricata*), alugbati (*Basella alba*), aratilis (*Muntingia calabura*), bignay (*Antidesma bunius*), and banaba (*Lagerstroemia speciosa*), are extensively used and cultivated as medicinal herbs with a wide range of ethnomedicinal applications in the literature (Stuart, 2017). Guyabano, alugbati, banaba, bignay, aratilis are popular herbal plants not only in the Philippines but also globally. For instance, traditional applications of guyabano involve making a poultice and preparing teas from the leaves, roots, and stems to relieve symptoms of rheumatism and arthritis and lessen inflammation. Modern studies have confirmed that the medicinal and therapeutic applications of the plant, as alcoholic extracts, showed moderate antibacterial activity against Gram-positive bacteria, and acetogenins and alkaloids were cited as antimicrobial agents (Zubaidi et al., 2023; Mutakin et al., 2022; Acharjee et al., 2023). The ethnomedicinal and ethnovaluative aspects of guyabano

have been documented, and its nutritive and socio-economic aspects have been cited (Kedari & Khan, 2014). Other studies reported various phytochemicals with antibacterial and antioxidant activities (Vijayameena et al., 2013). Later research focused on its medicinal uses, including as an anti-inflammatory and anticancer agent. *Annona muricata*, also locally known as guayabano, has been widely studied for its pharmacological properties. Kottila & Hena (2024) conducted a detailed review of guayabano and reported that extracts from the plant exhibit antibacterial and anti-inflammatory properties. Isolated metabolites from the plant include flavonoids, terpenoids, tannins, saponins, coumarins, and phenolic compounds, which contribute greatly to its antimicrobial effectiveness. Its availability as a locally sourced fruit product can increase in both nutritional and economic value.

Basella alba, locally known as alugbati, was also used to help reduce swelling in wounds and to serve as a laxative to treat ulcers and soothe burns. The ethnobotanical records have also documented multiple uses to promote suppuration and reduce swelling. However, laboratory studies showed that the effects on wound healing varied with the solvents and pathogens used, and that pathogen inhibition was also affected (D'Souza et al., 2023; Bose et al., 2023; Zhou et al., 2023). Recent phytochemical studies on alugbati confirmed the presence of flavonoids, saponins, and other bioactive compounds. The stem extracts showed positive results for in vivo assays related to wound healing, along with other studies and tests containing antimicrobial and antioxidant activities (D'Souza et al., 2023, Adegoke & Ojo, 2017; Das et al., 2026; Kumar et al., 2024) and in vivo tests; bioactive stem extracts showed wound healing properties (D'Souza et al, 2023). Alugbati, which has great ethnomedicinal value, is a green leafy vegetable cultivated throughout tropical countries. It is used as a substitute for true spinach (*Spinacia oleracea* L.). The abundant phytoconstituents and great ethno-medicinal value of alugbati are evident in the high levels of vitamins A and C and in phytochemicals such as flavonoids, saponins, carotenoids, and amino and organic acids (Reddy et al., 2023).

Lagerstroemia speciosa Pers., or banaba, found throughout the Philippines, Malaysia, and India. It is rich in triterpenoid compounds, specifically corosolic acid, which promotes glucose absorption and regulates insulin-dyslipidemia and glucose-related pathways. Banaba is often referred to as an insulin plant because of its hypoglycemic effects (Cannarella et al., 2023). It is also known locally as a natural remedy for high blood sugar, diabetes, and kidney and urinary problems. Its bark is often used to treat diarrhea, bladder inflammation and dysuria (Park & Lee, 2024; Ramos et al., 2025). Inhibition zones against *E. coli* are also observed for methanolic and aqueous extracts from banaba leaves, indicating that they contain bioactive compounds such as corosolic acid and ellagitannins, and thus have potential as alternative solutions and antibacterial substitutes in inhibiting bacterial growth (Laruan et al., 2014).

Antidesma bunius, known locally as bignay, and is one of the more bountiful shrub species in the Philippines. Its fruit looks like a cluster of smaller, grape-sized, oval fruit, and it often tastes sour, sometimes sweet. It can be eaten after being washed

and mixed with salt. The leaves are also used for flavoring, and the fruit is processed into jams, jellies, and fermented drinks like wine. Bignay's bioactive compounds, such as alkaloids, phenolics, flavonoids, and terpenoids, contribute to its growing popularity due to their antioxidant, anti-inflammatory, and antibacterial properties. Traditionally, bignay has been used to ease indigestion, loss of appetite, and snakebites. It has been used to prepare tea or juice, and other studies have noted its cardioprotective and antimicrobial effects. Some studies have shown that fruit extracts in ethanol and methanol exhibit antimicrobial activity against foodborne pathogens such as *E. coli* and have antimicrobial screening potential (Nguyen-Ngoc et al., 2024; Lizardo et al., 2015).

Another important fruit, especially to children, is aratilis, due to its potential pharmacological properties. The fruit, leaves, and flowers of aratilis have antiseptic and emollient effects and help to ease abdominal cramps and spasms (Saud et al., 2023; MosaChristas et al., 2022). The fruit and leaves of aratilis have been shown to have anti-inflammatory and anti-spasmodic properties. The methanolic fruit extract showed inhibition against *E. coli* and other microbes, and the ethyl acetate extract showed inhibition against *E. coli* and *C. albicans* (Indraningrat et al., 2025; Sekar & Jalil, 2017). The presence of flavonoids, tannins, saponins, and steroids in the phytochemicals has been cited as responsible for these effects, owing to their ability to disrupt bacterial membranes and inhibit enzymatic activity. The survey conducted by Kuchekar et al. (2021) on aratilis compiled data on its phytochemistry and pharmacology. Leaves, bark, flowers, and fruits of aratilis were found to contain diverse bioactive compounds such as flavonoids, tannins, saponins, triterpenes, polyphenols, and alkaloids. These components were found to possess numerous pharmacological activities. These include strong antioxidants, antimicrobial, anti-inflammatory, analgesic and antidiabetic activities, as well as cytotoxic and anticancer activities, in addition to hepatoprotective and cardioprotective activities. From a nutritional and therapeutic perspective, aratilis is a valuable medicinal plant. The ethnomedicinal applications of aratilis are well-documented, and the reviewed plant materials are the first of their kind to be validated as medicinal by modern science. Considering the ethnomedicinal applications of aratilis, combined with the value of the laboratory confirmation, aratilis stands out as a plant of great potential for further studies.

In support of the use of traditional medicine, the Department of Health initiated the Traditional Medicine Program and established the first validated list of traditional medicinal plants. The plants were akapulco (*Cassia alata*), ampalaya (*Momordica charantia*), bawang (*Allium sativum*), bayabas (*Psidium guajava*), lagundi (*Vitex negundo*), niyug-niyogan (*Quisqualis indica*), sambong (*Blumea balsamifera*), tsaang gubat (*Ehretia microphylla*), ulasimang bato (*Peperomia pellucida*), and yerba buena (*Clinopodium douglasii*). This study is conducted in compliance with the government's policy regarding the use of traditional medicine to treat infections and address health-related issues and diseases, and to discover ways to incorporate it, if found effective, into new methods and/or products that can potentially help improve

our healthcare system. This study is made towards the use of less-studied plants that have potential for microbial inhibitions, if not elimination and treatments of bacterial infections, specifically the ones that cause urinary tract infections (UTI), to reduce the current threats of antibiotic resistance (World Health Organization, 2019; Ong & Bodeker, 2021; Ramos et al., 2025).

Most existing studies rely on laboratory-optimized or solvent-based extracts of plant samples. Such extracts are not available to households that utilize these plants. Unprocessed leaf samples are the most utilized sample type in community studies. Although organic solvents may extract a broader range of phytochemicals, cold-water extraction was selected in the present study because it closely mimics traditional preparation practices and enables evaluation of bioactivity under conditions accessible to rural communities, where medicinal plants are often consumed as aqueous preparations. Water is a safe, inexpensive, and environmentally friendly solvent capable of extracting a wide range of polar bioactive compounds, including phenolics, flavonoids, glycosides, and tannins (Abubakar & Haque, 2020; Nn, 2015; Goti & Dasgupta, 2023).

This is not only a difference in extraction research techniques. There is a significant practical gap, as the equipment and/or reagents used in solvent extraction techniques are not readily available in most households. Therefore, this study aimed to report on the phytochemical profiling and to evaluate the antibacterial potential of fresh leaf extracts of five selected medicinal plants against the microorganism *E. coli*. Specifically, the study sought to determine the inhibitory effects of the extracts and assess their potential as sources of antimicrobial agents.

2. Methodology

2.1 Research Design

The study employed a completely randomized design (CRD) to evaluate the antibacterial effects of five plants: guyabano (*Annona muricata*), alugbati (*Basella alba*), aratilis (*Muntingia calabura*), bignay (*Antidesma bunius*), and banaba (*Lagerstroemia speciosa*) against *Escherichia coli*. The experiment was arranged using a completely randomized design with seven treatments and three replicates per treatment. The zone of inhibition was measured after 24 hours of incubation. This design was quantitative to facilitate precise measurement of the inhibition zones (in mm) and statistical treatment of the data. Descriptive statistics were used to summarize central tendencies and variability and to test the hypotheses. Other treatments that did not show an inhibitory response were not included in the final comparative analysis because the goal of this study was to assess the effectiveness of the treatments in inhibiting *E. coli*. Hence, the inferential analysis was geared towards the two treatments with zones of inhibition that yielded measurable data, and it involved an

independent-samples t-test to evaluate whether the mean effect of the two treatments differed significantly.

2.2 Research Materials

Collected samples of plants leaves and extracts were processed at Nueva Vizcaya State University's MECO TECO laboratory in the town of Bayombong, Province of Nueva Vizcaya. Matured leaves of five (5) herbal plants; guayabano, aratilis, alugbati, bignay, and banaba were used for this experiment. Analytical graded chemicals and reagents were also used. The test organism used was a standard laboratory strain of *Escherichia coli* that is laboratory-maintained culture and routinely employed in antimicrobial susceptibility and antibacterial activity studies. A standard compliant with 0.5 McFarland, equivalent to $\sim 1.5 \times 10^8$ CFU/ml, is obtained from Saint Mary's University's Center for Natural Sciences. The bacterial strain was cultured on Mueller-Hinton agar for 24 hours before testing for bacterial activity. To ensure ethical standards and methodological consistency, no actual urine samples were used.

2.3 Data Gathering and Analysis Procedure

There were five (5) phases to complete the experimental procedure. Before leaf collection for extraction, sample leaves from the five herbal plants were authenticated by the Nueva Vizcaya State University, College of Agriculture, with a certificate of plant identity. The mature leaves of the five herbal plants were harvested early in the morning at Lamo, Dupax del Norte, Nueva Vizcaya. The herbal plant leaves were checked for contamination, then washed with cold tap water and air-dried for 15 minutes. In the second phase, 500 g of each dried and powdered leaf sample was macerated in distilled water. The mixture was filtered through filter paper, and the filtrate was concentrated using a rotary evaporator at 43 °C and 100 rpm to obtain the crude extract. This method was consistently applied throughout the study to minimize variability among preparations. The extraction yield was expressed in terms of milliliters of extract per gram of fresh leaf, and the yields were: 45 ml alugbati (0.09 ml/g), 5 ml banaba (0.01 ml/g), 7ml bignay (0.014 ml/g), 7ml guayabano (0.014 ml/g), and 9 ml aratilis (0.018 ml/g). The extracts were stored in clean amber bottles and kept in the refrigerator.

Thirdly, qualitative phytochemical analysis was done through test tube methods as follows: for tannins, 3 grams of each plant extract were prepared using the test tube method; for saponins, 1 gram of each extract was subjected to the froth test; Dragendorff's was used for the detection of alkaloids with 1 ml of each extract; Shinoda's test was used for flavonoids using 2 ml of each extract; and for steroids, the Liebermann-Buchard Reaction was used with 1 ml of each extract (Guevarra, 2005; Dahiru et al., 2006; Gerardi & Giovinazzo, 2022). Fourth, for the evaluation of antibacterial activity, the Kirby-Bauer disc diffusion method was used (Mehmood et al., 2024; Bauer et al., 1966). Sterile 6-mm filter paper discs that were impregnated

with 10 µL of each plant extract, allowed to dry under sterile conditions, and placed on Mueller–Hinton agar plates previously inoculated with *E. coli* (0.5 McFarland). Cefuroxime (30 µg/disc) was used as the positive control, while sterile distilled water served as the negative control. Plates were incubated at 37°C for 24 h, after which inhibition-zone diameters were measured. Measuring the diameter of the inhibition zone involved measuring the clearance extending beyond the edge of the discs. Measurements were expressed in millimeters to provide measurement reliability. For each plant sample, extracts were prepared and evaluated using the disc diffusion assay. Three discs were tested for each plant extract as technical replicates. Thus, three inhibition zone measurements were obtained for each plant extract. The three measurements were used to calculate the mean and standard deviation.

Inhibition zone diameter data were analyzed descriptively for each treatment. In the inferential analysis, an independent-samples t-test was used only on treatments with clear zones of inhibition to determine the effects of the treatments on inhibiting the growth of *E. coli*. These methods were used to ensure the comparative effectiveness of the extracts against *Escherichia coli* while maintaining compliance with standards.

2.4 Ethical considerations

The study was conducted within the scope of safe, ethical, and scientific practices. No humans or animals were used during the experiment for ethical compliance. The medicinal plant leaves used were identified and authenticated (Table 1) to ensure the study's accuracy and reproducibility. Laboratory biosafety standards were followed and maintained throughout the experiment, including during the culturing, handling, and disposal of *Escherichia coli* and other biological materials. The study's goal is to provide additional information to support the development of plant-based antimicrobial solutions to help manage urinary tract infections in the future.

Table 1. Herbal plant samples and their scientific names.

Common Name	Scientific Name
Guyabano	<i>Annona muricata</i> Linn.
Alugbati	<i>Basella alba</i> Linn.
Aratilis	<i>Muntingia calabura</i> Linn.
Bignay	<i>Antidesma bunius</i> (Linn.) Spreng.
Banaba	<i>Lagerstroemia speciosa</i> (Linn.) Pers.

Note: Plant authentication was conducted by the College of Agriculture, Nueva Vizcaya State University, which issued certification of identity for all samples. Scientific names follow standard botanical nomenclature conventions (Guevarra, 2005).

2.5 Statistical Analysis

The experiment was arranged using a completely randomized design with seven treatments and three replicates per treatment. Preliminary analysis revealed that several treatments consistently produced identical 6-mm measurements across all replicates, resulting in zero within-treatment variance. To facilitate a valid inferential comparison, the analysis focused on the two treatments that exhibited measurable and variable responses. Because only two independent treatment groups were ultimately compared, an independent-samples t-test was employed to determine whether a significant difference existed between their mean responses. Descriptive statistics, including individual replicate measurements, means, standard deviations, minimum and maximum values, ranges, and the frequency of detectable inhibition, were also calculated. All data processing and statistical analyses were performed using IBM SPSS Statistics version 26. This provided a clear framework for evaluating differences among the extracts and the evolving nature of their inhibition over the specified periods. This also improved the overall validity of the study. It was also hoped that the study would be reproducible and reliable in its evaluation of the inhibitory properties of the herbal constituents.

3. Results

3.1 Phytochemical Analysis

Phytochemical screening of the fresh leaf extracts revealed distinct profiles of secondary metabolites across the five species examined: guyabano (*Annona muricata*), alugbati (*Basella alba*), aratilis (*Muntingia calabura*), bignay (*Antidesma bunius*), and banaba (*Lagerstroemia speciosa*). The qualitative assessment indicated the presence of alkaloids, flavonoids, and tannins in aratilis, and of alkaloids and tannins in bignay. Banaba indicated the presence of alkaloids, steroids, saponins, saponins, and tannins. Saponins and tannins were identified in guyabano, and the remaining species, alugbati, and the other evaluated phytochemicals were absent. Saponins, tannins, and alkaloids have been reported to be responsible for the antimicrobial and antioxidant activities, providing a rationale for additional testing of the extracts. The distinct phytochemical characteristics of the five species in the comparative analysis of their profiles helped interpret their antibacterial activity (Table 2).

Table 2. Qualitative phytochemical profile of fresh leaf extracts of selected medicinal plants.

Phytochemicals	Guyabano	Alugbati	Aratilis	Bignay	Banaba
Alkaloids	–	–	+	+	+
Flavonoids	–	–	+	–	–
Steroids	–	–	–	–	+
Saponins	+	–	–	–	+
Tannins	+	–	+	+	+

Note. (+) indicates presence of the phytochemical constituents; (–) indicates no positive reactions of the phytochemical constituents. Screening was performed using qualitative test tube assays: Dragendorff's Test for alkaloids, Shinoda's Test for flavonoids, Liebermann–Burchard Reaction for steroids, Froth Test for saponins, and ferric chloride test for tannins (Guevarra, 2005; Dahiru et al., 2006; Gerardi & Giovinazzo, 2022).

3.2 Antibacterial Assay

Using the Kirby-Bauer disc diffusion method to determine the antibacterial activity of the extracts, the measured zones of inhibition (ZOI) were used to indicate their antibacterial activity against *E. coli*. The ZOI was measured in diameter, showing clear zones extending beyond the disc edge, with each replicate recorded in millimeters after 24 hours. Table 3 revealed that the positive control (cefuroxime) consistently produced the largest inhibition zones across replications, ranging from 14.905 mm (R1) to 12.895 mm (R2) to 12.975 mm (R3) at 24 hours. Aratilis exhibited modest activity at all replicates, with inhibition zones increasing from 7.145mm (R1), 7.620 (R2) to 7.710 mm at 24 hours across replicates. Although the inhibition zones of aratilis were consistently smaller than those of cefuroxime, its activity remained measurable and statistically distinct from the inactive treatments. In contrast, guyabano, alugbati, bignay, banaba, and the negative control (distilled water) consistently showed no inhibition throughout the study, confirming their lack of antibacterial effect under the conditions tested.

Treatment-specific descriptive statistics are presented in Table 3. Five treatments produced measurements equal to the 6mm disc diameter in all three replicates, indicating no detectable inhibition beyond the disc margin. Treatment 1, cefuroxime produced the largest observed mean diameter of 13.59 ± 1.14 mm, while Treatment 5 (aratilis) produced a mean diameter of 7.49 ± 0.30 mm. Both treatments showed detectable inhibition in all three replicates. Four of the five herbal plant extracts produced measurements equal to the 6-mm disc diameter in all replicates, indicating no detectable inhibition beyond the disc margin under the conditions tested. Since these treatments produced no detectable inhibition zone, they were therefore considered inactive under the conditions of this study. The individual measurements and descriptive statistics are presented in Table 3.

Table 3. Antimicrobial activity of the treatments.

Treatment	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean $\pm$ SD, mm	Range, mm	Detectable inhibition
T1 – Distilled water	6.00	6.00	6.00	6.00 $\pm$ 0.00	6.00–6.00	0/3
T2 – Cefuroxime	14.905	12.895	12.975	13.592 $\pm$ 1.138	12.895–14.905	3/3
T3 – Guyabano (<i>A. muricata</i>)	6.00	6.00	6.00	6.00 $\pm$ 0.00	6.00–6.00	0/3
T4 – Alugbati (<i>B. alba</i>)	6.00	6.00	6.00	6.00 $\pm$ 0.00	6.00–6.00	0/3
T5 – Aratilis (<i>M. calabura</i>)	7.145	7.620	7.710	7.492 $\pm$ 0.304	7.145–7.710	3/3
T6 – Bignay (<i>A. bunius</i>)	6.00	6.00	6.00	6.00 $\pm$ 0.00	6.00–6.00	0/3
T7 – Banaba (<i>L. speciosa</i>)	6.00	6.00	6.00	6.00 $\pm$ 0.00	6.00–6.00	0/3

Note: The sterile discs measured 6 mm in diameter. A total measured diameter of 6 mm indicated no detectable inhibition beyond the disc margin. Measurements greater than 6 mm indicated detectable inhibition. Values are presented as mean $\pm$ SD from three discs tested using the same plant-extract preparation.

Table 4 presents the descriptive statistics of diameter across the two treatments showing clear zones of inhibition. Cefuroxime produced a higher mean diameter (13.592 $\pm$ 1.38) than aratilis (7.492 $\pm$ 0.304). The observed difference in means suggests that aratilis promoted greater diameter development compared with aratilis.

Table 4. Descriptive statistics of diameter by effective treatments.

	Treatment	N	Mean	Std. Deviation	Std. Error Mean
Diameter	Cefuroxime	3	13.592	1.38	.657073
	Aratilis	3	7.492	0.304	.175270

Note: Values represent the mean diameter $\pm$ standard deviation (SD) based on three replicates per treatment ($n = 3$). Standard error of the mean (SEM) is also presented.

The results of the independent-samples t-test comparing the mean diameter of the two selected treatments are presented in Table 5. The analysis revealed a statistically significant difference between the treatments, $t(4) = 8.970$, $p < .001$. The mean difference between treatments was 6.10 mm, with a 95% confidence interval ranging from 4.21 to 7.99 mm. Since the p-value was less than the 0.05 level of significance, the null hypothesis of no difference between treatments was rejected. These findings indicate that among the two treatments that exhibited a measurable zone of inhibition, one treatment produced significantly greater diameter measurements than the other.

Table 5. Independent samples t-test comparing diameter between treatments.

t-test for Equality of Means									
		t	Df	Significance		Mean Difference	Std. Error Difference	95% Interval of Difference	
				One-sided p	Two sided p			Lower	Upper
diameter	Equal variances assumed	8.97	4	<.001	<.001	6.1000	.680047	4.211887	7.98813
	Equal variances not assumed	8.97	2.283	.004	.008	6.1000	.680047	3.495669	8.704331

Note: $p < .05$ indicates a significant difference between treatments. Degrees of freedom (df) = 4. Values reported are based on the "Equal variances assumed" row.

4. Discussion

This study analyzed the phytochemical content and antibacterial activity of five herbal plants against *E. coli*: guyabano (*Annona muricata*), alugbati (*Basella alba*), aratilis (*Muntingia calabura*), bignay (*Antidesma bunius*), and banaba (*Lagerstroemia speciosa*). Understanding how these plants have been used in everyday life helps frame the significance of the present findings.

In traditional medicine, guyabano has been relied upon to ease rheumatism, arthritis, and other painful inflammatory conditions, and recent ethnopharmacological surveys continue to highlight its role in pain relief and infection management (Zubaidi et al., 2023; Mutakin, et al., 2022). Alugbati leaves are commonly used to treat burns, ulcers, and wounds, and recent studies confirm their wound-healing, anti-inflammatory, and antioxidant properties (Bose et al., 2023; Sagar et al., 2022). Banaba, one of the most recognized Philippine medicinal plants, has long been used for diabetes and kidney ailments, with recent pharmacological work validating its hypoglycemic and nephroprotective effects (Park & Lee, 2024; Ramos et al., 2025). Bignay has folkloric applications for anemia and hypertension, and newer studies document its antioxidant, cardioprotective, and antimicrobial potential (Nguyen-Ngoc et al., 2024). Aratilis is valued for relieving abdominal cramps and for its antiseptic properties, and recent investigations confirm its antimicrobial, anti-spasmodic, and anti-inflammatory activities (Cheong et al., 2022; Menon et al., 2025; Indraningrat et al., 2025). Importantly, recent ethnopharmacological reviews emphasize that Philippine medicinal plants remain under-explored despite their rich phytochemical diversity, and that systematic validation is needed to translate traditional claims into therapeutic applications (Atanasov et al., 2021; Coy-Barrera et al., 2023).

While most of the plant samples analyzed in this study contained secondary metabolites such as alkaloids, tannins, saponins, and steroids, only the aratilis leaf extract exhibited significant antibacterial activity (Table 2). Therefore, these findings

support the cited hypothesis regarding the contribution of phytochemical constituents in the leaf extract to antibacterial activity, with aratilis being the most significant.

When contextualized within the broader literature, the phytochemical profiles observed in this study align with previous reports. Phytochemical screening revealed that guyabano and banaba contained saponins and tannins, consistent with earlier findings in methanolic and aqueous extracts (Mutakin et al., 2022; Hernandez-Fuentes et al., 2024). Aratilis contained alkaloids, flavonoids, and tannins, corroborating prior studies highlighting its antimicrobial and antioxidant properties (Cheong et al., 2022; Menon et al., 2025; Sowjanya et al., 2023). However, no positive reactions were observed for the tested phytochemical groups in alugbati using the qualitative assays employed, in contrast to earlier reports of flavonoids and saponins in ethanolic extracts (Zhang et al., 2024), suggesting that solvent choice and extraction method may influence phytochemical yield. Alkaloids and tannins detected in bignay, and alkaloids, steroids, tannin, and saponins in banaba, corroborate earlier reports of their cytotoxic and antibacterial activity (Nguyen et al., 2024; Park et al., 2024). Collectively, these findings align with broader phytochemical surveys documenting alkaloids, flavonoids, tannins, saponins, and steroids in medicinal plants, highlighting their antimicrobial, anti-inflammatory, and anticancer properties (Acharjee et al., 2023; Ndezo Bisso et al., 2022; and Sicalan et al., 2023).

The antibacterial assay results further highlight the potential of aratilis as compared to the positive control. Table 3 presents the standard deviation, mean zone of inhibition (mm), and the frequency of inhibition for seven treatments tested against *E. coli* at 24 hours, showing measurable activity of cefuroxime and aratilis in inhibiting *E. coli* growth. Whereas guyabano, alugbati, bignay, and banaba showed no inhibition, unlike the negative control. The lack of activity in guyabano, alugbati, bignay, and banaba is consistent with the literature. According to Maramba-Lazarte et al. (2020), guyabano leaf extract had poor inhibitory activity against *E. coli* at all test concentrations. This may be due to the nature of *E. coli* being gram-negative bacterium. The results herein were first considered in the context of existing studies on the antibacterial activity and phytochemical composition of medicinal plants in the Philippines. do Nascimento and da Costa (2025) reported that the outer membrane of *E. coli* had a lipopolysaccharide (LPS) layer that acts as a barrier. The lack of measurable zones of inhibition of *E. coli* by the extracts of guyabano, banaba, alugbati, and bignay may be due to the permeability barrier posed by the LPS layer and lipopolysaccharides of *E. coli*.

Therefore, the findings indicate only preliminary antibacterial activity of fresh aratilis extract under the specific experimental conditions used. The strong statistical values and consistent groupings highlight the robustness of the observed differences, supporting the conclusion that antibacterial activity was present but limited to specific treatments (Sekar & Jalil, 2017).

The higher mean diameter observed in cefuroxime indicates that this treatment was more effective in enhancing diameter growth than aratilis (Table 4). The independent-samples t-test confirmed that the difference between treatments was

statistically significant ($t(4) = 8.970$, $p < .001$). This finding suggests that positive control, cefuroxime had a substantial positive effect on diameter compared with aratilis. The relatively lower standard deviation of aratilis indicates more uniform responses among replicates, while cefuroxime exhibited slightly greater variability. Nevertheless, the magnitude of the difference between treatment means was sufficiently large to yield a highly significant statistical result.

The significant difference observed between the two treatments suggests that they differed in effectiveness. Based on the descriptive statistics, cefuroxime had a higher mean diameter (13.592 mm) than aratilis (7.492 mm), yielding a mean difference of 6.10 mm. This finding indicates that the positive control was more effective in promoting the measured response than the experimental treatment (Table 4).

The narrow confidence interval (4.21-7.99 mm) further supports the consistency of the observed difference between treatments. Although only three replicates were used per treatment, the large difference in mean diameter was sufficient to produce a highly significant t-test result. Therefore, the observed effect is unlikely to have occurred due to random variation alone. These results demonstrate that cefuroxime has greater potential for achieving the desired biological effect under the conditions of the study. Values are based on three replicates per treatment ($n = 3$). Statistical significance was assessed at $\alpha = 0.05$. Results were interpreted using the "equal variances assumed" row, where $t(4) = 8.970$, $p < .001$, indicating a significant difference between treatments (Table 5). The statistical comparisons reflect differences among active treatments only and do not represent the full range of responses observed in the experiment.

These findings demonstrate that cefuroxime exerted antibacterial effects against *E. coli*, and aratilis exhibited measurable but weak antibacterial activity under the extraction conditions employed in this study. This inhibition zone is supported by the study conducted by Zakaria (2006), which also reported antibacterial activity of aratilis against *E. coli*. Indraningrat et al. (2025) reported that the ethyl acetate leaf extract of aratilis demonstrated clear inhibitory effects against *E. coli* and other standard pathogenic strains, attributing this activity to the presence of flavonoids, tannins, saponins, and steroids. The methanolic extracts showed inhibition zones between 3.33 and 14.60 mm against *E. coli* (Sekar and Jalil, 2017), and the solvent extracts of aratilis were found to be effective against *E. coli*, compared with standard antibacterials (Sowjanya et al., 2023). The results obtained agree with the present study, which showed that crude aratilis leaf extracts showed a moderate but measurable inhibition zone, with phenolics, tannins, and flavonoids identified as the primary bioactive constituents.

Independent samples t-test revealed that cefuroxime produced significantly larger inhibition zones than aratilis across the observed replicates, indicating superior antibacterial activity. While aratilis exhibited measurable antibacterial activity, the inhibition zones remained considerably smaller than those observed for cefuroxime. Therefore, the antibacterial effect of aratilis under the extraction conditions used in

this study should be regarded as limited relative to the standard antibiotic. The modest activity of aratilis suggests it could be a candidate for a natural antimicrobial agent. This is due to the activity potentially being attributed to flavonoids and tannins, which have been shown to disrupt bacterial membranes, inhibit intracellular components, and affect the integrity of the bacterial cell wall (Marouf et al., 2022; Shamsudin et al., 2022). Flavonoids and tannins may exert antibacterial effects through multiple mechanisms, some of which may be complementary. Disruption of bacterial membranes is one of the many effects of flavonoids. Flavonoids permeate the lipid bilayer of membranes. This leads to intracellular leakage and ultimately bacterial cell death. Flavonoids disrupt bacterial equilibrium, making them susceptible to lysis. Flavonoids limit bacterial survival via several methods. These methods include disrupting membrane integrity, perturbing nucleic acid and protein synthesis, disrupting cell wall biosynthesis, restricting efflux pump and biofilm activities, and interfering with metabolic processes and ATP production (Zhang et al., 2025). Zhang et al. (2025) and Sharma et al. (2025) discuss the diversity of the antibacterial mechanisms of flavonoids, beyond membrane damage, to include the restriction of protein and nucleic acid synthesis, cell wall biosynthesis, efflux pump activity, biofilm formation, and inhibition of energy metabolism. Flavonoids target pivotal enzymes in the respiratory chain and efflux pumps, thereby inhibiting bacterial growth and metabolism (do Nascimento & da Costa, 2025).

Liu et al. (2025) further highlighted the role of flavonoids in combating multidrug-resistant pathogens, noting their ability to synergize with conventional antibiotics by weakening bacterial defense systems. These diverse mechanisms explain why flavonoids are consistently associated with broad-spectrum antimicrobial activity and why their presence in Aratilis is particularly relevant to its observed inhibition of *E. coli*.

Alkaloids, another major class of phytochemicals detected in aratilis, also contribute to antimicrobial activity through distinct pathways. Many alkaloids act as enzyme inhibitors, blocking bacterial metabolic processes such as protein synthesis and cell wall formation (Zhang et al., 2026). Some alkaloids intercalate into DNA, disrupting replication and transcription, while others interfere with cell division by targeting microtubule assembly or related structures. Recent surveys (Thawabteh et al., 2024) confirm that alkaloids exhibit potent antibacterial activity at low MIC values, with mechanisms including DNA intercalation, enzyme inhibition, and synergistic interactions with other phytochemicals. These actions collectively impair bacterial viability and replication. Importantly, alkaloids have also been shown to act synergistically with flavonoids and tannins, enhancing overall antimicrobial potency.

Tannins, which were also detected in aratilis, complement these mechanisms by precipitating bacterial proteins, forming complexes with cell wall components, and reducing nutrient availability. Their ability to bind to adhesins and enzymes further limits bacterial colonization and virulence. Recent reviews (Huang et al., 2024) highlight the importance of tannins in a broad-spectrum antimicrobial defense system,

and, through synergistic interactions with other phytochemicals, they can inhibit nucleic acid synthesis and strengthen plant cell walls.

The combined presence of flavonoids, alkaloids, and tannins in aratilis thus represents a phytochemical profile with multiple overlapping antibacterial strategies, consistent with the moderate but observably significant inhibition zones observed at different time points. This indicates that naturally occurring phytochemicals can achieve preliminary antibacterial activity under certain conditions, but such statistical similarity should not be interpreted as therapeutic equivalence. This is especially useful in ethnopharmacological studies in regions with limited access to pharmaceutically synthesized antibiotics (Atanasov et al., 2021; Coy-Barrera et al., 2023).

The results of this study possess theoretical and practical implications. Theoretically, the results of this study provide further evidence for the existing body of literature linking phytochemicals to antimicrobial activity. Practically, this study demonstrates that the presence of significant phytochemicals in aratilis may offer an opportunity to supplement pharmacologically synthesized antimicrobials. This was especially noted, as aratilis exhibited significant antibacterial activity.

Several limitations must be considered. First, the study used crude leaf extracts, which may contain lower concentrations of active compounds compared to standardized or solvent-based extracts. The yield of phytochemicals in this study may have been influenced by the extraction method, as Sagar et al. (2022) and Zhang et al. (2024) reported the presence of flavonoids and saponins in alugbati extracted with ethanol. The non-significant effect of guyabano and banaba on microbial growth also corroborates Maramba-Lazarte et al. (2020), who reported that the poor inhibitory activity of *Annona muricata* extracts against *E. coli* is attributed to the choice of solvents and extraction method.

Second, only a single bacterial strain was tested in this study. Although measurable activity was shown against *E. coli*, it is possible that the activity was exhibited against several other gram-negative and gram-positive bacteria. Most extracts showed low activity, which may be due to some compounds' inability to penetrate the Gram-negative barrier of lipopolysaccharides (LPS), as noted by do Nascimento & da Costa (2025).

Third, the modest inhibition zones observed suggest that higher concentrations or purified compounds may be necessary to achieve potentially relevant effects. A methodological limitation of this study is that all inactive treatments (distilled water, guyabano, alugbati, bignay, and banaba) consistently produced 6.00 mm inhibition zones across replicates, resulting in zero variance. The extraction process employed in this study may have facilitated the recovery of all phytochemical constituents, particularly compounds that are more readily extracted with organic solvents or by heat-assisted methods. Although measurable antibacterial effects were observed, additional studies employing standardized bacterial strains, comprehensive phytochemical analyses, optimized extraction methods, and larger sample sizes are recommended to further verify and strengthen the reproducibility of the findings.

Another limitation of the present study is the use of a standard laboratory-maintained *Escherichia coli* strain rather than a characterized uropathogenic (*E. coli*) (UPEC) strain or a clinical isolate obtained from patients with urinary tract infections. Laboratory strains are often used in initial antibacterial screening due to their consistent and reproducible results. Still, they may differ in virulence phenotypes, antimicrobial resistance, and genetic diversity typically found among UPECs in clinical isolates. Therefore, the antibacterial activity exhibited by the plant extracts in this study must be interpreted as activity against the laboratory-maintained *E. coli* strain and cannot be directly extrapolated to UTI-causing or antibiotic-resistant strains of *E. coli*. The results should be confirmed with well-characterized UPEC reference strains and clinical isolates to more accurately evaluate the extracts' use in the management of UTIs.

Based on this, research can be directed towards optimizing extraction methods, quantifying active ingredients, and testing across several bacterial strains. Alternative statistical approaches, such as non-parametric tests or mixed-effects models, may also be considered to validate findings under conditions of zero variance. Also, testing the combined effects of phytochemicals and synergistic conventional antibiotics could help improve knowledge in the field, especially given the rise in antimicrobial resistance. Lastly, the study concludes that aratilis leaf extracts demonstrate preliminary antibacterial potential against *E. coli*, reinforcing the medicinal value of Philippine herbal plants and underscoring the need for continued phytochemical and pharmacological research.

5. Conclusion

The study sets out to determine the phytochemical composition and antibacterial efficacy of five herbal plants- guyabano (*Annona muricata*), alugbati (*Basella alba*), aratilis (*Muntingia calabura*), bignay (*Antidesma bunius*), and banaba (*Lagerstroemia speciosa*) against *Escherichia coli*, a primary pathogen of urinary tract infections. Screening revealed the presence of secondary metabolites: alkaloids, flavonoids, tannins, saponins, and steroids. These metabolites are known to exhibit antimicrobial activity. The lack of inhibition of guyabano, alugbati, bignay, and banaba in fresh-extract conditions highlights the importance of extraction methods and phytochemical availability in determining antibacterial efficacy. These findings underscore that crude extracts may not always capture the full therapeutic potential of plants, and standardized extraction techniques are essential for reliable evaluation. Among the tested species, only aratilis demonstrated measurable inhibitory activity, producing zones of inhibition that, while modest compared to the antibiotic control, still inhibit *E. coli* growth. This activity may be associated with water-soluble bioactive constituents present in the extract.

While aratilis exhibited measurable but weaker antibacterial activity than cefuroxime, its performance suggests potential as a plant-based antibacterial

candidate, particularly in resource-limited settings where antibiotic resistance is a growing concern. Future studies are recommended to focus on optimizing the extraction method, isolating and identifying the antimicrobial active components, and testing on a variety of bacterial strains. To confirm the connection between phytochemical constituents and antibacterial activity, a larger sample size and standardized bacterial strains are recommended, as well as other extraction processes. Also, many benefits could come from testing combinations of plant extracts with other antibiotics. Based on the evidence, it would be reasonable to investigate aratilis further to support its traditional use in medicine and to test its antimicrobial effects on additional bacterial strains.

However, the findings should be interpreted within the limitations of the study. The antibacterial activity was assessed using a standard laboratory-maintained *E. coli* strain rather than a characterized uropathogenic or antibiotic-resistant clinical isolate. Therefore, the results cannot be directly extrapolated to UTI-causing or multidrug-resistant *E. coli* strains. Further studies involving well-characterized uropathogenic *E. coli* (UPEC) strains, clinical isolates, larger sample sizes, and advanced phytochemical analyses are recommended to validate and expand upon the findings of this study. Despite these limitations, the results provide a basis for future investigations into the antibacterial potential of the evaluated plant extracts.

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Conflict of Interest Statement

The authors declare no financial or non-financial conflicts of interest related to this study.

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