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# Phytochemical Screening and Antibacterial Activity of *Ficus thonningii* Stem Bark Extracts

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The increasing emergence of antimicrobial resistance necessitates the search for new antibacterial agents, particularly from plant sources. This study investigates the phytochemical constituents and antibacterial activity of the stem bark of *Ficus thonningii* Blume, a traditionally used medicinal plant in Nigeria. Sequential extraction was conducted using n-hexane, ethyl acetate, and ethanol. . The extracts were subjected to qualitative phytochemical screening. The antimicrobial activity of the extracts was tested on *Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Streptococcus pyogenes* using agar well diffusion and broth dilution methods. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides and anthraquinones. The antibacterial activities of the extracts were tested against *Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Streptococcus pyogenes*. The ethyl acetate extract showed the highest antibacterial activity, with inhibition zones ranging from 1.50 mm to 17.00 mm particularly against Gram-positive bacteria. MIC and MBC tests confirmed its potency. These findings support the traditional use of *Ficus thonningii* in treating bacterial infections and highlight its potential as a source of plant-based antimicrobial agents.

**Keywords:** *Ficus thonningii*, Antibacterial activity, Phytochemicals.

## 1. Introduction

The global rise of antimicrobial resistance (AMR) has become one of the most critical challenges facing modern medicine, with the World Health Organization identifying it as a major public health threat (WHO, 2021). The extensive, irregular, and indiscriminate use of antibiotics has accelerated the emergence of drug-resistant pathogens, rendering many conventional therapies ineffective (Tortorella et al. 2018). This trend poses a significant risk to global health, increasing both mortality rates and the economic burden of treatment. In response to this crisis, researchers are intensifying efforts to identify novel antimicrobial agents, particularly from natural sources. Approximately 50% of current pharmaceuticals and nutraceuticals are derived from natural products (Chavan et al. 2018), making medicinal plants a promising reservoir of bioactive compounds. Over the years, numerous plant-derived compounds have demonstrated antimicrobial properties, yet a vast number remain underexplored (accessed August, 2021). Traditional medicine has long relied on plants for the treatment of various ailments, and up to 75% of the global population especially those in rural regions still depend on herbal remedies for primary healthcare (Gabhe et al. 2006). These remedies have proven effective in managing conditions such as metabolic, allergic, and cardiovascular diseases (Igoli et al. 2005). The widespread use of medicinal plants is attributed not only to their accessibility and affordability but also to their complex phytochemical profiles and synergistic mechanisms that enhance antimicrobial efficacy (Zaynab et al. 2018). Among such medicinal plants, the genus *Ficus* (family *Moraceae*) includes over 800 species globally, with around 65 species identified in Nigeria alone (MPNS, 2021). *Ficus thonningii*, a fast-growing tree reaching up to 10 meters in height, is commonly used in African ethnomedicine. Its leaves, roots, fruits, and stem bark have been traditionally employed to treat various conditions including diarrhea, epilepsy, wounds, and mental illness (MPNS, 2021). The ethanolic leaf extract has shown analgesic activity, while the stem bark is reputed for treating infections associated with diarrhea and dysentery.

## 2. Materials and Methods

### 2.1 Collection and Identification of Plant Material

Fresh stem bark of *Ficus thonningii* Blume was collected from Arkilla area in Wamakko Local Government Area of Sokoto State, Nigeria. The plant was identified authenticated at the Department of Pharmacognosy and Ethnopharmacy, Faculty of

  

Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. A voucher specimen (PCG/UDUS/MORA/0009) was deposited at the herbarium of the department.

### 2.2 Preparation and Extraction of Plant Material

A total of 250 g of powdered *Ficus thonningii* stem bark was subjected to maceration using three solvents of increasing polarity: n-hexane, ethyl acetate, and ethanol. Each solvent was kept in contact with the sample for 24 hours, and the resulting extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated to dryness under reduced pressure using a rotary evaporator and further dried at room temperature. The percentage yield of each extract was calculated.

### 2.3 Phytochemical Screening

Qualitative phytochemical tests were carried out on the three extracts to determine the presence of secondary metabolites including alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, and anthraquinones using standard procedures as described by (Harborne, 1998).

### 2.4 Microbial Strains and Antibacterial Assay

Four bacterial isolates (*Escherichia coli*, *Salmonella typhi, Staphylococcus aureus,* and *Streptococcus pyogenes*) were used. Antibacterial activity was tested using agar well diffusion method. Mueller-Hinton Agar plates were inoculated, and wells were filled with extract solutions. Standard antibiotic (Ciproflaxacin 2.5 mg/mL) served as a positive control. Inhibition zones were measured after 24 hours of incubation at 37°C.

### 2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined using the broth dilution method. Tubes were inoculated with bacterial suspensions and incubated at 37°C for 24 hours. The lowest concentration that showed no visible growth was recorded as the MIC. For MBC, samples from tubes showing no growth were sub-cultured on fresh MHA plates and incubated. The lowest concentration that produced no bacterial growth was noted as the MBC (Usman and Osuji, 2007).

## 3. Results and Discussion

### 3.1 Results

#### *3.1.1 Extraction*

Ethyl acetate gave the highest yield (7.22%), followed by ethanol (6.90%). Hexane gave the lowest yield (5.33%). This shows that semi-polar and polar solvents extracted more compounds than the non-polar solvent.

Table 1: Mass of Extract and Percentage Recovery of *F. Thonningii* Stem Bark Extracts

| Solvent | Original mass (g) | Mass of extract (g) | Percentage recovery (%) |
|---|---|---|---|
| **N-hexane** | 225.32 | 12.00 | 5.33 |
| **Ethyl acetate** | 198.75 | 14.35 | 7.22 |
| **Ethanol** | 177.56 | 12.26 | 6.90 |

#### *3.1.2 Qualitative Phytochemical Screening*

The phytochemical screening result of the stem bark extracts of *Ficus thonningii*. result shows that Alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, and anthraquinones were detected in the ethanol and ethyl acetate extract, while the n-hexane extract showed only a few and tested negative for others.

Table 2: Qualitative Phytochemical Screening of *F. Thonningii* Stem Bark Extracts

| Phytochemicals | N hexane | Ethyl acetate | Ethanol |
|---|---|---|---|
| **Alkaloids** | | | |
| a. Mayers test | - | + | + |
| b. Wagners test | - | + | + |
| c. Dragendorffs test | - | + | + |
| d. Confirmatory test | - | + | + |
| **Flavanoids** | | | |
| a. Sodium hydroxide test | - | + | + |
| b. Iron (III) Chloride test | - | + | + |
| c. Shinodas test | - | + | + |
| **Tannins** | | | |
| a) Iron (III) Chloride test | - | + | + |
| b) Lead acetate | - | + | + |
| **Saponins** | | | |
| a. Foam test | - | + | + |
| **Anthraquinones** | | | |
| a. Bontragers test | + | + | + |
| **Cardiac glycosides** | | | |
| a. Keller-killianis test | + | + | + |
| **Steroids** | | | |
| a) Salkowkis test | + | + | + |
| b) Lieberman-Buchards test | + | + | + |

**Key:** Present (+), Absent (-)

#### *3.1.3 Antibacterial Activity*

Table 3 below shows the antibacterial activity of the n-hexane extract of *Ficus thonningii* stem bark. The extract showed mild to moderate inhibition, with zones ranging from 1.00 mm to 17.00 mm, and was less effective compared to the ethyl acetate extract.

  

Table 3: Antibacterial activity (zone inhibition (mm) ) value of N-hexane fraction of *F. Thonningii* stem bark extract against isolates

| Concentration (mg/mL) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***S. typhi*** | ***S. aureus*** | ***S. pyogens*** |
| 31.25 | 1.00 | 4.50 | 0.00 | 0.00 |
| 62.50 | 2.50 | 7.50 | 1.00 | 0.50 |
| 125.00 | 3.00 | 9.00 | 2.00 | 2.00 |
| 250.00 | 5.00 | 13.00 | 3.50 | 5.00 |
| 500.00 | 7.00 | 17.00 | 5.00 | 7.00 |
| **Control (2.5 mg/mL)** | 14.50 | 22.00 | 11.00 | 9.00 |

Table 4 below shows the antibacterial activity of the ethyl acetate extract of *Ficus thonningii* stem bark. The extract exhibited the highest activity among all tested extracts, with zones of inhibition ranging from 1.50 mm to 17.00 mm across all bacterial strains and concentrations.

Table 4: Antibacterial activity (zone inhibition (mm) ) value of ethyl acetate fraction of *F. Thonningii* stem bark extract against isolates

| Concentration (mg/mL) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***S. typhi*** | ***S. aureus*** | ***S. pyogens*** |
| 31.25 | 1.50 | 9.00 | 4.00 | 4.00 |
| 62.50 | 3.50 | 11.00 | 6.00 | 6.50 |
| 125.00 | 4.00 | 14.00 | 7.00 | 7.00 |
| 250.00 | 6.50 | 16.00 | 9.00 | 9.00 |
| 500.00 | 9.00 | 17.00 | 13.00 | 11.00 |
| **Control (2.5 mg/mL)** | 7.00 | 24.00 | 7.00 | 7.00 |

Table 5 below shows the antibacterial activity of the ethanol extract of *Ficus thonningii* stem bark. The extract demonstrated moderate activity on all tested bacterial strains, with inhibition zones between 1.00 mm and 17.00 mm, but overall less than the ethyl acetate extract.

Table 5: Antibacterial activity (zone inhibition (mm) ) value of ethanol fraction of *F. Thonningii* stem bark extract against isolates

| Concentration (mg/mL) | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | ***E. coli*** | ***S. typhi*** | ***S. aureus*** | ***S. pyogens*** |
| 31.25 | 1.50 | 3.50 | 0.00 | 1.00 |
| 62.50 | 3.00 | 5.00 | 2.00 | 2.50 |
| 125.00 | 5.00 | 10.00 | 3.00 | 5.00 |
| 250.00 | 7.00 | 13.00 | 6.00 | 7.00 |
| 500.00 | 9.00 | 17.00 | 8.50 | 11.00 |
| **Control (2.5 mg/mL)** | 8.50 | 24.00 | 17.00 | 16.00 |

### *3.1.4 MIC and MBC Results*

The results in Table 6 shows that the n-hexane extract had an MIC of 250 mg/mL on *E. coli*, *S. typhi*, and *S. aureus*, and 125 mg/mL on *S. pyogenes*. MBC values were 250 mg/mL for *E. coli* and *S. aureus*, 62.5 mg/mL for *S. typhi*, and 125 mg/mL for *S. pyogenes*.

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of N-hexane fraction of *F. Thonningii* stem bark extract against isolates

| Test Organism | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| *E. Coli* | 250.00 | 250.00 |
| *S. Typhi* | 250.00 | 62.50 |
| *S. Aureus* | 250.00 | 500.00 |
| *S. Pyogens* | 125.00 | 125.00 |

  

Table 7 shows that the ethyl acetate extract had MIC and MBC values of 250 mg/mL on *E. coli*, 125 mg/mL on *S. typhi,* 250 mg/mL on *S. aureus*, and 62.5 mg/mL on *S. pyogenes*, indicating broad bactericidal activity.

Table 7: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethyl acetate fraction of *F. Thonningii* stem bark extract against isolates

| Test Organism | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| *E. Coli* | 250.00 | 250.00 |
| *S. Typhi* | 125.00 | 125.00 |
| *S. Aureus* | 250.00 | 250.00 |
| *S. Pyogens* | 62.50 | 62.50 |

As shown in Table 8 below, the ethanol extract had an MIC of 250 mg/mL on *E. coli*, *S. typhi*, and *S. pyogenes*, and 500 mg/mL on *S. aureus*. MBC values were 250 mg/mL for *E. coli* and *S. pyogenes*, 125 mg/mL for *S. typhi*, and 500 mg/mL for *S. aureus*.

Table 8: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanol fraction of *F. Thonningii* stem bark extract against isolates

| Test Organism | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| *E. Coli* | 250.00 | 250.00 |
| *S. Typhi* | 250.00 | 250.00 |
| *S. Aureus* | 500.00 | 500.00 |
| *S. Pyogens* | 250.00 | 250.00 |

## 3.2 Discussion

### *3.2.1: Extraction*

Medicinal properties of plants are a function of their phytoconstituents as plants rich in phytochemicals have been proven to exhibit varying pharmacological activities (Tuwangun et. al, 2020). The extraction yields of the stem bark of Ficus thonningii using solvents of different polarities are presented in Table 1. Ethyl acetate produced the highest recovery (7.22%), followed by ethanol (6.90%), while n-hexane gave the lowest yield (5.33%). This variation is consistent with the influence of solvent polarity on the solubility of phytochemicals. Solvent polarity plays a critical role in determining the efficiency of extraction, as different classes of secondary metabolites differ in solubility (Dai and Mumper, 2010). Ethyl acetate, being semi-polar, was able to dissolve both moderately polar and some non-polar compounds, thereby extracting a wider range of metabolites, such as phenolics, flavonoids, and glycosides. Ethanol, a highly polar solvent, also gave a relatively high yield due to its efficiency in solubilizing polar phytochemicals, including tannins, alkaloids, and saponins (Cowan, 1999). In contrast, n-hexane, a non-polar solvent, extracted mainly lipophilic compounds such as steroids, waxes, and non-polar terpenoids, resulting in a much lower recovery. Similar patterns, where semi-polar and polar solvents outperform non-polar solvents in plant extractions, have been reported in other medicinal plants (Cushnie and Lamb, 2011). Overall, these results suggest that semi-polar and polar solvents are more efficient in extracting bioactive compounds from F. thonningii stem bark, which aligns with its ethnomedicinal uses and provides a basis for further biological evaluation.

### *3.2.2: Qualitative Phytochemical Screening*

The results of the qualitative phytochemical screening of the extracts are presented in Table 2. The ethanol and ethyl acetate extracts contained a broad spectrum of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, anthraquinones, steroids, and cardiac glycosides. In contrast, the n-hexane extract showed strong reactions only for steroids and slight presence of anthraquinones, while other phytochemicals were absent. The broad presence of phytochemicals in the ethanol and ethyl acetate extracts correlates with their higher extraction yields compared to n-hexane. Ethanol, due to its high polarity, strongly extracted flavonoids and tannins, which are well-known antioxidants and antimicrobial agents (Do and Ju, 2014, Okwu, 2004). Ethyl acetate, being semi-polar, also recovered diverse metabolites including alkaloids and glycosides, reflecting its ability to extract compounds of intermediate polarity [90]. On the other hand, the strong reactions observed for steroids in the n-hexane extract are consistent with the non-polar nature of the solvent, which favors dissolution of lipophilic molecules (Okwu, 2004). The detection of diverse classes of compounds in ethanol and ethyl acetate extracts is particularly significant since these metabolites are associated with important pharmacological properties. Alkaloids are reported to exhibit antimicrobial and analgesic activities, while flavonoids and tannins possess strong antioxidant and antibacterial (Stalikas, 2007). Saponins have been linked with antibacterial, antifungal, and membrane-permeabilizing effects, whereas cardiac glycosides and steroids are known for cardioprotective and anti-inflammatory properties. The presence of anthraquinones, although in smaller amounts, is also notable since they are associated with antimicrobial and cytotoxic activities (Tiwari et al., 2011).

### *3.2.3: Antibacterial Activity*

The antibacterial assays (Tables 3 to 5) demonstrated that all extracts exhibited measurable

  

inhibitory effects against Escherichia coli, Salmonella typhi, Staphylococcus aureus, and Streptococcus pyogenes, though their potency varied with the solvent and test organism. The ethyl acetate extract displayed the strongest antibacterial activity, producing inhibition zones ranging from 1.50 mm to 17.00 mm across all tested strains. Its highest activity was observed against S. typhi (17.00 mm at 500 mg/mL), followed by significant inhibition of S. aureus and S. pyogenes. The ethanol extract showed moderate activity (1.00–17.00 mm), while the n-hexane extract had the weakest effect, with inhibition zones between 0.50 mm and 17.00 mm, and in some cases no inhibition at lower concentrations.

### *3.1.4 MIC and MBC*

The MIC and MBC data (Tables 6 to 8) further confirmed this trend. The n-hexane extract required relatively high concentrations (125–250 mg/mL) to inhibit or kill bacterial growth, indicating weak activity compared to standard antibiotics such as ciproflaxacin. By contrast, the stronger effects of the ethyl acetate and ethanol extracts can be linked to the broader spectrum of phytochemicals present in these fractions, particularly flavonoids, tannins, and saponins. These findings are consistent with earlier reports that semi-polar and polar extracts of Ficus thonningii possess significant antimicrobial activity. (Abbah *et al*., 2009) Reported that ethanolic extracts of F. thonningii exhibited broad-spectrum antibacterial effects, while (Olayinka *et al*., 2020) observed that ethyl acetate fractions showed higher activity compared to non-polar extracts. The results also align with general phytomedicine literature, where crude plant extracts often show moderate activity compared to synthetic antibiotics, but serve as important leads for bioactive compound discovery (Savoia, 2012). The greater susceptibility of S. typhi compared to E. coli and S. aureus may be due to differences in cell wall structure and permeability. Although Gram-negative bacteria typically show higher resistance due to their outer membrane, some plant-derived compounds can penetrate or destabilize this barrier, leading to effective inhibition (Savoia, 2012).

## 4. Conclusion

Based on the phytochemical screening of *Ficus thonningii* stem bark extracts using n-hexane, ethyl acetate, and ethanol, it can be concluded that the plant contains alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, and anthraquinones. Among the three extracts, the ethyl acetate extract exhibited the highest antibacterial activity, with inhibition zones ranging from 1.50 mm to 17.00 mm, and the lowest MIC (62.5 mg/mL) and consistent MBC values across all tested bacterial strains.

## Conflict of Interest

The authors declare no conflict of interest.

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