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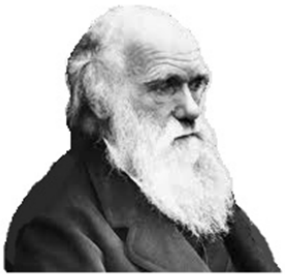

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Comparative Anti-Bacterial Effect of Aqueous and Ethanolic Stem Barks of *Tamarindus indica* Linn on *Staphylococcus aureus*

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ABSTRACT

This study focuses on *Tamarindus indica*, a plant belonging to family Fabaceae commonly found in Nigeria and the Asian continent, and explores the potential comparative antibacterial activity of its aqueous and ethanolic stem barks against *Staphylococcus aureus*, a Gram-positive bacterium. The stem barks of *Tamarindus indica* were obtained from a live Tamarind tree located at Bayero university Kano, old campus, was dried under shade away from direct sunlight in the laboratory, pounded to fine powder using mortar and pestle and extracted through maceration method using distilled water and ethanol solvents. Phytochemical analysis was carried out following standard procedures and it revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, steroids, glycosides, and carbohydrates in the extracts. Agar well diffusion method was employed to assess the antibacterial activity. The results showed the aqueous extracts exhibited bigger zones of inhibition compared to the ethanol extracts. Furthermore, the lowest MIC was 15.625 mg/ml for ethanolic Stem bark extract revealing how effective the stem bark ethanol extracts were in combating the bacteria. The study demonstrated that *Tamarindus indica* stem barks possesses antibacterial properties which may be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and phenols and highlights the potential of *Tamarindus indica* as an affordable and accessible natural remedy against *Staphylococcus aureus* infections and diseases.

***Keywords*:** Antibacterial, *Tamarindus indica*, bactericidal, inhibitory, *Staphylococcus aureus*

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1. INTRODUCTION

Plants have long been used as natural remedies for a variety of ailments, including bacterial infections (Sarker and Nahar, 2004). Many plants contain compounds that have been shown to have antibacterial properties, including essential oils, tannins, and alkaloids (Mohamed *et al.,* 2020). These compounds can be used to treat bacterial infections both topically and internally (Ghurghure *et al.,* 2019) (Silén *et al.,* 2023). For example, tea tree oil has been used to treat skin infections, while garlic has been used to treat respiratory infections (Kairey *et al.,* 2023). In addition, some plants, such as eucalyptus, have been found to have broad-spectrum antibacterial activity (Korpinen *et al.,* 2021), meaning they can be effective against a wide range of bacteria. Finally, some plants, such as oregano, have been found to have strong antibacterial activity against certain types of bacteria, such as E. coli and Salmonella (de Almeida *et al.,* 2023). Medicine made from herbs or plants (herbal medicine) is a very important part of both traditional and modern medicine. Nigeria is a very blessed nation with rich heritage of plant kingdom due to its geo-location and tropical weather. Plants have always played a key role in the treatment of different diseases and ailments in human and animals from ancient times. *Staphylococcus aureus* is a type of bacteria that can cause a variety of infections, including skin infections, food poisoning, and respiratory infections (Tigabu and Getaneh, 2021). It is one of the most common causes of hospital-acquired infections. It is also a common cause of food poisoning, as it can survive in food for long periods of time (Algammal *et al.,* 2020). Treatment for *S. aureus* infections typically involves antibiotics (Urish and Cassat, 2020). *S. aureus* is a Gram-positive, spherical bacterium that is found in the environment, on the skin, and in the nose of healthy individuals (Nandhini *et al.,* 2022). It is a facultative anaerobe, meaning that it can survive in both aerobic and anaerobic environments (Asif et al, 2021). It is also a facultative pathogen, meaning that it can cause disease in humans and other animals. *S. aureus* is a highly contagious organism and can be spread through contact with infected individuals or contaminated surfaces. It is also spread through the air, and can be found in the environment, on the skin, and in the nose of healthy individuals.

Tamarindus indica L. (Family: Fabaceae), commonly known as Tamarind grows naturally in the tropical and sub-tropical regions of Nigeria, India and Asia (www.kccil.us). Traditionally in Nigeria, Tamarind has wide array of uses and some of these uses include inflammation, constipation, indigestion, pneumonia and diabetes. In fact, in northern Nigeria, the fresh stem bark and leaves have been in use in the form of decoction mixed with potash for treatments of jaundice, stomach disorder, general body pain, yellow fever, skin cleansers and as blood tonics (Doughari, 2006).

Medicinal uses of *Tamarindus indica* are numerous. The fruits extracts are used as refrigerants in fevers and as laxatives and carminatives alone or combinations with lime juice, honey, milk, dates and spices (Abubakar *et al.,* 2008). The pulp is used in digestive as remedy for biliousness and bile disorders (Jayaweera, 1981). The tamarind leaves present an array of nutrients like a good level of protein, fat, fibber and some vitamins such as thiamine, riboflavin, ascorbic acids and B-carotene (El-siddiq *et al.,* 2006). The leaves and stem barks of *Tamarindus indica* have been found to possess antibacterial properties (Soni and Singh, 2019). Studies have shown that the extracts of the leaves and stem barks of *Tamarindus indica* have the ability to inhibit the growth of a wide range of bacteria, including *Staphylococcus aureus*, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi (Yadima *et al.,* 2017). The antibacterial activity of the extracts is attributed to the presence of various compounds, such as tannins,

flavonoids, and phenolic acids (Alrasheid *et al.*, 2019). These compounds are known to possess antimicrobial properties, which help to inhibit the growth of bacteria.

In this study therefore, *Tamarindus indica* leaves and stem bark were screened for phytochemical constituents and antibacterial activity against *Staphylococcus aureus* and compared.

Because of the resistance developed by some bacteria to antibiotics, the side effects of this synthetic antibiotics over long usage and its lack of availability and high cost nature to the rural communities which has necessitated more interest in biologically active compounds present in *Tamarindus indica*.

An estimate of 75-90% of the rural population of the world still relies on herbs for their healthcare. Therefore, in many village markets in Africa, Asia and Latin America, medicinal herbs are sold alongside vegetables and other wares (Lai and Roy, 2004).

More so, not much scientific research has been conducted in these areas to prove the validity of the traditional methods or claims which brought about this research to prove and provide necessary data as to whether the plants are capable of those activities they are being utilized for traditionally.

The study aim is to compare antibacterial effects of aqueous and ethanolic stem barks extract of *Tamarindus indica* on *Staphylococcus aureus* to see how much they can be used.

2. RESULT/EXPERIMENTAL MATERIALS AND METHODS

2. 1. 1. EXPERIMENTAL SITE

The experiment was conducted at the plant pathology laboratory of the Department of Plant Biology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, Old Bayero University Kano Campus in Kano State. The location of the lab is 11.9794° Latitude and 8.47838° Longitude.

2. 1. 2. COLLECTION OF SAMPLES

In the month of December, between the hours of 4-6 pm, the *Tamarindus indica* stems were collected from Bayero university, Kano old campus Kano state, Nigeria and was identified and certified at the herbarium by the curator, Mallam Baha'udeen Said Adam of the department of Plant Biology with Accession number BUKHAN0074. Bayero University's Department of Microbiology provided the bacterial sample (*Staphylococcus aureus*) which were verified by the chief technologist Mallam Idris. All chemicals used was of analytical grade.

2. 1. 3. PREPARATION OF EXTRACTS

The method of (Gidado *et al.*, 2005) as described by (Ekeleme *et al.*, 2017) was slightly modified for the preparation of the extract. The freshly collected stem barks were thoroughly rinsed with distilled water and spread out on a flat dry surface to air dry at room temperature for ten (10) days. The dried stem barks were pounded using a mortar and pestle until fine powders were achieved and sieved too using a mesh and packed into an airtight container.

Employing maceration method of extraction, aqueous and ethanol solvents were used: 80g of finely powdered stem barks was weighed and transferred to a clean air tight container and 500 ml of distilled water was measured and poured into the container and tightly closed.

80g of finely powdered stem barks was weighed and transferred to a clean air tight container and 500 ml of ethanol was measured and poured into the container and tightly closed.

All containers were clearly and appropriately labelled and subjected to agitation 5 times daily for five days after which each of the extracts was then filtered using Whatman No: 1 filter paper. The resulting filtrates were then concentrated to dryness using water bath at 50 °C. to achieve the required concentration 1 gramme of each of these extracts were subsequently dissolved in 1 milliliter of dimethyl sulfuroxide (DMSO).

2. 1. 4. MAINTENANCE OF BACTERIAL ISOLATE

The bacteria were kept in nutrient agar slant and refrigerated at 4 °C until they were needed.

2. 1. 5. STANDARDIZATION OF INOCULUM DENSITY OF THE TEST BACTERIA

The bacteria isolates was standardized by a method involving a loop full of confirmed test bacteria been picked by a sterile wire loop and emulsified into 10ml of sterile normal saline to match with the turbidity of the 0.5 Macfarland Standard for the sensitivity tests as described by Perez *et al.,* (1990) and Kirby Bauer, (1996).

2. 1. 6. PHYTOCHEMICAL ANALYSIS

The freshly prepared extracts were subjected to standard phytochemical analysis to test for the presence of the phytochemicals which include alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, steroids, glycosides and carbohydrates.

Test for Alkaloids

Dragendorff's test was carried out through the follow procedures;
The extract was diluted in 1ml of water and 1ml of dilute hydrochloric acid (HCl) was added and drops of dragendorff reagent and the presence of orange-red precipitate confirmed the presence of alkaloids.

Test for Flavonoids

Decolorisation test was used for the confirmation.
Extract was treated with few drops of NaOH. Formation of intense yellow color that became colorless on addition of few drops of dilute HCl confirmed the presence of flavonoids.

Test for Tannins

A small quantity of the extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicated the presence of tannins.

Test for Saponins

Foam test was used for this confirmation.
The extract was shaken vigorously with equal volume of water and the persistent foaming was observed, which indicated the presence of saponins.

Test for Phenols

Ferric chloride test was used to confirm presence of phenols.

5 ml of extract was treated with few drops of ferric chloride solution. Formation of bluish black color confirmed the presence of phenols.

Test for Terpenoids

Salkowaski's test procedures was used as follows;
10ml of the extract was mixed with 2 ml of chloroform and then added carefully to it was 3 ml of concentrated H_2SO_4 to form a layer. The appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

Test for Steroids

Salkowaski's test procedures was used as follows;
Some of the extract was mixed with 2ml of chloroform and then added carefully to it was 3ml of concentrated H_2SO_4 and shaken gently. The appearance of reddish brown colour indicates the presence of steroidal ring.

Test for Glycosides

The extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of ferric chloride. Then the mixture was poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring formed at the interphase which indicated the presence of cardiac glycosides. (Yadav and Munin, 2011).

Test for Carbohydrates

1g of the extract will be dissolved in water and filtered. 1 ml of benedict solution was added to the filtrate and heated gently in water bath and the formation of red precipitate of copper (I) oxide indicated the presence of reducing sugar. (Sofowora, 1993).

2. 1. 7. ANTIBACTERIAL ASSAY

The effect of two plant extracts on *Staphylococcus aureus* were assayed by Agar well diffusion method and the minimum inhibitory concentration (MIC) of the extracts on the bacteria was carried out using tube dilution technique as described by Cheesbrough, (2002). The minimum bactericidal concentration (MBC) was also carried out.

Agar Well Diffusion method

The antibacterial activities of the extracts and the reference drugs Ampiclox were determined according to the method described by Agyare *et al.*, (2013).
Petri-plates containing 20 ml of Nutrient Agar were seeded with the standardized test organism (*Staphylococcus aureus*). In each of those bacteria seeded plates, wells of 6mm were cut using a sterile cork borer and different concentrations of the plant extract and the reference drug was dissolved in dimethyl sulfoxide (DMSO) and added to the wells that has been dug on the plates and allowed to diffuse at room temperature (28-30 °C) for 1 hour and then incubated at 37 °C for 18-24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well using a transparent ruler in millimeters. (NCCLS, 1993). Antibiotic used was Ampiclox as positive control and Dimethyl Sulfoxide (DMSO) as negative control.

2. 1. 8. Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the antibacterial agent that inhibits the bacterial growth and was carried out using the tube dilution technique as described by Cheesbrough, (2002).

The plant extracts that showed significant antibacterial activity by Agar well diffusion method was subjected to minimum inhibitory concentration assay by using the tube dilution technique, using Dimethyl Sulfoxide (DMSO) to arrive at concentrations of 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml and 7.8125 mg/ml.

Two milliliters (2ml) of each extract and Nutrient Broth was mixed and 0.1 ml of standardized inoculum (3.33×10^6 cfu/ml) was added to each of the test tubes. The test tubes were incubated at 35 °C for 24 hours. One tube containing broth and the extract served as the positive control while another tube containing broth and the inoculum served as the negative control for the comparison.

Record of the presence of growth (turbidity) or absence of growth (clear solution) was taken at the end of the 24 hours in comparison with the controls. The lowest concentration that showed no evidence of growth (turbidity) was regarded as the minimum inhibitory concentration (MIC), (Baker *et al.*, 1993, Vallekobia *et al.*, 2001). This was done for all the different plant extracts (Aqueous stem bark and ethanolic stem bark).

2. 1. 9. MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Minimum Bactericidal Concentration (MBC) of the aqueous and ethanolic extracts was determined by the sub culturing from each of the MIC test tubes that showed no evidence of growth (turbidity).

Fresh Nutrient Agar was prepared and 20ml of it was put into petri-plates. Sub culturing of the MIC test tubes that showed no evidence of growth (turbidity) was carried out on the petri-plates with clear partitions. The plates were further incubated at 37 °C for 24 hours to determine the minimum Bactericidal Concentration (MBC), that is, the lowest concentration that yielded no single bacterial colony on the solid media (Baker *et al.*, 1993, Vallekobia *et al.*, 2001).

2. 2. RESULTS

2. 2. 1. PHYTOCHEMICAL SCREENING OF AQUEOUS AND ETHANOLIC STEM BARKS EXTRACTS OF *TAMARINDUS INDICA*

The phytochemical constituents of the aqueous and ethanol extract of *Tamarindus indica* stem barks showed that they are highly rich in phytochemicals which are recorded in Table 2.1 below. Aqueous stem bark and ethanol stem bark all showed the presence of alkaloids, phenols, tannins, terpenoids, glycosides, carbohydrates and steroids with exception of aqueous stem bark showing the absence of flavonoids and saponins.

2. 2. 2. ANTIBACTERIAL ASSAY AND ZONE OF INHIBITION

The antibacterial assay of aqueous and ethanol extract of *Tamarindus indica* stem barks were all sensitive at the various concentrations at which they were studied. The result of this study showed Stem barks Aqueous extracts having 17.07 ± 3.86^{a} and Stem Barks Ethanol extracts with the lowest mean of 13.18 ± 2.97^{b} and presented in table 2.2.

2. 2. 3. MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC).

The minimum inhibitory concentration was carried out on the isolates to determine at what concentrations the extract will inhibit or kill the organism.

The results on MIC of aqueous and ethanol extract of the stem barks were presented in Table 2.3. The MIC values were obtained thus, for Stem Bark Aqueous Extract it is 62.50 mg/ml and for Stem Bark Ethanol Extract it is 15.625 mg/ml. With Regards to the MBC, it was observed that for Stem Bark Aqueous Extract and for Stem Bark Ethanol Extract the values were the same, 62.50 mg/ml.

Table 2.1. Phytochemical screening of aqueous and ethanolic stem bark extracts of *Tamarindus indica*

S. No	Phytochemical Constituents	Stem Bark Aqueous	Stem Bark Ethanol
1.	Alkaloids	+	+
2.	Phenols	+	+
3.	Tannins	+	+
4.	Glycosides	+	+
5.	Terpenoids	+	+
6.	Flavonoids	-	+
7.	Saponins	-	+
8.	Carbohydrates	+	+
9.	Steroids	+	+

Key: +, presence of phytochemicals while -, absence of phytochemicals.

Table 2.2. Antibacterial activity of the Aqueous and Ethanol Stem bark extracts of *Tamarindus indica* against *Staphylococcus aureus.*

Sources of Variation	Mean (mm) ± Standard Error
500 mg/ml	22.92 ± 1.19 [a]
250 mg/ml	19.08 ± 1.21 [b]
125 mg/ml	16.50 ± 1.03 [c]
62.5 mg/ml	13.17 ± 0.87 [d]
+ Control	18.75 ± 0.00 [b]
- Control	0.0 ± 0.00 [e]
LSD	2.15
Plant Source	
SBAE	17.07 ± 3.86 [a]
SBEE	13.18 ± 2.97 [b]
LSD	1.76
INTERACTION	NS

Key: SBAE, Stem Bark Aqueous Extract; SBEE, Stem Bark Ethanol Extract, LSD, Least Significant Difference; DMSO, Dimethyl Sulfoxide as - Control; + Control as Ampiclox (125 mg/ml); NS, Not Significant.
Values having different superscript are considered to be significantly at P<0.05.

Table 2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Aqueous and Ethanol Stem bark extracts of *Tamarindus indica* against *Staphylococcus aureus.*

Extracts	Minimum Inhibitory Concentration (MIC) (mg/ml)	Minimum Bactericidal Concentration (MBC) (mg/ml)
SBAE	62.50	62.50
SBEE	15.625	62.50

Key: SBAE, Stem Bark Aqueous Extract; SBEE, Stem Bark Ethanol Extract.

2. 3. DISCUSSION

Phytochemical constituents such as alkaloids, flavonoids, tannins and other compounds are referred to as secondary metabolites and they serve as a defense mechanism for the plant against predation and destruction by microorganisms, insects, herbivores etc. This could be the reason for its antibacterial activities (Doughari, 2006).

The presence of the above phytochemicals especially alkaloids, flavonoids, saponins, tannins and glycosides in plant parts have been said to be responsible for its antibacterial activities. Saponins possess antibacterial capabilities, tannins play an important role in wound healing and control of infection on them (Gonzalez-Lamothe *et al.,* 2009) (Cowan MM, 1999) and flavonoids also acts as anti-inflammatory and has antimicrobial activity (Madubunyi, 1995). More so, alkaloids which is also very present is known to play some metabolic roles as well as anticancer anesthetics because of its large group of nitrogenous compounds. Hence, all the above mentioned compounds been confirmed present in light of this study has therefore proven and accounted for the use of *Tamarindus indica* as an antibacterial treatment, used for skin infections, constipation diarrhea and diabetes. The result of this study conforms with that of Sravanthi *et al.,* 2017 who reported that the results of the phytochemical studies revealed the presence of tannins, saponins, alkaloids and terpenoids. The antibacterial activity of the stem bark could be attributed to the presence of phenol in them in line with the findings of (Pelczar *et al.,* 1998), In fact, phenol itself was the first antiseptic used in surgery.

Alkaloids which has been confirmed to be readily available in the leaves and stem barks has a type of it Vinblastine being used as a treatment on diabetes and high blood pressure and as disinfectant, also as a cancer fighter (Moudi, *et al.,* 2013).

The demonstration of antibacterial activity of stem bark of *Tamarindus indica* goes to show a wider and more broad channels of antibiotic compounds and adds an advantage to the fight against reoccurring infections, resistant bacteria and also the availability of this antibiotics to the common man at affordable prices too. The experiment evaluated the antibacterial potential of two different extracts: stem bark aqueous extract (SBAE), and stem bark ethanol extract (SBEE) and were tested at various concentrations: 500 mg/ml, 250 mg/ml, 125 mg/ml,

and 62.5mg/ml. Additionally, there are two control groups: a negative control using Dimethyl Sulfoxide (DMSO) and a positive control using Ampiclox at a concentration of 125 mg/ml.

From the results, it can be observed that the antibacterial activity of the extracts varies depending on the concentration and the source of the extract. The stem bark ethanol extract (SBEE) and – control (DMSO) showed the lowest mean antibacterial activity. Additionally, the result shows that there is no significant difference (NS) between the means of the interaction and control groups, indicating that the antibacterial activity of the extracts is comparable to the positive control (Ampiclox).

The MIC values obtained for the different extracts are as follows: 62.50 mg/ml for Stem Bark Aqueous Extract (SBAE), and 15.625 mg/ml for Stem Bark Ethanol Extract (SBEE).

Similarly, the MBC values are as follows: 62.50 mg/ml for both SBAE and SBEE.

These results indicated the concentration at which each extract is effective in inhibiting and killing *Staphylococcus aureus*. A lower MIC value suggests greater potency, as it takes a lower concentration to inhibit bacterial growth. Similarly, a lower MBC value indicates a stronger bactericidal effect, requiring a lower concentration to kill the bacteria.

From the results, it can be observed that both the aqueous and ethanolic stem bark extracts exhibit antibacterial activity against *Staphylococcus aureus*. The Stem bark aqueous extract (SBAE) demonstrates the lowest MIC and MBC values at 15.625 mg/ml, suggesting it has the most potent inhibitory effects against the tested bacteria.

Furthermore, it is worth noting that the MIC and MBC values for the stem bark aqueous extract (SBAE) are the same, indicating that this extract is equally effective in inhibiting and killing the bacteria at a concentration of 62.50 mg/ml.

Although different solvents have been reported to have different capacity to extract different and varying amounts of phytoconstituents present in the plant depending on their polarity of the solvent and its solubility (Doughari, 2006). The demonstration of water based extracts during this study has very good results too and provides scientific basis for use of this plant parts traditionally in treatment of diseases and infections seeing that most traditional medicine practitioners uses water as their solvent while making decoctions.

The positive control drug Ampiclox used was found to be statistically similar to the 250 mg/ml concentration of the extracts used and the 500 mg/ml concentration of the extract had a higher antibacterial activity against *Staphylococcus aureus* and that the leaves and stem bark extracts of *Tamarindus indica* possess antibacterial properties against *Staphylococcus aureus* therefore, signifying that the extracts can be recommended for treatment of diseases and infections caused by the bacteria. These results suggest the aqueous extract demonstrated stronger antimicrobial activity compared to the ethanolic extracts.

3. CONCLUSION

The study revealed the phytochemicals present in the extracts screened were Alkaloids, Phenols, Tannins, Glycosides, Terpenoids, Flavonoids, Saponins, steroids and Carbohydrates and The minimum inhibitory concentration and minimum bactericidal concentration of the extracts on *Staphylococcus aureus* were determined and the result shows that the aqueous extract had a stronger effect in inhibiting and killing the bacteria than the ethanolic extract. This study provides scientific evidence supporting the traditional use of *Tamarindus indica* stem bark as an antibacterial agent and fever reducer.

These findings contribute to the exploration of new antibiotic sources and highlight the potential of *Tamarindus indica* extracts as affordable and accessible treatments for diseases and infections caused by bacteria, particularly *Staphylococcus aureus*.

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