



Article Info

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Phytochemical Screening and Antibacterial Activity of *Ficus platyphylla* Leaves ExtractsIbrahim Muhammad¹, Aliyu A. Jabo^{1,2}, Asmau B. Riskuwa¹, Jamilu Usman¹

Plants are rich in bioactive compounds, including alkaloids and phenolic compounds such as phenolic acids, coumarins, flavonoids, and tannins. These protective compounds in plants also have significant therapeutic benefits for humans. The study was conducted at Sokoto State University, Nigeria, the research involved collecting sample at Waziri Maccido road, Bazza Area, Sokoto and extracting the sample using n-hexane, ethyl acetate and ethanol (cool extraction) and aimed to investigate the phytochemical and antibacterial activities of the extracts (n-hexane, ethyl acetate and ethanol). The bioactive compounds were analyzed using method adapted from Ahmad and the antibacterial analysis was carried out against standard laboratory strains of *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (gram positive), *Escherichia coli* (Gram negative) and *Pseudomonas aeruginosa* (Gram negative) using Broth Dilution method. From the result obtained, the mass and the percentage yield of the three fractions obtained from n-hexane, ethyl acetate and ethanol were 9.35g, 3.79%, 6.55g, 2.91% and 24.17g, 11.35% respectively. Ethanolic fraction contains more of the fraction than n-hexane and ethyl acetate. Saponins, phenols, flavonoids, tanins, alkaloids were all present in ethyl acetate and ethanol extract but absent in n-hexane extract. Anthraquinones was absent in all the three extracts but there was presence of steroid and cardiac glucosides in all the three extracts. The result of antibacterial deduced that n-hexane and ethyl acetate fractions have more activity on bacterial strains with zone of inhibition ranging from 2-21mm while the ethanol fractions show less activity ranging from 2-12mm. These extracts have shown the zone of inhibition of the commercial antibiotic Ampicilin (positive control) to be 13-27mm. The MIC/MBC result of n-hexane was 500/500, ethyl acetate 500-62.5/500-62.5 and ethanol 250-62.5/250-62.5 against all the isolates respectively.

Keywords: *Ficus platyphylla*, Phytochemicals, Anti-bacterial, Extracts.

1. Introduction

Bacteria have a natural ability of transferring from one person to another and also fight against the drugs that are used for the prevention of diseases (Yakub *et al.*, 2018). Even though pharmaceutical industries have done many efforts to address this issue by producing various forms of antibiotics during the past decades, there is still an increased in resistance to these drugs by the microorganisms (Kumar, 2019) hence the need to develop new drugs. In response to these challenges, medicinal plants are used since ancient times to manage and treat various diseases, because they constitute a renewable source of nutrients and bioactive compounds (Kumar, 2019).

Ficus platyphylla is a medicinal plant belonging to the *Moraceae* family. It is widely distributed throughout the savannah region of the West African coast. The plant *Ficus platyphylla* has been used in Nigeria's folk medicine for many years to treat

epilepsy, depression, psychosis, pain, and inflammation (Béné *et al.*, 2016) Its effectiveness is widely recognized among rural communities in Northern Nigeria, where it is locally known as 'Gamji' (Dar *et al.*, 2017). High-performance liquid chromatography (HPLC) and preliminary phytochemical screening of the methanol extract from the stem bark of *Ficus platyphylla* have identified the presence of saponins, flavonoids, and tannins (Njila *et al.*, 2017) Previous studies have also demonstrated the analgesic and anti-inflammatory effects of this methanol extract through various assays, including acetic acid-induced writhing, formalin-induced nociception, and albumin-induced edema in mice (Haile, 2007). The behavioural and anticonvulsant effects of the standardized extract from *Ficus platyphylla* stem bark have also been known (Chindo *et al.*, 2014). Additionally, studies have shown that the oral

administration of the methanol extract is safe for rodents (Chindo *et al.*, 2012). *Ficus platyphylla* has been shown to contain bioactive constituents such as tannins, saponins, and flavonoids, which have anti-inflammatory potential and have demonstrated relative safety in previous toxicological studies (Sabele *et al.*, 2012). Pharmacological studies on *Ficus platyphylla* have demonstrated its antinociceptive, antimalarial, antibacterial, antifungal, anti-inflammatory, and gastrointestinal activities (Redondo *et al.*, 2014). While previous studies have examined stem bark extracts, there is limited data on the antibacterial activity of the leaves extracts. In this context, the present study was designed to investigate the phytochemical constituents of *Ficus platyphylla* leaves extract and to test its antibacterial activity against some selected bacteria for the possibilities of using them for new drug development.

2. Materials and Methods

2.1 Sample collection and Identification

The leaves of *Ficus platyphylla* were collected from Waziri Maccido Road (Bazza Area) Sokoto North Local Gov't, Sokoto State, Nigeria. The sample was identified and authenticated at the herbarium of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. The voucher specimen with number (VN: PCG/UDUS/MORA/0003) was deposited at the herbarium for reference purposes.

2.2 Sample Preparation

The collected sample was thoroughly washed with clean water to remove any sand or debris. The sample was shade dried to prevent degradation of active compounds. Once fully dried, the sample was grounded into a fine powder using a mortar and pestle, and then sieved. The powdered sample was stored in an airtight, moisture-free polyethylene bag until used for further extraction and analysis.

2.3 Extraction Procedure

The powdered sample (246.8g) was subjected to extraction in a 1000 cm³ conical flask using three different solvents. First, N-hexane (900 cm³) was used to remove non-polar substances. After that, the marc was dried and soaked in ethyl acetate (900 cm³) to remove moderately polar substances. Finally, ethanol (900 cm³) was used to extract polar compounds. The extraction was done in the order of increasing solvent polarity. Each solvent stayed in contact with the sample for 24 hours, with intermittent stirring to help the extraction. After 24 hours, the mixture was filtered using Whitman filter No.1 and the filtrates were transferred to dishes for evaporation (Saleh *et al.*, 2015).

2.4 Test Organisms

Standard laboratory strains of *Staphylococcus aureus* (Gram-positive), *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative), and *Pseudomonas aeruginosa* (Gram-negative) were obtained from the Department of Microbiology, Sokoto State University, Sokoto. All the isolates were checked and maintained in Muller Hinton Agar.

2.5 Qualitative Phytochemical Screening of Extracts

The following tests were performed to screen for phytochemicals in the leaf extracts:

2.5.1 Test for flavonoid

(a). Sodium hydroxide test

Sodium hydroxide 10% (1 cm³) was added to 3 cm³ of the aliquot of the filtrate. The formation of yellow color indicates the presence of flavonoids (Saleh *et al.*, 2015).

(b). Shinodas test

Magnesium chips 2 cm³ was added to 2 cm³ of the extract followed by addition of few drops of concentrated HCl, a pink, orange or red to purple coloration was taken as a confirmation for presence of flavonoids (Saleh *et al.*, 2015).

(c). Ethyl acetate test

Ethyl acetate 1 cm³ was added to 2 cm³ extracts. The mixture was shaken and allowed to settle. Formation of yellow color in the organic solvent layer was taken as positive for free flavonoids c

2.5.2 Test for tannins

(a). Iron (iii) chloride test

Ferric chloride solution 5% ferric chloride solution was added drop by drop to 2-3 cm³ of the extract and the coloured produced is noted. Condensed tannins usually give a dark green color, hydrolysable tannins give blue black colour (Saleh *et al.*, 2015).

(b). Lead acetate test

The extract 2 cm³ was treated with few drops of lead acetate solution. Formation of a yellow precipitate indicates the presence of tannins (Saleh *et al.*, 2015).

2.5.3 Test for saponin

(a). Frothing test

Water 5 cm³ was added to the extract and shakes vigorously for 5 minutes. The formation of honey comb froth which lasted for several minutes indicates the presence of saponins (Saleh *et al.*, 2015).

2.5.4 Test for alkaloids

(a). Dragendorff's test

The extract 2 cm³ was stirred with 2 cm³ of 10% aqueous hydrochloric acid. 1 cm³ was treated with a few drops of Dragendorff's reagent. Precipitation with this reagent was taken as preliminary evidence for the presence of alkaloids (Saleh *et al.*, 2015).

(b). Wagner's test

The extract 2 cm³ was stirred with 2 cm³ of 10% aqueous hydrochloric acid. 1 cm³ was treated with a few drops of Wagner's reagent. Precipitation with this reagent was taken as preliminary evidence for the presence of alkaloids (Saleh *et al.*, 2015).

(b). Mayer's test

The extract 2 cm³ was stirred with 2 cm³ of 10% aqueous hydrochloric acid. 1 cm³ was treated with a few drops of Mayer's reagent. Precipitation with this reagent was taken as preliminary evidence for the presence of alkaloids (Saleh *et al.*, 2015).

2.5.5 Test for Cardiac glycoside (Keller killianis test)

To 1 cm³ of the extract, 2 cm³ of 0.5% of ferric chloride solution was added and allowed to stand for 1 minute. 1 cm³ of concentrated H₂SO₄ was carefully added down the wall of the tube so as to form a lower layer. A reddish browning at the interface indicates the presence of cardiac glycoside (Saleh *et al.*, 2015).

2.5.6 Test for steroid

(a). Salkowskis test

The extract 0.5g was dissolved in 2 cm³ of chloroform, 2 cm³ of sulphuric acid will be carefully added to form lower layer. A reddish browning of the interface indicates the presence of a steroidal ring (Saleh *et al.*, 2015).

(b). Lieberman buchard test

The extract 2 cm³ was treated with 2 cm³ of acetic aldehyde and then 2 cm³ of concentrated sulphuric acid was added and formation of blue or green ring at the interface indicate the presence of steroid (Truchan *et al.*, 2015).

(c). Test for Anthraquinones

The extract 0.5 cm³ was shaken with 10 cm³ of benzene and 5 cm³ of 10% ammonia solution was added. The mixture was shaken, the presence of pink, red, or violet colour in the ammonia (lower) phase indicates the presence of anthraquinones (Halilu *et al.*, 2013).

2.6 Antibacterial Tests

The antibacterial test was conducted using the cup plate method described (Garrod *et al.*, 1968). The Nutrient Agar plates prepared according to manufacturer's instruction and allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at 37 °C to ensure the sterility of the medium. The dilution ratio for Gram-positive bacteria and Gram-negative bacteria will be 1:1000 and 1:5000, respectively, using normal saline water. The Nutrient Agar plates were beflooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. Four wells (cups) of about 4 mm in diameter will be cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs will be removed using sterile ampoule file. The extract solution (0.5 ml) was placed in each of the wells and was allowed to settle for two hours at room temperature and then incubated for 24 hours at 37 °C. The inhibition zone was observed and recorded in millimeters using a transparent ruler. Standard antibiotic was used.

2.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC was determined using the broth dilution technique (Usman, 2007). This was carried out as described by (Usman, 2007). Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration where no visible turbidity will be observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, the micro-organisms were prepared using the broth dilution technique. The stock extract concentration of 5 mg/ml, 10 mg/ml, 15 mg/ml and 20 mg/ml was made by dissolving 0.05 g, 0.1 g, 0.15 g and 0.2 g, respectively, of the extract in 10 ml of sterile distilled water and the working concentrations prepared by two-fold serial dilution technique that ranged from 0.098 mg/ml to 50 mg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 24 hours incubation at 37 °C, the tubes will be observed for turbidity. The lowest concentration where no turbidity was observed was determined and noted as the Minimum Inhibitory Concentration (MIC). The lowest concentration of the extract that inhibits visible bacterial growth was noted.

The minimum bactericidal concentration was determined from broth dilution test resulting from the MIC tubes by inoculating the content of each test tube on a nutrient agar plate. The plates were incubated at 37 °C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration (Usman, 2007).

3. Results and Discussion

3.1 Results

3.1.1 Extraction

The percentage yield of the three extracts obtained from n-hexane, ethyl acetate and ethanol are represented in Table 1. The ethanolic extracts has the highest yield than n-hexane and ethyl acetate.

Table 1: Mass of extract and percentage recovery of *F. platyphylla* leaves extracts.

Extract	Original Mass (g)	Mass of Extract (g)	Percentage Recovery (%)
N-hexane	246.80	9.35	3.79
Ethyl acetate	225.48	6.55	2.91
Ethanol	213.04	24.17	11.35

3.1.2 Qualitative Phytochemical Screening of *Ficus platyphylla* Leaves Extracts

The results for the phytochemical screening of *F. platyphylla* leaves extracts are presented in Table 2.

Table 2: Qualitative phytochemical screening of *F. platyphylla* leaves extracts.

Phytochemical	N-hexane	Ethyl acetate	Ethanol
Saponins	-	+	+
Phenols	-	+	+
Flavonoids	-	+	+
Tannins	-	+	+
Alkaloids	-	+	+
Anthraquinones	-	-	-
Steroids	+	+	+
Cardiac Glycosides	+	+	+

Key: (+): present, (-): absent

3.1.3 Determination of antibacterial activity of *F. platyphylla* leaves extracts

The results of the antibacterial activity of *F. platyphylla* leaves extracts are presented in Table 3, 4, 5, 6, 7 and 8.

Table 3: Zone of inhibition (mm) of n-hexane fraction of *F. platyphylla* leaves against selected bacteria.

Test Isolates	500	250	125	62.5	31.25	Control
						2.5 mg/ml Cipro
<i>E. coli</i>	9.0	6.0	4.0	4.0	2.0	22.0
<i>P. aeruginosa</i>	21.0	19.0	17.0	13.0	11.0	27.0
<i>S. aureus</i>	8.0	4.00	2.0	2.0	0.0	18.0
<i>B. subtilis</i>	14.0	12.0	8.0	6.0	4.0	19.0

Table 4: Zone of inhibition (mm) of ethyl acetate fraction of *F. platyphylla* leaves against selected bacteria.

Test Isolates	500	250	125	62.5	31.25	Control
						2.5 mg/ml Cipro
<i>E. coli</i>	9.0	6.0	4.0	4.0	2.0	22.0
<i>P. aeruginosa</i>	21.0	19.0	17.0	13.0	11.0	27.0
<i>S. aureus</i>	8.0	4.0	2.0	2.0	0.0	18.0
<i>B. subtilis</i>	14.0	12.0	8.0	6.0	4.0	19.0

Table 5: Zone of inhibition (mm) of ethanolic fraction of *F. platyphylla* leaves against selected bacteria.

Test Isolates	500	250	125	62.5	31.25	Control
						2.5 mg/ml Cipro
<i>E. coli</i>	8.0	6.0	4.0	2.0	0.0	13.0
<i>P. aeruginosa</i>	12.0	8.0	6.0	4.0	0.0	19.0
<i>S. aureus</i>	9.0	5.0	3.0	2.0	0.0	13.0
<i>B. subtilis</i>	8.0	6.0	4.0	2.0	0.0	13.0

Table 6: MIC and MBC (mg/mL) of n-hexane fraction of *F. platyphylla* leaves

Test Isolates	MIC	MBC
<i>E. coli</i>	500	500
<i>P. aeruginosa</i>	500	500
<i>S. aureus</i>	500	500
<i>B. subtilis</i>	500	500

Table 7: MIC and MBC (mg/mL) of ethyl acetate fraction of *F. platyphylla* leaves.

Test Isolates	MIC	MBC
<i>E. coli</i>	250	250
<i>P. aeruginosa</i>	500	500
<i>S. aureus</i>	62.5	62.5
<i>B. subtilis</i>	250	125

Table 8: MIC and MBC (mg/mL) of ethanolic fraction of *F. platyphylla* leaves.

Test Isolates	MIC	MBC
<i>E. coli</i>	250	250
<i>P. aeruginosa</i>	62.5	62.5
<i>S. aureus</i>	125	125
<i>B. subtilis</i>	125	125

3.2 Discussion

The results of percentage yield obtained from the extraction of *F. platyphylla* leaves showed the mass and percentage yield of the extracts. The mass and the percentage yield obtained from n-hexane, ethyl acetate and ethanol were 9.35g, 3.79%, 6.55g, 2.91% and 24.17g, 11.35% respectively. Ethanolic extract has the highest yield of 11.35% while ethyl acetate yielded the least percent of 2.91%. This is in line with report of Saleh *et al.* (2015).

The results of the qualitative phytochemical screening of the extracts obtained showed that saponins, phenols, flavonoids, tannins, alkaloids were all present in ethyl acetate and ethanol extracts, but absent in n-hexane extract. Anthraquinones were absent in all the three extracts (n-hexane, ethyl acetate and ethanol), but there was presence of steroids and cardiac glycosides in all the three extracts.

The antibacterial activity of the extracts was tested against standard laboratory strains of *Staphylococcus aureus* (Gram-positive), *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative), and *Pseudomonas aeruginosa* (Gram-negative). Table 3, 4 and 5 above showed the details of the results obtained from the antibacterial activity test of the n-hexane, ethyl acetate and ethanol extracts of *F. platyphylla*. The results deduced that n-hexane and ethyl acetate extracts have more activity on bacterial strain with zone of inhibition ranging between 2-21 mm while the ethanol fraction shows less activity ranging from 2-12 mm, being it polar and many strong antibacterial phytochemicals are semi-polar to non-polar (terpenoids, phenolics, flavonoids) which ethanol may not extract as efficiently. This indicates that the active phytochemical constituents in the plant with antibacterial properties are in the non-polar and moderately polar solvents (n-hexane and ethyl acetate). The above result obtained is higher when compared with work of Konaré *et al* (2020) on *F. platyphylla* stem bark.

4. Conclusion

Ficus platyphylla contain bioactive compounds with antibacterial potential, particularly in ethyl acetate and n-hexane extracts. These findings support the plant's ethnomedicinal use and warrant further studies including isolation of active compounds and in vivo testing.

Conflict of Interest

The authors declare no conflict of interest.

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