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Research article

Isolation and Antimicrobial Investigation of Extract and Fractions Obtained from the Stem Bark of *Ficus sycomorus* (Moraceae)

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ABSTRACT

Microbial infections are very common in developing countries like Nigeria. It is the cause of poverty as well as many deaths in developing countries, while the current treatments are encountering resistance by these pathogens. However, natural products can help to overcome the problems associated with pathogenic diseases. Ethanolic extract of Ficus sycomorus stem bark was evaluated phytochemically for its constituents and antimicrobial potential on selected Gram-positive and Gram-negative organisms. The extract was fractionated by column and pooled into four fractions (A–D), these were evaluated for their antimicrobial activity against *E. coli, S. typhi, S. aureus* and *K. pneumonia* at different concentrations. The fractions A–C were active at concentration of 50-100 mg/mL. The low MIC values of the crude extract against S. aureus and *K. pneumonia* holds potential for use as antimicrobial drug leads.

Keywords: *Ficus sycomorus, stem-bark, extracts, isolation, antimicrobial investigation*

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INTRODUCTION

Millions of people are affected by several pathogenic diseases including *S. typhi* and *S. aureus* which are easily gotten through contaminated water and food. These food-borne pathogens are among the leading causes of high morbidity and mortality rate in developing countries. Treatment for gastroenteritis and other bacteria-caused infectious disorders is greatly improved by medicinal herbs. There may be advantages to exploring newer antimicrobials in plants since it results in a different strategy for reducing antibiotic resistance (Dubale *et al*., 2023).

Plants used as antimicrobial agents may inhibit bacteria through a different method than antibiotics. Consequently, may aid in the treatment of microbial diseases that are resistant. As a result, several studies on the screening of plants as natural antimicrobials have been reported by authors (Stuper-Szablewska *et al*., 2023; Dos Santos, *et al*., 2022; Manandhar *et al*., 2019; Aljeldah, 2022; Salim *et al*., 2023; Adamczak *et al*., 2020). Treatment for gastroenteritis and other bacterially-caused infectious disorders is greatly improved by medicinal herbs. The quest for more recent antimicrobial agents in plants reveals new compounds with complicated structure which the synthetic chemist may modify through synthesis for more potent activity.

Ficus sycomorus is a member of the family moraceae, which has approximately 1,400 species of trees, vines, and herbs and roughly 40 genera. These plants frequently produce milky latex fluids (Wanjala Wafula *et al*., 2023; Al-Shabibi *et al*., 2022; Kone *et al*., 2022). It is commonly known as fig mulberry. The Hausa people of Northern Nigeria call it Farin Baure or Bore and is known among the Ichen people of Taraba state as tû-engətsən. The leaves are said to treat jaundice and act as an antidote for snakebites. The milky latex produced by a sycomorus tree is applied to inflamed areas or used to treat dysentery, chest conditions, and colds. Concoction of stem bark and milky latex is used in the treatment of ringworm. Stem bark remedies are used in treatment of coughs, throat infections and chest pains (Saheed *et al*., 2020; Toma *et al*., 2019). The roots of *Ficus sycomorus* have anthelmintic and laxative effects (Nawaz *et al*., 2020). Additionally, it has been reported that the plant is an effective antibacterial agent against *Salmonella typhi* that is resistant to ciprofloxacin (Saheed *et al*., 2020).

However, according to other researchers, *F. benghalensis* and *F. racemosa* roots had mild antibacterial action against

isolates of *S. aureus, P. aeruginosa*, and *K. pneumonia* at doses of 25, 50, and 75 mg/ml (Olawuwo *et al*., 2022). According to another study, Ficus spp. stem bark extract showed significant antibacterial activity against the pathogens *P. aeruginosa, E. coli, P. vulgaris, B. subtilis*, and *S. aureus* (Salehi *et al*., 2021; Saheed *et al*., 2020). The leaf acetone *F. tsiela* extract, however, was observed to have the highest inhibitory action (11 mm) against isolates of *S. aureus, P. aeruginosa*, and *K. pneumonia* (Adeyemi *et al*., 2021; Jassal and Sharma, 2019).

Since these pathogens were associated with antibiotic-resistant bacterial infections and a problem in developing nations like Nigeria, the current study sought to evaluate the phytochemical components and antibacterial spectrum of an ethanol stem bark extract of *F. sycomorus*.

MATERIALS AND METHODS

Sample Collection: October 20, 2022, fresh stem-bark of the medicinal plant *F. sycomorus* L. was taken from its native habitat in Didan area of Kurmi LGA, Taraba State, Nigeria. The Taraba State University's Biological Science Department conducted a taxonomical examination to identify the plant.

Sample Preparation and Extraction: The stem-bark were chopped into small portions and air-dried under shade. The dried stem-bark was pulverized into powder to increase the surface area. One thousand five hundred grams (1.5kg) of the crushed sample material was extracted with 96% ethanol using Soxhlet extractor. The crude extract was exposed to 25°C air after being concentrated over a water bath at 100°C. The dry extract was weighed, tagged and kept in a desiccator, subject to further analysis.

Qualitative phytochemical screening: The freshly made crude extract underwent qualitative phytochemical screening to check for the presence of proteins, phenols, tannins, alkaloids, flavonoids, steroids, glycosides, saponins, and steroid-like compounds according to Vishnoi *et al., (*1979), Markhan, (1982), Trease and Evans (2002), Silver *et al.* (1998), Sofowora, (1993), and Brain and Turner, (1975).

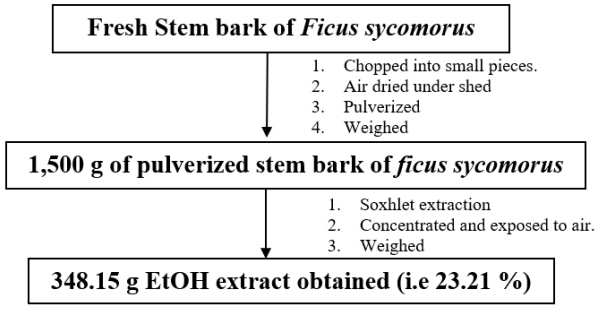

Figure 1
Scheme of extraction

Column Chromatography and Purification: The column was packed with silica gel (stationary phase) of appropriate mesh size and the extract was spread over the column. Eluting solvents (mobile phase) were applied to the column from the top down, starting with a less polar solvent mixture (70:30 hexane to ethyl acetate) and ending with a more polar solvent mixture (70:30 ethyl acetate to methanol) as modified from (Igoli *et al.,* 2005). Different mixture components with differing degrees of interactions with the stationary and mobile phases were clearly separated as the mobile phase dripped down by gravity. The separated components were collected sequentially and carefully labeled for further analysis. Purification of compounds isolated was carried out using thin layer chromatography. Chromatography plate were obtained and prepared by spotting it with extract solution at about 2cm above the lower edge of the plate using a micro-pipette. The plate was then developed by placing the lower edge of the plate in a chromatographic tank containing a suitable solvent system benzene/hexane (1:1) and letting the spotted area stay just above the solvent surface. The sample components went up the plate at varied rates depending on their solubility and degree of retention by the stationary phase as the solvent was permitted to migrate up the plate through capillary action. Isolation was identified after drying the plate, exposing it to sulfuric acid and heating in an oven.

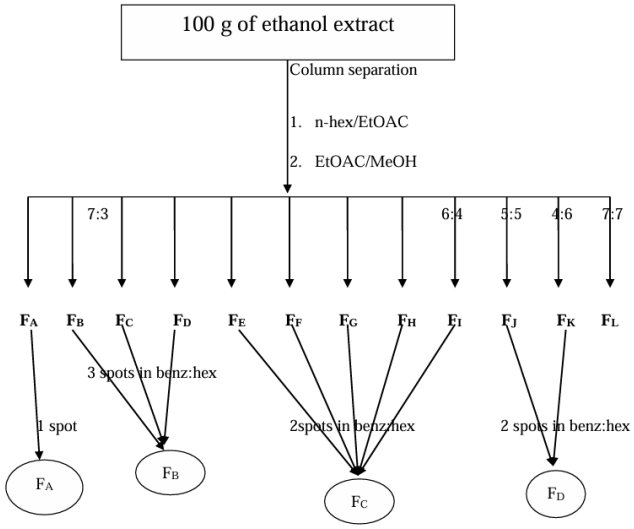

Figure 1
Column Separation and TLC profile of Ethanol Extract of Ficus sycomorus

Microorganisms and growth conditions: Test microorganisms used include *Salmonella typhi, Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus.* All the organisms were pure clinical isolates obtained from National Vetenary Research Institute Vom, Plateau State. Standard antibiotic (Ciprofloxacin 500mg) was used as standard.

In a sterile bottle filled with pure broth, test organisms that had been grown for 24 hours were suspended. It was gradually infused with normal saline, and the turbidity was measured and compared to that of the 0.5 McFarland standard, which equals to 108 cells/mL. Then, this was diluted to create 10^6 cells/mL, and used in the studies. The dilution ratios for Gram-positive and Gram-negative organisms were 1:1000 and 1:1500, respectively (Usman *et al*., 2009).

Antimicrobial Susceptibility Assay (agar well diffusion method): These tests used the agar plates that had been

inoculated with test organisms. A sterile cork borer was used to make wells on the agar that were 6 mm in diameter and 4 mm deep. Each of the extracts was prepared in a two-fold serial dilution of 200, 100, 50, 25 and 12.5 mg/mL. A measure of 0.5 mL of each concentration was pipette into the holes bored on the agar plates. Negative control consisted of 0.5mL of the extraction's pure solvent, while positive control consisted of 0.5mL of ciprofloxacin 500 mg in a 5µg/mL solution. The plates was allowed to dry, and then incubated at 37^0C for 24hrs. Measurements of the diameters of growth inhibition zones in triplicates were used to assess the antibacterial activity, and the findings were provided as Mean±SEM (Emeruwa, 1982).

Determination of Minimum Inhibitory Concentration (MIC): Stock solution of the extracts was prepared by dissolving 10g of extract in 10mL of distilled water, and 1000mg/mL solution was obtained. The broth dilution method was used to determine the minimal inhibitory concentration. The stock solutions of the extracts were diluted to 400, 200, 100, 50, and 25mg/mL in nutrient broth. Each dilution's duplicate tubes were inoculated with 1mL of the test organism and then incubated at 37^0C for 24 hours. Each experiment included a solution of ciprofloxacin (5 µg/mL), whereas the extraction solvent alone served as the negative control. The lowest extract concentration that prevented any observable growth was the minimum inhibitory concentration.

Determination of Minimum Bactericidal Concentration (MBC): Every broth tube that failed to grow during the minimum inhibitory concentration assay had a culture of 2 mL removed from it, and it was then inoculated onto brand-new agar plates. These were kept at 37°C for 24 hours. The growth on the plates was monitored, and the smallest concentration that revealed no growth was noted as the minimum bactericidal concentration.

Statistical Analysis

Mean and Standard error of the Mean (SEM) were used to present the generated data. The mean and standard error of the mean were tested using one-way analysis of variance (ANOVA), and a significance level of $P<0.05$ was deemed significant.

RESULTS

Phytochemical Investigation for Ethanol Extract of *Ficus sycomorus:* Carbohydrates, tannins, cardiac glycosides, cardinolides, saponins, alkaloids, terpenoids, and flavonoids were all detected in the ethanol extract. There were no anthraquinones or combined anthraquinones as shown in Table 1.

Antimicrobial Evaluation: The antibacterial activity of ethanol SBE of F. sycomorus L and fractions obtained from column against pathogenic species such as E. coli, S. typhi, S. aureus and K. pneumonia was evaluated based on ZIs, MIC and MBC parameters. Zone of inhibition (ZIs) varied based on the tested bacteria pathogens and fractions of extract. The results are presented in Table 2. This value ranged between 13.4 –15.0 mm and 9.67 - 18.0 mm against *E. coli* and S. *aureus*, respectively for crude ethanol extracts. *K. pneumonia* isolate was found to be the pathogen that the extracts inhibited the most. This value was noted in this regard as 17, 30, 25, 20 mm ZIs for crude ethanol SBE, Fraction A, B and C against K. pneumonia; while it was recorded to be 15, 15.12, 10, 10 mm ZIs for crude ethanol SBE, Fraction A, B and C respectively against E. coli. Fraction D showed no activity at all concentrations. This investigation shows that fraction A possesses a higher antibacterial activity than that of the crude, fraction B, C and D which may be a result of differences in phytochemical constituents.

Table 1
Phytochemical Evaluation for Ethanol Extract of *Ficus sycomorus.*

Phytoconstituent	Result
Alkaloids	+
Free- anthraquinones	-
Anthraquinones	-
General carbohydrates test	+
Monosaccharide	+
Free reducing sugar	+
Reducing sugar	+
Ketoses	+
Soluble starch	+
Cardenolides	+
Steroidal nucleus	+
Steroidal nucleus	+
Terpenoids	+
Flavonoids	+
Saponins glycosides	+
Tannins	+
Phlobatannins	+

Key: *+ = present, - = absent*

Minimum Inhibitory Concentrations (MIC) for Ethanol Stem Bark Extract of *F. sycomorus* Against Susceptible Organisms: The pathogens' susceptibility to the crude ethanol SBE was determined through the minimum inhibitory concentration (MICs) evaluation. Estimated MIC values were in the range of 50–100 mg/mL (Table 3). The most antibacterial activity was observed in *S. aureus* and *K. pneumonia* isolates (50 mg/mL).

Minimum Bactericidal Concentrations (MBC) for Ethanol Stem Bark Extract of *F. sycomorus* Against Susceptible Organisms: The minimum bactericidal concentration (MBC) estimation method was used to determine the efficiency of stem bark extract of *F. sycomorus* in killing bacteria. In this regard, *S. aureus* and *K. pneumonia* isolates had the highest antibacterial activity (100 mg/mL) (Table 4), whereas *E. coli* and *S. typhi* had the maximum antibacterial activity at 200 mg/mL.

Table 2
Antimicrobial activity of the crude ethanol stem-bark extract and pooled column fractions of *F. sycomorus* L. against selected Gram-negative and Gram-positive organisms using agar-well method (zone of inhibition in mm).

	Extract mg/mL	*E. coli*	*S. typhi*	*S. aureus*	*K. pneumonia*
Crude extract	25	0.00±0.00	8.00±1.63	0.00±0.00	0.00±0.00
	50	0.00±0.00	9.00±0.66	9.67±2.04	10.00±1.45
	100	13.40±0.43	11.00±0.26	12.00±1.04	13.00±1.62
	200	15.00±0.15	16.10±0.98	18.00±0.53	17.00±0.81
Fraction A	12.5	0.00±0.00	0.00±0.00	7.00±0.00	8.10±0.52
	25	8.00±1.11	12.41±0.41	9.04±1.02	14.00±0.23
	50	12.33±0.36	18.00±0.76	12.40±0.64	22.00±0.31
	100	15.12±0.19	25.10±0.06	15.00±0.40	30.00±0.00
Fraction B	12.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	8.00±0.00	0.00±0.00	13.40±0.34
	50	0.00±0.00	12.00±0.81	9.00±0.60	18.10±0.14
	100	10.00±0.00	15.00±0.85	15.00±0.00	25.00±0.00
Fraction C	12.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	8.53±0.00	9.00±0.00
	50	0.00±0.00	12.50±0.00	12.00±0.00	14.30±0.35
	100	10.00±0.00	20.10±0.10	15.00±0.00	20.00±0.50
Fraction D	12.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ciprofloxacin	5 µg/mL	30.43±0.00	30.54±0.57	25.00±0.00	40.14±0.14

Table 3:
Minimum inhibitory concentration of crude ethanol stem bark extract of *ficus sycomorus.*

Organism	400	200	100	50	25
Escherichia coli	-	-	β	+	+
Salmonella typhi	-	-	β	+	+
Staphylococcus aureus	-	-	-	β	+
Klebsiella pneumonia	-	-	-	β	+

Key: *β = MIC, - = no activity, + = there is activity*

Table 4:
Minimum Bactericidal Concentrations (mg/mL) for Ethanol Stem Bark Extract of *F. sycomorus* Against Susceptible Organisms.

Organisms	400	200	100	50
Escheria coli	-	α	+	+
Salmonella typhi	-	α	+	+
Staphylococcus aureus	-	-	α	+
Klebsiella pneumonia	-	-	α	+

Key: α = MBC, - = no activity, + = there is activity

Table 5:
One-way ANOVA between the Means of Fraction A and Ciprofloxacin

Variable source	Sum of squares (SS)	Degree of freedom (df)	Mean of squares	F-cal
Between	224.5812	1	224.5812	**5.96**
Within	301.2324	8	37.6541	**Fcri=5.32**

DISCUSSION

This investigation shows that fraction A possesses a higher antibacterial activity than that of the crude, fraction B, C and D which may be a result of differences in phytochemical constituents.

The zone of inhibition by crude ethanol extract of F. sycomorus was found to vary between 15 and 23.5 mm, which is comparable to studies published by Isah et al., 2020 and Josephus et al., (2020) indicating the ZIs of acetone extract as between 16 to 27 mm. According to another study, F. tsiela leaf extract in diethyl ether had the most inhibitory effect against K. pneumoniae (20 mm), E. coli (12 mm), and P. aeruginosa (12 mm), with S. aureus showing the least amount of activity (10 mm) (Jassal and Sharma, 2019; Adeyemi et al., 2021). The antibacterial activity of methanolic extract of F. carica against five bacterial strains, including B. cereus, E. aerogens, K. pneumoniae, B. subtilis and S. epidermidis, at various concentrations (30, 40, 50, and 60 g/ml) was also reported by Tkachenko et al., (2022) and Belattar et al., (2021). The later study showed that for the five diseases under investigation, the predicted ZIs values ranged between 12.5 and 14.0 mm.

In general, antibiotic and antibacterial activity showed that applied antibiotic was more potent than crude extract and pooled column fractions against the pathogens under investigation (Tab. 2); this could be because these extracts contain a variety of compounds that act antagonistically against one another. Our findings showed that the lowest Ciprofloxacin activity was observed against isolates of S. aureus bacteria. According to another investigation, Ciprofloxacin recorded ZIs against S. aureus is between 13 and 24 mm (Saleh et al., 2015).

MIC values of ethanol leaf extract of F. exasperate against E. coli was previously reported to be 300 mg/ml and S. albus' was 700 mg/ml [18]. However, other research discovered that the MIC values for ethanol F. sycomorus L. extract ranged from 13.5 to 17.5 mg/mL (Tanoğlu et al., 2019); while, F. platphylla's ranged from 1.97 to 7.81 mg/ml (Saleh et al., 2015). Whereas lupenol, an isolated compound from F.

deltoidea leaves, was discovered to have antibacterial properties against S. aureus, E. coli, and B. subtilis. E. coli, B. subtilis, and S. aureus each have MICs of 150, 220, and 130 µg/ml, respectively (Abdulrahman, 2023). However, it has been reported that the MIC values of the methanolic extract of F. carica against the five tested bacterial strains ranged from 3 to 7 µg/ml (Abdou et al., 2021).

On the other hand, the minimum bactericidal concentration (MBC) estimation method was used to determine the efficiency of stem bark extract of F. sycomorus in killing bacteria. In this regard, S. aureus and K. pneumonia isolates had the highest antibacterial activity (100 mg/mL) (Table 4), whereas E. coli and S. typhi had the maximum antibacterial activity at 200 mg/mL. The MBC by ethanol F. sycomorus L. extracts was previously reported to be between 200 and 250 mg/ml, whereas that of F. platphylla against S. aureus and S. typhi isolates ranged from 3.9 to 62.5 mg/ml (Saheed et al., 2020; Isah et al., 2020; Sheidu et al., 2020). Another report suggests that MBC values of methanolic extract of F. carica against five bacterial strains examined ranged between 6 to 11 µg/ml (Jabbari and Firoozabad, 2021). One way analysis of variance between the activity of FA and the standard drug used (Ciprofloxacin 5 µg/mL) was calculated to compare the means and determine the difference between the means. Table 5 showed that there is no significant difference as $p \geq .05$ between the activity of FA and the standard drug used as positive control, hence the use of Ficus sycomorus stem bark by traditional medicine practitioners to cure diseases caused by the selected microorganisms is confirmed.

According to the antimicrobial investigation, column fraction A of the extract was observed to be more effective against all tested pathogens compared to the other extracts. On the other hand, 50 and 100 mg/mL MIC and MBC values against S. aureus and K. pneumonia was more potent than that of E. coli and S. typhi (100 and 200 mg/mL MICs and MBC values). The difference in observed biological activity between fraction A and crude ethanol SBE could be attributed to the presence of mixture of many compounds that may act antagonistically compared to a purer compound FA. The highest antibacterial activity was recorded in S. aureus and K. pneumonia isolates with 50 mg/mL MIC and 100 mg/mL MBC. Results from the MBC assay supported the data obtained from the MIC determination assay. Otherwise, the lowest antibacterial activity of the standard drug used (ciprofloxacin) was observed for S. aureus pathogen.

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