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# Phytochemical Screening and Antibacterial Activity of Pandakaki-Puti (*Tabernaemontana pandacaqui Poir*) Ethanolic Plant Extracts

**Manuel Aulreck Siville V. Sicalan*, Alysa Vianca A. Agpalo, Jerleen Tefina R. Meliston, Thea Sofia Cassandra A. Pancho, Ian Jay P. Saldo, Jevoy Gumanyao, Mary Jade Peñafiel-Dando**

Integrated Basic Education Department, San Isidro College, Malaybalay City, Bukidnon, 8700 Philippines
*Corresponding author: sicalanmanuel@gmail.com

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**Abstract** Resistance of microorganisms, primarily bacteria, to antimicrobial treatments is a major potential global health risk, especially in developing countries. Investigating and developing new antibacterial agents is crucial to combat this antibacterial-resistant microorganism. T. pandacaqui plants have been known as herbal medicine and are widely distributed in the Philippines, but limited studies have been done on this plant species. Thus, this study aims to conduct phytochemical screening and determine the antibacterial activity of Tabernaemontana pandacaqui Poir. The biologically active constituents of the plant were identified using qualitative phytochemical screening, while the Kirby-Bauer method was used for the antibacterial test. The study's findings indicate that phenols and tannins were only present on the leaves and stem, while triterpenoids were present in all plant parts. The study also found that the T. pandacaqui plant extract exhibited a zone of inhibition for both bacterial strains but mostly against Pseudomonas aeruginosa. The root extracts were observed to have a zone of inhibition against P. aeruginosa in all concentrations for all concentrations (14.8mm at 25mg/ml, 13.8mm at 50mg/ml, 10mm for 75mg/ml, and 11.8 for 100mg/ml) while the leaf (75mg/ml with 10mm) against P. aeruginosa and stem extracts (8mm at 25mg/ml for S. aureus, and 8.8mm at 25mg/ml for P. aeruginosa). The results indicated a statistically significant difference in the stem and root extract's efficacy against P. aeruginosa (p=0.000). The current findings indicate that T. pandacaqui extracts have antimicrobial activity against both bacterial strains but mostly against P. aeruginosa. Additional research is needed to determine the antimicrobial potential of the crude extracts against other infectious agents.

***Keywords:*** *antibacterial activity, pandakaki-puti, phytochemical components, plant extracts, Pseudomonas aeruginosa, Staphylococcus aureus, Tabernaemontana*

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## 1. Introduction

The Philippines, specifically the province of Bukidnon, is one of the world's 17 mega-biodiverse nations, containing two-thirds of the planet's biodiversity and between 70 and 80 percent of its plant and animal species. There are 9,250 plant species in the Philippines, making up about 5% of the world's flora [1]. Among those plants found in the country, several medicinal plants have yet to be reviewed because their use is either poorly regulated or not regulated, despite several having shown substantial promise for wound healing. One of these exciting plants is the *Tabernaemontana pandacaqui Poir*, one example of such a plant with a high potential for its antibacterial and antioxidant benefits [2].

The pandakaki-puti plant (*Tabernaemontana pandacaqui Poir*) belongs to the family *Apocynaceae*, which can be found all across the tropics, but it is most common in countries of Southeast Asia. The *Apocynaceae* family has been known and used to treat various diseases because they possess great medicinal qualities. Additionally, a native Filipino species of this genus, *T. pandacaqui* (also called "t. kampupot" or "t. pandakaki-puti"), has been used for both medicinal purposes for quite some time. The plant is employed as an ethnobotanical source in countries such as Thailand, where the roots of the plant are utilized in the treatment of conditions including fever, discomfort, and diarrhea. However, some studies point out that more research needs to be done on *T. pandacaqui* and its possible medical applications. Given that this plant is also native to the Philippines—more specifically, the province of Bukidnon—where it serves as a popular decorative element thanks to its unique shape and lovely flowers. There must be a more extensive study on this plant's advantages, especially local varieties [3].

Given this, it has been studied that these medicinal plants, often made from natural materials, are used to treat various illnesses ranging from moderate to chronic, as stated by [4]. There have been many studies about this medicinal plant and the effects of the *Apocynaceae* family; however, further evidence of their safety and efficacy is needed to be considered [5]. Although the medicinal characteristics of the *Tabernaemontana* genus and other species in this family have been studied, from roots to flowers, *T. pandacaqui's* bioactive constituents, particularly its antibacterial and antioxidant activity, have not been broadly examined to the same level and are scarcely limited. Additionally, bioactive compounds in plants of the same genus can vary widely in concentration and identity due to cultivation and regional diversity [6]. Further research into this plant species could lead to revelations concerning this problem and the identification of additional antibacterial-rich plant species. Thus, these differences need to be researched so that ideally, the plant materials used for potential pharmacological benefits can be chosen.

There has been a rise in infectious diseases, which has become a significant public health concern, especially in underdeveloped countries [7]. The increase of microorganisms resistant to antimicrobials, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, is a significant threat to global public health and a major cause of nosocomial infections [8]. More people have died from illnesses caused by microorganisms that are antibacterial resistant than from any other infectious disease, and this problem is likely to get worse if the proper steps aren't taken to fix it. Clinical outcomes and death rates due to contagious infections are poor due mainly to bacterial resistance, as reported by [9]. The ability of microorganisms (particularly bacteria) to resist antimicrobial treatments has been identified as one of the top ten potential global health risks [10]. In connection, phytochemicals, including alkaloids, flavonoids, phenols, saponins, tannins, and triterpenoids, can be found in their natural plant and plant-based product forms. The presence of these phytochemicals is known to be responsible for their antibacterial activity, as previously described in the study conducted by [11]. In order to develop novel, efficient antibacterial and antioxidant drugs, it is crucial to perform scientific investigations to establish the efficacy of indigenous medicinal plants. This could reduce the high cost of healthcare while increasing patient access to necessary treatments.

The beneficial potential of *Tabernaemontana pandacaqui Poir* needs more research. The purpose of this research is to identify the specific phytochemical compounds found in *T. pandacaqui* present in its various vegetative parts; evaluate the antioxidant activity; test the biological activity of *T. pandacaqui* extracts as an antibacterial agent, and compare the biological activity of the leaves and stems of *T. pandacaqui*. Several of these plants are endemic. Therefore, learning as much as possible about their chemical makeup and medicinal potential is important. Research into the Pandakaki species could reveal new insights into the therapeutic potential of endemic species. This could lead to groundbreaking new treatments for many different diseases.

# 2. Methodology

## 2.1. Research Design

This study was quantitative research that would investigate the phytochemical compounds present and the antibacterial activity of Pandakaki-Puti (*Tabernaemontana pandacaqui Poir*) on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study used different phytochemical tests to determine their presence on the plant extracts and would select the different concentrations of *T. pandacaqui* plant extracts as the independent variable and the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and phytochemical presence as the dependent variable. This research would be experimental as it used many experimental procedures, concentrations, and controls.

## 2.2. Method of Data Analysis

To answer the problem statement, the study aims to analyze the relationship between the dependent and independent variables by collecting primary data. The data will include the mean, standard deviation, and one-way analysis of variance (ANOVA). The study utilizes a statistical approach that entails the comparison of means from three distinct groups to establish whether there is substantial proof that the corresponding population means are dissimilar. The action being taken aims to assess the hypothesis put forward in the study.

## 2.3. Phytochemical Screening Procedure

Ethanol solvents were used for the extraction of the leaves and stem of *T. pandacaqui*. Slight modifications in extract preparation, individual extracts were examined for the presence of secondary metabolites [12]. In the funnel, extracts were filtered using Whatman no. 1 filter paper. During the various tests, filters were used in combination with certain reagents.

### 2.3.1. Alkaloids

The presence of alkaloids was determined by Wagner's test. 1mL of the extract was taken and placed into a test tube. Then 1mL of potassium iodide (Wagner's reagent) was added and shaken. The appearance of a reddish-brown precipitate signifies the existence of alkaloids.

### 2.3.2. Flavonoids

The presence of flavonoids was determined using Shinoda's test. 400 mg (0.4 g) sample was extracted with 10 ml, ethanol was then filtered. 1 mL filtrate was added with magnesium ribbon and conc. HCl (5 drops) and allowed reaction. A pink or red color indicated the presence of flavonoids.

### 2.3.3. Phenols and Tannins

The presence of phenolics and tannins was determined using a ferric chloride test. 500 mg (0.5 g) sample was boiled in 30 mL distilled water and filtered. 2 mL filtrate was mixed with 1 mL 5% $FeC1_3$. The formation of blue, green, or violet color indicated the presence of phenolic and tannin compounds.

#### 2.3.4. Saponins

The presence of saponins was indicated using Foam Test. 1 g powdered sample was boiled in 10 mL distilled water for 15 min. After cooling, the extract was shaken vigorously to record froth formation. The presence of honeycomb froth above the surface after 30 minutes indicated the presence of saponins.

### 2.4. Antibacterial Activity Procedure

Analysis of the antibacterial activity of *T. pandacaqui* plant extracts was done using the agar disk diffusion assay or the Kirby-Bauer test [12,13]. The research samples will proceed to the Central Mindanao University College of Veterinary Medicine Laboratory for testing. Test organisms included one Gram-Positive, *Staphylococcus aureus,* and one Gram-Negative, *Pseudomonas aeruginosa*. These test organisms were provided by Central Mindanao University. Organisms were cultivated at 30°C for 24 hours on Nutrient agar, while the stock culture was maintained at 4°C.

Petri dishes and bent glass rods had been prepared and sterilized. Mueller-Hinton agar had been mixed and stirred with distilled water in a beaker. The solution would then be sterilized in an autoclave. The agar solution had been poured into the petri dish and it had been left for five minutes to harden. To introduce the test organisms, cultured *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the spread plating method was used. The following treatments were applied to sterile paper discs: ethanol (95%) as the negative control, streptomycin as the positive control, and four different concentrations of each plant extract; each treatment had 5 replicates: 25mg/ml, 50mg/ml 75mg/ml, 100mg/ml.

After the discs had dried, they were placed in four distinct corners. The Petri dishes had been labeled and the plates were incubated at 37°C to allow for the growth of bacteria. In order to determine the antibacterial activity of the test agent, the diameter of the zone of inhibition was measured in millimeters using a caliper. The susceptibility of extracts is assessed when the zone of inhibition is bigger than 7 mm, statistical analysis will be conducted [14].

### 2.5. Data Gathering Procedures

In this study, the data gathering procedure would include an antibacterial test that would be made use of disk diffusion by the Kirby-Bauer method. This test would require the following materials and equipment such as Petri dishes, Mueller-Hinton agar, beaker, bent glass rod, ethanol (99%), paper discs, incubator, and autoclave. Meanwhile, the phytochemical screening had been conducted using different phytochemical tests for each component. This test required certain materials such as test tubes, beakers, droppers, stoves, stirring rods, plant extracts, and certain reagents.

### 2.6. Statistical Treatment

The study conducted a qualitative phytochemical analysis on each ethanolic *T. pandacaqui* plant extract to identify the biological components present in each plant part. The aim was to compare the results of the analysis for each plant part. For the antibacterial test, after the incubation period, data was collected on the inhibition of the plant extracts. The study utilized Zone of Inhibition Testing, or Kirby Bauer's Test, to measure the potential of plant extracts to inhibit the growth of selected microorganisms. The measurement of the inhibition zone was conducted to determine the effectiveness of the extract in preventing the growth of these microorganisms. The recorded mean diameter of the zone of inhibition has been interpreted using the Kirby-Bauer disk diffusion susceptibility test protocol [15]. The evaluation of extract susceptibility involves conducting statistical analysis when the zone of inhibition exceeds 7 mm [14]. The study aims to analyze the relationship between the dependent and independent variables by collecting primary data. The data will include the mean, standard deviation, and one-way analysis of variance (ANOVA). The study utilizes a statistical approach that entails the comparison of means from three distinct groups to establish whether there is substantial proof that the corresponding population means are dissimilar. The action being taken aims to assess the hypothesis put forward in the study.

## 3. Results and Discussion

### 3.1. Phytochemical Screening

Phytochemical screening is beneficial for several reasons, including the discovery of bioactive molecules that can be employed in the synthesis of effective medications as well as the identification of plant extracts whose contents are most abundant [16]. The relative chemistry and quantities of the components in the plant extract are revealed by the results of the phytochemical screening. To better comprehend the pharmacological significance and health dangers, it is of the utmost importance to analyze medicinal plants to identify their phytochemical components.

**Table 1. Phytochemical Screening of *T. pandacaqui* plant extracts**

| *T. pandacaqui* Ethanolic Extracts | Phytochemicals | | | | | |
|---|---|---|---|---|---|---|
| | Alkaloids | Flavonoids | Phenols | Tannins | Triterpenoids | Saponins |
| Leaves | - | - | +++ | +++ | ++ | - |
| Stem | - | - | +++ | +++ | +++ | - |
| Roots | - | - | - | - | +++ | - |

Legend: negative (-), slight (+), moderate (++), abundant (+++)

Tabulated in Table 1 are the Phytochemical Results from *T. Pandacaqui Poir* Plant Extracts. Extracts from the plant's leaves, stems, and roots are shown in the table, along with their corresponding Phytochemical activity reactions and outcomes. Six phytochemicals were examined—alkaloids, flavonoids, phenols, tannins, triterpenoids, and saponins. The leaves exhibit a lack of presence of Alkaloids, Flavonoids, and Saponins. Triterpenoids indicate a moderate level of occurrence within the leaves. The extracts of leaves exhibit a significant abundance of Phenols and Tannins. There are no detectable alkaloids, flavonoids, or saponins in the analyzed stem samples; however, there is a high concentration of phenols, tannins, and triterpenoids. Finally, it should be noted that Triterpenoids are the only compounds abundantly present in roots. Conversely, the absence of Alkaloids, Flavonoids, Phenols, Tannins, and Saponins yielded a negative outcome.

The study demonstrates the existence of phytochemical constituents within the *T. Pandacaqui* plant, specifically in the phenol, tannin, and triterpenoid components. It is possible that some of the phytochemical components do not contain alkaloids, flavonoids, phenols, tannins, and saponins. The content of plant materials can be affected by various factors, including plant species, genetic background, a portion of plant studies, growing conditions, agronomic practices, and post-harvest handling [17]. They can be found in a wide variety of shapes and sizes, and these variations are not limited to individual plants but also to seasonality, growth stage, latitude, and longitude [18]. It has been observed that drying causes a reduction in the amount of plant bioactive components [19]. The observed presence of phytochemicals may be attributed to the drying process employed in the oven, which was conducted at a temperature of 120°C and has contributed to reducing phytochemicals and other constituents. It has been shown that the phytochemical content of a plant is greatly diminished when dried at a higher temperature [20].

## 3.2. Antibacterial Test

One of the most significant potential global health risks for 2019 has been named by the World Health Organization (WHO) as the resistance of microorganisms, primarily bacteria, to antimicrobial treatments. Antibiotic-resistant organisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* are on the rise and pose a severe risk to public health globally [8]. They are also increasingly to blame for nosocomial infections. Table 2 summarizes the recorded measurements of the inhibition zone diameter resulting from various treatments and concentrations of *T. pandacaqui* plant extracts against *S. aureus* and *P. aeruginosa.*

The presented data in Table 2 display the outcomes of an antimicrobial susceptibility test, specifically the zone of inhibition. The study was conducted to evaluate the efficacy of varying concentrations of *T. pandacaqui* extracts derived from its leaves, stem, and roots against two bacterial strains, namely *S. aureus* and *P. aeruginosa*, and the zone of inhibition (mm) was measured for each concentration and bacterial strain. By the guidelines for antibiotics, the primary data obtained from this test is typically presented as either a zone size or Minimum Inhibitory Concentration (MIC) [21]. Kirby-Bauer disk diffusion susceptibility test protocol was followed to determine whether or not the isolates were susceptible, intermediate, or resistant based on the size of the diffusion zones they produced [15]. The positive control, amikacin, produced a zone of inhibition of 10mm or more against both bacterial strains at all four concentrations. This indicates that amikacin is an effective antibiotic against *S. aureus* and *P. aeruginosa*. The negative control, ethanol, did not produce any inhibition zone, suggesting it is not an effective antibiotic. The study results indicate that the extracts derived from the leaves stems, and roots of *T. pandacaqui* exhibited inhibitory effects against both bacterial strains. The roots showed a larger zone of inhibition than the leaves or stem, with a greater degree of inhibition observed for *P. aeruginosa* than for *S. aureus*. The findings indicate that the extracts derived from the roots of *T. pandacaqui* exhibit the most significant antimicrobial efficacy against both bacterial strains.

The zone of inhibition for the *T. pandacaqui* leaves was 0mm at all four concentrations for both bacterial strains, excluding the 75mg/ml concentration against *P. aeruginosa*. This indicates that the extracts from the leaves were not effective in inhibiting the growth of either *S. aureus* or *P. aeruginosa* but were also effective in specified concentrations. The leaves of *T. pandacaqui* had significant inhibitory activity against multidrug-resistant *Staphylococcus aureus* [22]. There are a few possible reasons why the leaf extracts may not have had any inhibitory effect against both bacterial strains. This could have been due to insufficient concentration and the difference in the preparation of the plant material, leading to the varying phytochemicals responsible for the plant extract's antibacterial activity. Different concentrations of plant extracts are needed to test for the zone of inhibition because the attention of the active compounds in the extract can vary. Plants use biological constituents such as flavonoids, alkaloids, tannins, saponins, and a variety of other secondary metabolites to defend themselves against invasion by various microorganisms, insects, and other herbivores [23].

**Table 2. Zone of Inhibition of *T. pandacaqui* Plant Extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa***

| *Plant Sample* | *Zone of Inhibition (mm)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | | | | *P. aeruginosa* | | | |
| | *25mg/ml* | *50mg/ml* | *75mg/ml* | *100mg/ml* | *25mg/ml* | *50mg/ml* | *75mg/ml* | *100mg/ml* |
| leaves | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| stem | 8 | 0 | 0 | 0 | 8.8 | 0 | 0 | 0 |
| roots | 0 | 0 | 0 | 0 | 14.8 | 13.8 | 10 | 11.80 |
| Amikacin | 10 | 10 | 10 | 10 | 34 | 34 | 34 | 34 |
| Ethanol | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The zone of inhibition for the *T. pandacaqui* stems was 8mm at 25mg/ml for *S. aureus* and 8.8mm at 25mg/ml for *P. aeruginosa*. This indicates that the extracts from the stems effectively inhibited the growth of S. aureus and *P. aeruginosa* at low concentrations but not at high concentrations. The extracts from the stems indicate varying concentrations resulting in different effects in the zone of inhibition. The impact of common plant extract concentration, relative to higher concentrations, varies depending on the plant extract and the tested microorganism. Other studies, however, have demonstrated that lesser concentrations of plant extract have a greater effect on the zone of inhibition than higher concentrations [24,25]. Lower concentrations appeared to have a better zone of inhibition than those with higher concentrations [26].

The roots of *T. pandacaqui* have shown a zone of inhibition against *P. aeruginosa*, 14.8mm at 25mg/ml, 13.8mm at 50mg/ml, 10mm for 75mg/ml, and 11.8 for 100mg/ml. This indicates that the extracts from the roots were effective in inhibiting the growth of *P. aeruginosa* at all concentrations and were not effective in inhibiting the growth of *S. aureus* at any concentrations. It is common for roots to possess more significant levels of active compounds in comparison to other plant components [27]. Certain investigations have indicated that the root extracts derived from *Tabernaemontana* plants, particularly *Tabernaemontana Stapfiana Britten* (Apocynaceae), have demonstrated remarkable antibacterial properties [28].

### 3.3. Significant Difference

**Table 3. One-Way Analysis of Variance (ANOVA) *T. pandacaqui* plant extracts against *Staphylococcus aureus***

| *T. pandacaqui* Samples | p-value |
|---|---|
| Leaves | - |
| Stem | - |
| Roots | - |

The zone of inhibition of *T. pandacaqui* plant extracts and the positive and negative controls had no significant difference in the susceptibility level against the gram-positive bacteria *Staphylococcus aureus*, as there was no yield in the results, as shown in Table 3. This means that the data collected was insufficient and that there are no differences between the groups given that the zone of inhibition, as shown in Table 2, where all variables had the same result, was zero zones of inhibition against *S. aureus*. This could have been because of the need for variations between the groups, that in return, becomes difficult to detect significant differences. This can happen when the data points within each group are very similar. Therefore, the study failed to reject the null hypothesis that the means of the groups are equal.

The stem extract in this study possesses a zone of inhibition of 8mm; similarly, another study reports that the stem bark of *Secondatia floribunda A. DC (Apocynaceae)* showed antibacterial activity against strains of S. aureus [29]. Additionally, Ethanolic extracts obtained from the leaves and roots of *T. pandacaqui* in different concentrations showed no antibacterial activity against *S. aureus*. This could have been because the plant collected did not contain enough active compounds responsible for the antibacterial activity. Some phytochemical compounds, specifically the alkaloidal fraction, demonstrated antibacterial activity against *S. aureus* but at a significantly lower concentration [30]. The absence or less effect of antimicrobial activity of plant extract indicates the lack of active ingredients in this solvent [31].

**Table 4. One-Way Analysis of Variance (ANOVA) *T. pandacaqui* plant extracts against *Pseudomonas aeruginosa***

| *T. pandacaqui* Samples | p-value |
|---|---|
| Leaves | - |
| Stem | .000 |
| Roots | .000 |

Based on the provided results, the stem and roots show highly significant results, p = .000. The associated p-value of .000 suggests that the observed difference is doubtful to occur by chance alone. However, the leaves show no result in the significance level of the analysis of variance in the zone of inhibition of *T. pandacaqui* leaves and both the positive and negative controls against the bacterium *P. aeruginosa*. This means that there is no variability or variance between the groups. In this case, the F-value and p-value will typically be undefined or not calculable because the denominator of the F-ratio becomes zero. In conclusion, the ANOVA results indicate strong evidence of a significant difference among the groups being compared in the stem and roots but not on the leaves.

Similar to another study, *T. pandacaqui* leaves had no inhibitory effect on *Pseudomonas aeruginosa* [22]. This could have been due to the presence of the cell wall structure of Gram-negative bacteria that may account for their resistance. Numerous studies indicate that the outer membrane of Gram-negative bacteria, such as *P. aeruginosa*, can be an effective permeability barrier against the penetration of toxic particles, such as antibiotics [32]. In addition, the stem and root extracts inhibited the bacteria *P. aeruginosa* which caused variation in the data and the p-value. Methanolic extracts of the stem and root bark of *Tabernaemontana Stapfiana* Britten (*Apocynaceae*) were effective against various bacterial strains, including those resistant to multiple antibiotics [28]. Other studies have also shown that stem bark extracts possess broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria [33]. Furthermore, several scholarly investigations have examined the existence of bioactive constituents, including alkaloids and flavonoids, within *Tabernaemontana flora* [34]. The elevated concentration of said compounds in the root extracts may plausibly contribute to their increased zone of inhibition.

## 4. Conclusion

Based on the thorough analysis and evaluation of the results and findings of the study the following conclusions are drawn:

Certain phytochemical components, namely phenols, tannins, and triterpenoids, are present in the plant extracts of *T. Pandacaqui Poir*. On the other hand, alkaloids, flavonoids, and saponins are all conspicuously absent from the leaves. Some of the plant's phytochemical components might not have been present due to the process of conducting the study. These findings support the possibility that *T. Pandacaqui* contains phytochemical components that help resist diseases and have nutrient value.

The extracts from *T. pandacaqui* leaves did not influence the zone of inhibition for either S. aureus or *P. aeruginosa*, although it did have an effect at a specified concentration tested. Additionally, extracts from the stems of *T. pandacaqui* were able to suppress the growth of *S. aureus* at low concentrations, but not at high concentrations, as measured by the zone of inhibition. Furthermore, *T. pandacaqui* root extracts showed a significant zone of inhibition against *P. aeruginosa* growth at all concentrations tested. The positive control, amikacin, exhibited a zone of inhibition or greater against both bacterial strains across all four concentrations. The data indicate that amikacin exhibits efficacy as an antibiotic agent against both *S. aureus* and *P. aeruginosa*. On the other hand, it appears that ethanol, which served as the negative control in this experiment, is not effective as an antibiotic because there was no zone of inhibition measured for it.

The findings of the research indicate that the extracts obtained from various sections of *T. pandacaqui Poir*, including the leaves, stems, and roots, exhibited qualities that inhibited the growth of both types of bacterial strains. The result of the study shows that there is a significant difference in the zone of inhibition of the roots and stem as a result of the ANOVA test. Root extracts suppressed *P. aeruginosa* at all concentrations. *P. aeruginosa* was more strongly inhibited than *S. aureus*, and the zone of inhibition was significantly larger in the roots than in the leaves or stems. Additionally, it shows a significant difference as it inhibited both bacterial strains in lower concentrations. The results show that the *T. pandacaqui* root extracts have the greatest antibacterial activity against *P. aeruginosa* and have a significant difference.

# 5. Recommendation

The phytochemical and antibacterial activities of *T. pandacaqui* plant extract raises the possibility that the plant contains bioactive substances with a strong potential for antibacterial activity. Additional studies can be done on other plants, particularly those in the same family and on several Gram-positive and Gram-negative bacteria. It is also advised to test the effectiveness of several extraction techniques, such as microwave- or ultrasound-assisted extraction, to extract bioactive substances from fruit rinds.

To determine the ideal formulation that can successfully suppress the growth of particular bacterial strains, it is also recommended to evaluate different quantities and combinations of bioactive chemicals taken from plants. This is because different bacterial strains may be resistant to different amounts of the antibacterial activity of the bioactive chemicals found in plants. Additionally, the promotion of organic and sustainable antimicrobial treatments can be advanced by using herbal plants as a natural substitute for conventional antibiotics. However, Additional research is needed to determine the antimicrobial potential of the crude extracts against other microorganisms. This will help ensure that the extracts can be used in antimicrobial treatments.

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