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Evaluation of the Antimicrobial Activity of Ethanolic Leaf Extract of *Tabernaemontana pandacaqui* Lam. against Wound-Infecting Pathogens

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Abstract

Wound infections caused by antibiotic-resistant microorganisms are widely recognized as a major public health concern, particularly in developing countries. To combat antibiotic-resistant microorganisms, it is critical to develop new antimicrobial agents. Local medicinal plants are viewed as potential candidates for the treatment of microbial infections. Thus, the purpose of this study is to determine the antimicrobial activity of *Tabernaemontana pandacaqui* Lam. leaves against wound-infecting pathogens. The biologically active constituents of the plant were identified using qualitative phytochemical screening and Fourier Transform Infrared (FTIR) analysis of the crude extract. The study's findings indicated that *T. pandacaqui* leaf extract was antimicrobial against a variety of pathogens tested. The highest zone of inhibition was observed against the pathogenic fungus *Candida tropicalis* at a mean value of 17.73 ± 2.42 mm, followed by *Staphylococcus aureus* at a mean value of 14.73 ± 3.17 mm. However, leaf extracts had no inhibitory effect on *Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus* (MRSA), and metallo-beta-lactamase *Pseudomonas aeruginosa* (MβL- *P.aeruginosa*). The bioactivity of leaf extracts is attributed to the active constituents contained within. The results indicated a statistically significant difference in the leaf extract's efficacy against *Staphylococcus aureus* (p=0.007) and *Candida tropicalis* (p=0.008). The current findings indicate that *T. pandacaqui* leaf extracts have antimicrobial activity against wound-infecting pathogens. Additional research is needed to determine the antimicrobial potential of the crude extracts against other infectious agents.

Keywords: antibiotic-resistance, antimicrobial activity, disc diffusion assay, natural products, *Tabernaemontana pandacaqui* Lam.

Introduction

Over the last few decades, wound infection has remained a significant clinical challenge in hospitals, particularly in developing countries where adequate health care delivery is hampered by resource constraints (Kihla et al., 2014). Barbosa and Martins (2018) define wound infection as the presence of replicating microorganisms within a wound that results in host injury. Generally, it is caused by a variety of microscopic organisms, including bacteria and fungi. According to Reddy et al. (2008), open wounds are susceptible to bacterial, fungal, and viral infections and act as an entry point for foundational infections, putting them at a higher risk for severe complications (Mummed et al., 2018; Tiwari et al., 2012). Infected wounds frequently produce noxious exudates and toxins, as

well as the death of regenerating cells (Flanagan, 2003; Jalalpure et al., 2008). To alleviate discomfort and pain associated with wounds, to prevent infection, and to activate tissue repair processes, it is necessary to promote wound healing and restore normal body functions to the affected area (Reddy et al., 2008).

Each year, approximately 3,300 people die directly as a result of an infection caused by antibiotic-resistant microorganisms, and the risk of such infections is comparable to that of infectious diseases such as influenza, tuberculosis, and HIV/AIDS combined (Cassini et al., 2019). Additionally, a World Health Organization-funded survey discovered a prevalence of nosocomial infections ranging from 3 to 21%, with wound infections accounting for 5 to 34 %. Wound infection was one of the top five causes of morbidity in the Philippines. This was confirmed by a study conducted at the University of the Philippines-Philippine General Hospital. According to the findings, wound infection is a significant cause of morbidity and mortality in patients, owing to the steady increase in antibiotic-resistant microorganisms (Abesamis & Cruz, 2019). Antibiotic-resistant microorganisms pose an alarming and growing threat to modern health care.

Antibiotic-resistant microorganisms are rapidly spreading across the globe, jeopardizing antibiotic efficacy (Golkar et al., 2014). As demonstrated by Lomazzi et al. (2019), microorganisms such as bacteria, fungi, and viruses that are highly exposed to antimicrobial drugs can develop the ability to resist the drugs intended to eradicate them, jeopardizing antibiotics' ability to treat common diseases. Mummed et al. (2018) discovered that bacteria and fungi such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida* spp., and *Aspergillus* spp. are frequently associated with wound infections, and several of them are antibiotic-resistant. These pathogens have the potential to significantly slow, if not completely halt, the wound healing process, resulting in functional limitations of the damaged tissue, which can exacerbate pain and discomfort (Annan & Houghton, 2008). Indeed, the emergence of antibiotic-resistant pathogens has grown to be a significant global threat. This highlights the critical need for new antibiotics to treat common infections such as wound infection.

Over the last few years, there has been an increase in the development of novel antibiotics capable of combating the global threat of antibiotic resistance. Local medicinal plants are being screened for antimicrobial activity and are being considered as promising candidates (Dan et al., 2018). Medicinal plants have been critical to human health and well-being since the dawn of civilization (Bag et al., 2012). They are typically made with natural ingredients and are used to treat minor or chronic ailments. Natural products are small molecules that biological organisms synthesize (Krause & Tobi, 2013). Numerous studies have concluded that natural products are more affordable, environmentally sustainable, and less toxic than commercially available antibiotics or have fewer side effects (Lirio et al., 2019; Moloney, 2016). The World Health Organization estimates that approximately 80% of the world's population lives in developing countries and is heavily reliant on medicinal plants for health care due to cultural traditions or a lack of alternatives. Plants contain an abundance of bioactive compounds with antimicrobial properties that can be used to treat or prevent microbial infections in a cost-effective and safe manner (Bag et al., 2012). Thus, it is critical to conduct scientific trials to determine the efficacy of indigenous medicinal plants in order to develop new and effective antimicrobial agents.

In the Philippines, the Department of Health-Philippine Institute of Traditional and Alternative Health Care has endorsed only ten medicinal plants following scientific validation to ensure their safety and efficacy (Boy et al., 2018; Principe & Jose, 2002). Although a number of medicinal plants in the Philippines have demonstrated promising potential for wound healing, many remain untested and their use is either poorly regulated or not regulated at all. One such plant is *Tabernae-*

montana pandacaqui Lam., commonly referred to as pandakaki-puti in the Philippines, is a member of the dogbane family, Apocynaceae. Members of this family, as Gruyal and Medina (2019) note, are exploited for their secondary metabolites, most notably alkaloids. Secondary metabolites have been shown to have a variety of biological effects, establishing a scientific basis for the use of herbs in traditional medicine, as well as possessing antimicrobial properties (Hussein & El-Anssary, 2019). Numerous studies on *T. pandacaqui* have been conducted, and the results and findings indicate that this species possesses antiprotozoal (Bradacs et al., 2011), anti-inflammatory, antipyretic, and antinociceptive (Taesotikul et al., 2003), cardiovascular effects (Taesotikul et al., 1989), antibacterial (Gruyal et al., 2019), and antihelminthic properties (Mandanas, 2018). Apart from the studies mentioned, limited attempts were made to explore the antimicrobial properties of *T. pandacaqui* leaves.

T. pandacaqui's various parts have been used medicinally in the past. There are, however, a few scientific studies that have evaluated the antimicrobial activity of *T. pandacaqui* leaves against wound-infecting pathogens. Thus, the purpose of this study is to examine the antimicrobial potential of ethanolic leaf extract of *T. pandacaqui* against common pathogens associated with wound infection and to serve as a natural source to supplement the growing need for effective antibiotics to combat the problem of antibiotic-resistant microorganisms.

Materials and Methods

Specimen Collection Plant Material

The leaves of *T. pandacaqui* were chosen for investigation of its antimicrobial properties. Morphologically, *T. pandacaqui* leaves are elliptical to narrowly elliptical in shape, as described by Leeuwenberg (1988). The apex is either obtuse or occasionally rounded. The secondary veins are usually pale green on both sides, and the petiole measures between 2 and 20 mm (Figure 1).

Specimen Collection, Preparation, and Identification

Fresh leaves of *T. pandacaqui* were collected in Brgy. San Eugenio, Natividad, Pangasinan, Region I, Philippines (latitude 16.0382°N, longitude 120.8384°E). The sample was placed in an airtight polypropylene-lock bag with the appropriate labeling and washed carefully with clean water to remove any dust, dirt, residue, or other contaminants. Until used, the cleaned leaves were stored and air-dried in a cool, dry location. To ensure proper identification, plant specimens were authenticated at the Institute of Biology Jose Vera Santos Memorial Herbarium (PUH), College of Science, University of the Philippines-Diliman (UPD), Diliman, Quezon City, Philippines.

Extraction of Plant Sample

The leaves of *T. pandacaqui* were dried for approximately 14 days at room temperature using shade drying (Chao et al., 2017). These dried samples were cut and crushed in an electric blender until a 90-gram fine powder was obtained. The fine powder was collected into a clean vessel and soaked in 95% ethanol at a ratio of 1:3 w/v (Mailoa, 2013), in which 270 mL of ethanol was added to 90 grams of plant fine powder and stored for three days with occasional shaking (Gupta et al., 2011). Following maceration, the homogenates were filtered through a filter No. 39 (Dent et al., 2013) and transferred to a 200 mL beaker. Excess solvent was evaporated from the filtered extracts by placing them in a water bath at a low temperature for more than an hour (Gupta & Dhawan, 2016). After the evaporation process was complete, the sample was scraped, collected, and transferred to a small glass tube, from which a 12 mL crude extract was obtained and stored aseptically in a laboratory refrigerator for subsequent use.

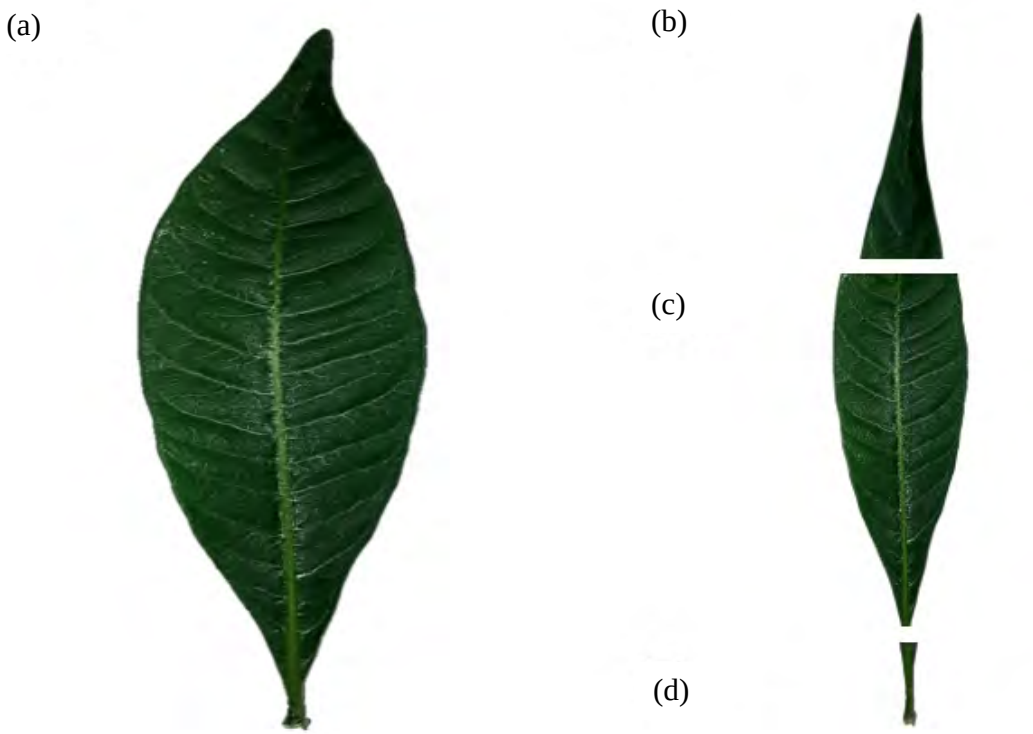

Figure 1. Tabernaemontana pandacaqui Lam. leaf external morphology: (a) elliptical shape, (b) obtuse apex, (c) pale green secondary veins, and (d) short petiole.

Test Microorganisms

The antimicrobial activity of *T. pandacaqui* leaves was evaluated against the common wound pathogens, including two multidrug-resistant bacteria, one gram-positive and negative bacteria, and one pathogenic fungus. They were selected on the basis of their clinical and pharmacological significance. The bacterial and fungal strains were provided by the Polytechnic University of the Philippines' Microbial Collection Culture (PUPMCC). While, the multidrug-resistant strains were obtained from the De La Salle University's Microbial BioBanks (DLSU-MB). Table 1 lists the pathogens and resistant phenotypes used in the study.

Table 1. Selected pathogens and resistant phenotypes used

Pathogens	**Resistant Phenotypes**
Multi- drug resistant bacteria	Trimethoprim-sulfamethoxazole, Cefoxitin, Oxacillin, Penicillin, Amikacin, Cefepime, Ceftadizime, Imipenem, Meropenem
MR- *Staphylococcus aureus*	
MβL- *Pseudomonas aeruginosa*	
Gram-positive bacteria	
Staphylococcus aureus (BIOTECH 1582)	
Gram-negative bacteria	
Pseudomonas aeruginosa (BIOTECH 1335)	
Fungus	
Candida tropicalis (BIOTECH 2085)	

Phytochemical Analysis

Following standard procedures, the ethanolic leaf extract of *T. pandacaqui* was subjected to qualitative phytochemical analysis to determine the presence and absence of biological constituents such as flavonoids, alkaloids, tannins, glycosides, saponins, resins, phytosterols, anthraquinones, carbohydrates, reducing sugars, and proteins with peptide bonds. The analysis was conducted at the National Institutes of Health's Institute of Pharmaceutical Sciences at the University of the Philippines Manila (UPM) in Manila, Philippines. Table 2 summarizes the test results.

Table 2. Qualitative phytochemical screening procedures and its positive indicator

Tests	**Positive Indicator**
For Carbohydrates	
Molisch Test	Violet ring at the junction
For Reducing Sugars	
Fehling's Test	Formation of brick red precipitate
For Flavonoids	
Alkaline Reagent Test	Yellow coloration which disappears upon the addition of dilute acid
Lead Acetate Test	Presence of yellow turbidity or precipitate
For Alkaloids	
Hager's Test	Yellow precipitate or turbid solution
Mayer's Test	White precipitate or turbid solution
Wagner's Test	Reddish brown or turbid solution
For Tannins	
Ferric Chloride Test	Blue solution- presence of gallic tannins Green to black solution- presence of catecholic tannins
For Glycosides	
Keller Killani Test	Reddish brown/purple ring at the junction
For Saponins	
Froth Test	Froth greater than 2 cm even after 30 seconds
For Resins	
Test for Resins	Turbid Solution
For Phytosterols	
Liebermann-Burchard Test	Brown ring at the junction Green upper layer- presence of sterols Deep red- presence of triterpenoids
For Anthraquinone	
Test for Anthraquinone	Red color
For Proteins (Peptide Bonds)	
Test for Resins	Purple violet color

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was performed on the ethanolic leaf extract of *T. pandacaqui* to determine the functional groups and types of chemical bonds present in the crude extract. The analysis was con-

ducted at the University of Santo Tomas-Research Center for Natural and Applied Sciences (UST-RCNAS), located in Manila, Philippines.

Antimicrobial Assay

The antimicrobial activity of *Tabernaemontana pandacaqui* Lam. leaf extract was determined using a modified Kirby- Bauer disk diffusion susceptibility test protocol (Bauer et al., 1966).

In a petri dish, Whatman filter paper discs were placed and autoclaved for 45 minutes (Kala et al., 2010). The sterile discs were inserted into sterile 96-well microplates and dispensed 100 µl of crude extracts using a sterile micropipette. The discs were then dried at room temperature. The test microorganisms prepared by growing the cultures in Mueller-Hinton broth (MHB) and incubated for 24 h, followed by turbidity level adjustment to match 0.5 McFarland standards, or 1.5 10^8 colony-forming unit (CFU)/ml. Standardized test organisms were swabbed over the sterile Mueller-Hinton agar (MHA) plate surface. Using a sterile pointed forceps, the discs containing *T. pandacaqui* leaf extracts were impregnated onto the plate.

The plates were inverted and allowed to set for approximately 1 h and incubated for 24 h. Antimicrobial activity was determined by observing and measuring the diameter of the zone of inhibition (ZOI) surrounding the discs using a millimeter-scale Vernier caliper. The following standard antibiotics were used as positive controls for bacteria: Rifampicin (5 mcg/ disc, TM Media), Trimethoprim (5 mcg/ disc, TM Media), Penicillin (10 units/ disc, TM Media), and Ofloxacin (5mcg/ disc, TM Media); Nystatin was used as a antibiotic control for fungi. Meanwhile, the discs containing sterile water served as the negative control. All tests were done in triplicates.

Statistical Analysis

The experimental results were expressed as the mean and standard deviation (SD) of the replicates. To determine significant group differences, mean values were compared between ZOIs of plant extracts and antibiotic control using one-way analysis of variance (ANOVA) and t-test using JASP version 0.14.1 (JASP Team 2020). Means were considered statistically significant if p-value is less than 0.005.

Results and Discussion

Antimicrobial Activity

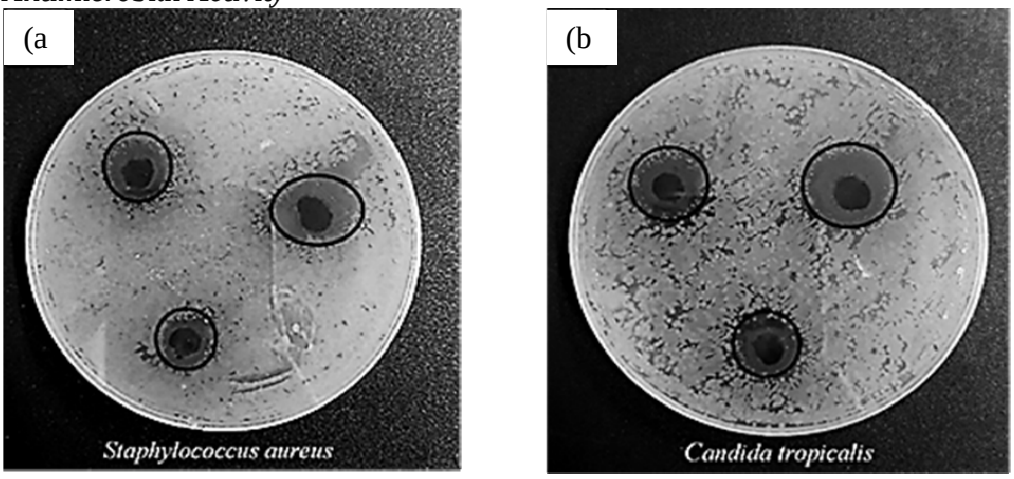

Figure 2. Disc diffusion assay results of ethanolic leaf extract of Tabernaemontana pandacaqui Lam. against wound-infecting pathogens: (a) leaf extract against Staphylococcus aureus, and (b) leaf extract against Candida tropicalis.

The efficacy of *Tabernaemontana pandacaqui* Lam. leaf extracts against several microorganisms associated with wound infection was determined using the disc diffusion method. Leaf extracts were found to have varying degrees of antimicrobial activity against test microorganisms. As illustrated in Figure 2, disc diffusion analysis revealed that *T. pandacaqui* leaf extract had a significant inhibitory effect on gram-positive bacteria and fungi, *Staphylococcus aureus* and *Candida tropicalis*, respectively. On the other hand, *T. pandacaqui* Lam. leaf extracts had no inhibitory effect against gram-negative bacteria, *Pseudomonas aeruginosa*, and multidrug-resistant bacteria, *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (MβL).

Five antibiotics were used as positive controls in this study: Rifampicin (5 mcg/ disc), Trimethoprim (5 mcg/ disc), Penicillin (10 units/ disc), Ofloxacin (5mcg/ disc), and Nystatin. Table 3 summarizes the antimicrobial activity of *T. pandacaqui* leaf extracts and antibiotic control against wound-infecting pathogens. The research findings indicate a statistically significant difference in the efficacy of the leaf extract compared to a positive control against *Staphylococcus aureus* (p=0.007) and *Candida tropicalis* (p=0.008), as determined by One-Way Analysis of Variance (ANOVA) and t-test at 0.05.

Table 3. Average diameter of the zone of inhibition of the plant extracts and antibiotic

	Treatment	**Antibiotics Control**					
Pathogens	**Leaf extract**	**Rifampicin**	**Trimethoprim**	**Ofloxacin**	**Penicillin**	**Nystatin**	**P value**
Staphylococcus aureus (MRSA)	0	18. 43 ± 0.23	20. 27 ± 0.46	19. 73 ± 0.92	19. 83 ± 1.62	0	0
Pseudomonas aeruginosa (MβL)	0	0	0	0	0	0	0
Staphylococcus aureus	14. 73 ± 3.17	15. 17 ± 0.98	16.93 ± 16. 1.44	17.40 ± 17. 0.52	17. 67 ± 3.58	0	0.007 ^{S}
Pseudomonas aeruginosa	0	17. 87 ± 1.04	22. 03 ± 0.40	18. 07 ± 19. 0.98	0	0	0
Candida tropicalis	17. 73 ± 2. 42	0	0	0	0	10. 83 ± 0.98	0.008 ^{S}

Note: ^{S} statistically significant p-value ($p \leq 0.05$)

In general, the present study's findings indicate that the ethanolic leaf extract of *T. pandacaqui* possesses antimicrobial activity against the microorganisms tested. The comparison of the efficacy of *T. pandacaqui* leaf extracts and antibiotic control against wound-infecting pathogens is presented in Figure 3. The ethanolic leaf extract of *T. pandacaqui* exhibited the highest zone of inhibition against *Candida tropicalis*, with a mean value of 17.73 2.42 mm, followed by *Staphylococcus aureus*, with a mean value of 14.73 3.17 mm. However, the leaf extract had no inhibitory effect on any of the other microorganisms tested.

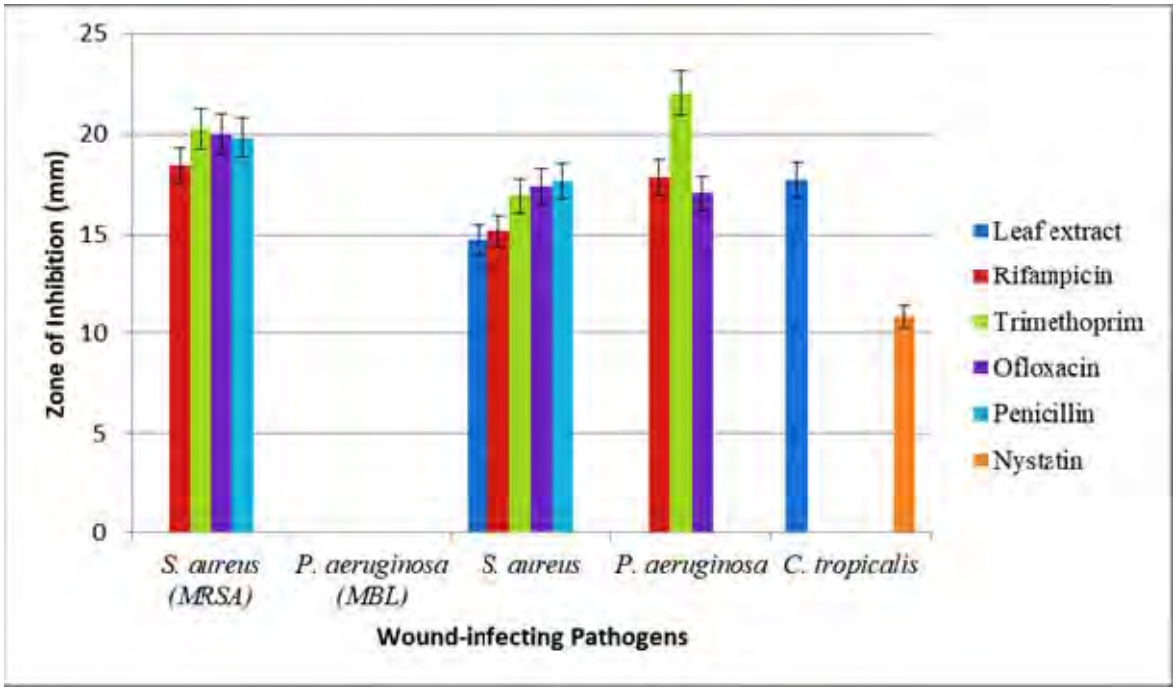

Figure 3. Comparison of antimicrobial activity of *Tabernaemonatana pandacaqui* Lam. leaf extracts and antibiotics control (Rifampicin, Trimethoprim, Ofloxacin, Penicillin, and Nystatin) against wound infecting pathogens.

The findings of this study are consistent with those of other *Tabernaemontana* species. Interestingly, Sathishkumar et al. (2012) discovered that the leaves of *Tabernaemontana heyneana* Wall. possessed a broad antimicrobial spectrum against microorganisms tested. While Ruttoh et al (2010) findings indicate that there were significant differences in the inhibitory activity of *Tabernaemontana stapfiana* between Gram-positive and Gram-negative bacteria, with Gram-positive bacteria exhibiting significantly greater inhibitory activity.

The resistance of Gram-negative bacteria may be attributed to the structure of their cell wall. Numerous studies have demonstrated that the outer membrane of Gram-negative bacteria such as *Pseudomonas aeruginosa* can act as an effective permeability barrier against the penetration of toxic particles such as antibiotics (Choi & Lee, 2019; Tegos et al., 2002). Additionally, several studies have demonstrated that Gram-positive bacteria are more susceptible, and even exhibit a greater effect, to antimicrobials derived from plants, than Gram-negative bacteria, which are generally more resistant due to the presence of the outer membrane (Koohsari et al., 2015; Rameshkumar et al., 2007; Tajkarimi et al., 2010,). Additionally, Ruttoh et al. (2010) stated that the thick, porous peptidoglycan layer within the Gram-positive semipermeable membrane allows for the passage of substances such as antibiotics and penetration into the cell, making it more susceptible.

Phytochemical Analysis

The presence of secondary metabolites such as flavonoids, alkaloids, tannins, glycosides, saponins, phytosterol, reducing sugars, and carbohydrates was determined by phytochemical analyses of *T. pandacaqui* leaf extract, as summarized in Table 4.

Table 4.Qualitative phytochemical analysis for the ethanolic leaf extract of *T. pandacaqui*

Tests	**Actual Results**	**Indication**
For Carbohydrates		
Molisch Test	Violet ring at the junction	Positive
For Reducing Sugars		
Fehling's Test	Brick red precipitate	Positive
For Flavonoids		
Alkaline Reagent Test	Light yellow-green solution	Positive
Lead Acetate Test	Yellow precipitate	Positive
For Alkaloids		
Hager's Test	Turbid yellow solution	Positive
Mayer's Test	Turbid yellow-green solution	Positive
Wagner's Test	Turbid reddish-brown solution	Positive
For Tannins		
Ferric Chloride Test	Greenish-black solution	Positive
For Glycosides		
Keller Killani Test	Brown ring at the junction	Positive
For Saponins		
Froth Test	Persistent froth formation	Positive
For Resins		
Test for Resins	Clear yellow solution	Negative
For Phytosterols		
Liebermann-Burchard Test	Brown ring at the junction	Positive
For Anthraquinone		
Test for Anthraquinone	Clear orange solution	Negative
For Proteins (Peptide Bonds)		
Test for Resins	Greenish-white precipitate	Negative

The phytochemicals identified in the extract may explain the inhibitory activity observed against the bacterial and fungal strains used in the study. According to Shahidi Bonjar (2004), plants use biological constituents such as flavonoids, alkaloids, tannins, saponins, and a variety of other secondary metabolites to defend themselves against invasion by a variety of microorganisms, insects, and other herbivores.

Flavonoids are hydroxylated phenolic compounds that possess antimicrobial activity against a broad spectrum of microorganisms. Flavonoids inhibit the function of the cytoplasmic membrane, nucleic acid synthesis, energy metabolism, attachment and biofilm formation, alter membrane permeability, and decrease pathogenicity (Xie et al., 2014). Flavonoids have been attributed their antimicrobial activity to their structural functionalities (Daglia et al., 2012). According to Wu et al. (2013), antimicrobial activity of flavonoids is dependent on their hydroxyl groups. Previously published research established a strong correlation between the chemical structure of flavonoids and their inhibitory activity against pathogens, demonstrating that removing the hydroxyl group from flavonoids significantly reduces their antimicrobial activity (Sichel, 1991; Tripoli et al., 2007).

Alkaloids are a diverse group of naturally occurring bioactive compounds found in plants, animals, bacteria, and fungi. This phytochemical played a critical role in the development of new antimicrobial agents. Thawabteh et al. (2019) state that the mechanism of action of alkaloid antimicrobial agents varies by class. Several classes act as respiratory inhibitors by inhibiting the enzyme dihydrofolate reductase, thereby inhibiting nucleic acid synthesis; others act as respiratory inhibitors by decreasing the treated microorganisms' oxygen consumption.

Additionally, tannins were identified in this study. These antimicrobial molecules exert their effect by inhibiting extracellular microbial enzymes, depriving bacteria of essential growth substrates, or directly affecting microbial metabolism via inhibition of oxidative phosphorylation (Scalbert, 1991). Saponins, on the other hand, possess antimicrobial properties due to the presence of a lipophilic portion (aglycon or sapogenin) and a hydrophilic core composed of one or more sugars (Costa, 2010). According to Ravi et al. (2016), saponin acts as a chemical barrier in the plant's defense system when pathogens are encountered. This phytochemical may result in the expulsion of proteins and enzymes from the cell.

Fourier Transform Infrared Spectroscopy Analysis

The FTIR analysis is critical for deciphering the chemical functionality of a compound found in a plant sample (Prabha et al., 2014). As illustrated in Figure 4 and Table 5, the FTIR analysis of *T. pandacaqui* leaf extract revealed the presence of functional groups such as N-O stretching for nitrogen compounds (1523.83 cm^{-1}), C=C stretching for alkene and conjugated alkene (1638.60 cm^{-1}), O=C=O stretching for carbon dioxide (2341.68 cm^{-1}), C-H and N-H stretching for alkane and amine salt, respectively (2929.03 cm^{-1}), and O-H. The FTIR spectroscopic analysis of *T. pandacaqui* ethanolic leaf extract revealed that major peaks at 3283.95 cm^{-1} were observed, which could be attributed to the O-H stretching. This indicates that the primary functional group in *T. pandacaqui* leaf extract is O-H, which is equivalent to alcohol and carboxylic acid.

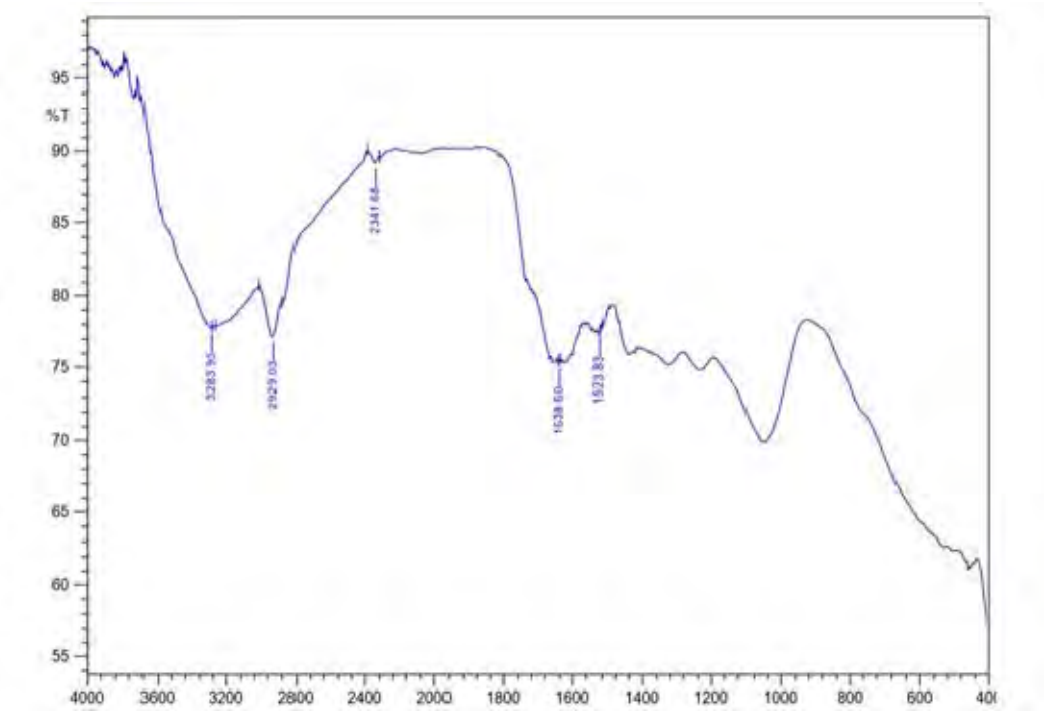

Figure 4. The FTIR spectrum analysis of ethanolic leaf extract of T. pandacaqui Lam.

Table 5.The FTIR spectral wave number values and functional group obtained from the ethanolic leaf extract of *T. pandacaqui*

Characteristic Absorption (cm^{-1})	Functional Group	Bond
1523.83	Nitro compound (Stretch)	N-O
1638.60	Alkene (Stretch) Conjugated Alkene (Stretch)	C=C
2341.68	Carbon Dioxide (Stretch)	O=C=O
2929.03	Alkane (Stretch) Amine Salt (Stretch)	C-H N-H
3283.95	Alcohol (Stretch) Carboxylic Acid (Stretch)	O-H

The FTIR spectroscopic analysis of ethanolic leaf extract of *T. pandacaqui* revealed that major peaks were observed at 3283.95 cm^{-1} which could be assigned to the O-H stretching. This indicates that the primary functional group present *in T. pandacaqui* leaf extract is O-H corresponding to alcohol and carboxylic acid.

The present study on plant samples revealed the presence of not only biologically active constituents, but also a variety of functional groups that contributed significantly to antibiotic action. Clearly, FTIR analysis of *T. pandacaqui* ethanolic leaf extract revealed the presence of nitro compounds, alkenes, conjugated alkenes, carbon dioxide, alkanes, amine salts, alcohol, and carboxylic acid as a major functional group. According to Burman et al. (2012), the plants' antimicrobial activity is attributed to functional groups such as alcoholic, aromatic, amine, and carbo-acids found in tannins, alkaloids, and flavonoids that can be extracted from the plant and used in herbal drug preparations for the treatment of a variety of bacteria-borne diseases.

The presence of functional groups confers a variety of beneficial properties (Prabha 2014). The major peak observed in the O-H stretch could be attributed to alcohol or carboxylic acid in this study. Prasanna & Anuradha (2016) demonstrated that carboxylic acids are critical metabolic products in the formation of fat in the body and act as potent antimicrobial agents. This organic compound was previously used in pharmaceuticals as a primary ingredient in the treatment of a variety of illnesses, including ulcers, nasal congestion, jaundice, headache, liver pain, edema, and rheumatic joint pains. Additionally, previous research has demonstrated that metabolites containing alkene, alcohol, and hydroxyl have significant antimicrobial activity (Chopra & Roberts, 2001). According to Moovendhan et al. (2014), these functional groups are responsible for cleavage of the bacterial cell wall and inhibition of cellular respiration and electron transport in their mode of action.

Interestingly, the FTIR analysis results obtained in this study also confirmed the presence of the detected phytochemical in the plants. For example, the presence of alkaloids is explained by the N-H stretch at the 2929.03 cm1 position. Meanwhile, the presence of flavonoids is a result of the peak at 3283.95 cm1, which revealed the presence of the O-H stretch (Jabamalairaj et al., 2015). The spectroscopic analysis revealed the presence of biologically active functional groups, indicating the presence of active phytochemicals that may contribute to the plant's bioactivities, such as bactericidal and antimicrobial activity.

Conclusions

In the current investigation, the efficacy of ethanolic leaf extract of *Tabernaemontana pandacaqui* Lam. against several microorganisms associated was evaluated. The findings of this study

indicate that the ethanolic leaf extracts of *T. pandacaqui* have significant inhibitory activity against multidrug-resistant *Staphylococcus aureus*, and pathogenic fungus, *Candida albicans*. The plant extracts have been found to possess a variety of biologically active phytochemicals and functional groups, all of which have been reported to have inhibitory properties. However, it is necessary to conduct additional research in order to determine the potential effectiveness of crude extracts as antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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