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Phytochemical and Antimicrobial Content of Sycamore Seed (*Ficus sycamorus*)

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ABSTRACT

The growing threat of antibiotic resistance has intensified the global search for alternative antimicrobial agents, particularly from natural sources. Ficus sycamorus, a traditionally used medicinal plant in Nigeria and other developing countries, was investigated for its phytochemical constituents and antimicrobial potential. This study evaluated the phytochemical composition, antimicrobial activity, and minimum inhibitory concentrations (MIC) of various solvent extracts (ethanol, methanol, n-hexane, petroleum ether, and aqueous) of F. sycamorus leaves. Phytochemical screening revealed the presence of bioactive compounds such as tannins, alkaloids, steroids, glycosides, and flavonoids. Antimicrobial assays demonstrated that the crude extracts exhibited varying zones of inhibition against selected pathogenic bacteria (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) and fungi (Penicillium chrysogenum, Aspergillus fumigatus). The MIC values ranged from 125 to 500 µg/mL, with ethanol and methanol extracts showing higher antimicrobial efficacy. These findings suggest that F. sycamorus possesses promising antimicrobial properties and may serve as a potential source for novel plant-based therapeutics.

Keywords: *Ficus sycamorus, phytochemicals, antimicrobial activity, minimum inhibitory concentration (MIC), medicinal plants*

1.0 Introduction

Plants have long served as valuable sources of medicine due to their rich diversity of secondary metabolites with therapeutic potential. With an estimated 250,000 to 500,000 plant species on Earth, only a small fraction—approximately 1–10%—have been systematically studied for their antimicrobial properties [1–4]. Plant materials were used to treat infectious diseases even before the understanding of microbial causative agents [5]. This practice persists in many parts of the world, including Nigeria, where traditional medicine remains a significant component of primary healthcare.

The renewed interest in medicinal plants is fueled by their accessibility, affordability, safety profile, and relatively minimal side effects. Medicinal plants, particularly those with a long history of ethnopharmacological use, are considered promising candidates for the discovery of new therapeutics, including anti-cancer [7] and antimicrobial agents [8]. According to the World Health Organization (WHO), medicinal plants are among the best sources for drug development, and it is estimated that approximately 80% of the population in developing countries rely on traditional remedies containing plant-derived compounds [9], many medicinal plants remain scientifically underexplored. Comprehensive investigation into their phytochemical compositions, pharmacological properties, and safety profiles is essential to validate traditional claims and support their integration into modern healthcare systems.

Plants have played a significant role in pharmaceutical research and continue to positively impact healthcare, particularly in the treatment of cancer and other chronic diseases [10]. They are capable of synthesizing a wide array of bioactive compounds, known as phytochemicals, which contribute to their therapeutic properties. These compounds, often found in high concentrations in fruits and vegetables, exhibit potent antioxidant activity that can help mitigate oxidative stress caused by free radicals [11].

Phytochemicals such as flavonoids, alkaloids, tannins, terpenes, and steroids have been shown to provide various health benefits. Flavonoids possess anti-inflammatory and antibacterial properties, alkaloids are known for their analgesic effects [15], while tannins exhibit healing, antiseptic, and antibacterial activities [16]. Terpenes and steroids offer analgesic and anti-inflammatory effects, and quinone derivatives have demonstrated notable antibacterial activity [17]. According to the World Health Organization (WHO), traditional medicine remains the primary source of healthcare for a large proportion of the population in developing countries [12]. Knowledge of the medicinal use of plants is typically passed down orally from generation to generation, often within families or communities. The advent of modern medicine has led to the erosion of this valuable traditional knowledge. Scientific validation and documentation of such practices are therefore crucial to preserving this cultural heritage and expanding the pharmacopeia of modern medicine [13,14].

Physiologically, the human body produces free radicals that play essential roles in various metabolic processes, including serving as defense mechanisms against invading pathogens, when produced in excess under conditions of oxidative stress, these free radicals become detrimental, damaging critical cellular components such as proteins, lipids, and DNA. Oxidative stress has been implicated in the pathogenesis of several age-related and chronic diseases, including cancer and neurodegenerative disorders [18].

The rise of antibiotic-resistant bacterial strains has emerged as a major global health challenge, emphasizing the urgent need for the discovery of new antimicrobial agents. Natural products, particularly those derived from plants, offer a promising avenue in the search for novel bioactive compounds.

Ficus sycomorus L., commonly used in various ethnomedicinal systems, has been employed by diverse ethnic communities for the treatment of numerous ailments. Traditional uses include employing its fruits, roots, stem bark, and leaves to manage gastrointestinal, respiratory, and cardiovascular disorders [19]. Notably, *Ficus sycomorus* is also recognized for its antimicrobial efficacy, particularly against fungal infections [20]. Given the well-documented therapeutic potential of phytochemicals such as polyphenols, flavonoids, alkaloids, and tannins, there is a compelling rationale for investigating the medicinal value of such plants. In regions like Senegal, where plant-based medicine remains integral to healthcare, systematic exploration and validation of indigenous medicinal plants are essential [21], herbal medicines continue to be used widely in both conventional and alternative medical practices, serving as complementary treatments in many developed and developing nations [22]. Plants not only provide inspiration for drug discovery but also contribute significantly to pharmaceutical innovation. Understanding the chemical constituents of medicinal plants is vital—not only for identifying therapeutic agents but also for uncovering new sources of pharmacologically active compounds [23].

1.2 Aim and Objectives of the Study

The aim of this study is to investigate the phytochemical constituents and antimicrobial properties of leaf extracts of *Ficus sycomorus* (Sycamore).

2.0 Materials and Methods

2.1 Collection of Plant Material

Fresh and healthy leaves of *Ficus sycomorus* were collected from mature sycamore trees located in Uturu, Isuikwuato Local Government Area, Abia State, Nigeria. The plant was identified and authenticated by a botanist in the Department of Plant Science and Biotechnology, ensuring the correct taxonomic classification.

2.2 Preparation of Plant Sample

The collected *Ficus sycomorus* leaves were thoroughly washed with distilled water to remove any debris or contaminants. The cleaned leaves were then air-dried at room temperature for several days until a constant weight was achieved. Once dried, the leaves were ground into a fine powder using an electric blender sterilized with 70% ethanol to prevent microbial contamination. The powdered sample was stored in airtight containers for subsequent extraction.

2.3 Extraction Method

The crude extracts were obtained using a cold maceration method. A total of 400 grams of the powdered *Ficus sycomorus* leaves were placed into separate 800 mL conical flasks, each containing 500 mL of one of the following solvents: methanol, ethanol, distilled water, *n*-hexane, and petroleum ether. The mixtures were allowed to stand for 72 hours at room temperature with intermittent shaking to enhance extraction. Afterward, the extracts were filtered using Whatman No.1 filter paper. The filtrates were concentrated using a rotary evaporator under reduced pressure to yield the crude extracts. These were then stored in sterile, airtight sample bottles at 4°C until required for phytochemical screening and antimicrobial analysis.

2.4 Phytochemical Analysis

Phytochemical screening was carried out to detect the presence of bioactive compounds in the crude extracts of *Ficus sycomorus* leaves. The screening focused on identifying major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, glycosides, and terpenoids, using standard qualitative methods.

2.5.1 Qualitative Test

The qualitative phytochemical tests were performed according to the standard procedures described by Trease and Evans [24] and Sofowora [25]. One gram of each crude extract (methanol, ethanol, *n*-hexane, petroleum ether, and aqueous) was dissolved in 100 mL of the respective solvent to prepare stock solutions. These solutions were then subjected to a series of qualitative tests to determine the presence or absence of specific phytochemicals, as outlined below:

- **Test for Alkaloids** (Mayer's and Wagner's reagents)
- **Test for Flavonoids** (Shinoda test)
- **Test for Tannins** (Ferric chloride test)
- **Test for Saponins** (Frothing test)
- **Test for Steroids** (Salkowski's test)
- **Test for Glycosides** (Keller-Killiani test)
- **Test for Terpenoids** (Liebermann–Burchard test)

The results were recorded based on the presence (+) or absence (−) of characteristic color changes or precipitate formations indicating each metabolite.

2.5.1.1 Test for Alkaloids

About 3 mL of each crude extract was pipetted into six different test tubes. To each, 1 mL of hydrochloric acid (HCl) was added. The mixtures were heated in a water bath for 20 minutes with intermittent shaking. After cooling, the mixtures were filtered into clean test tubes. Subsequently, 1 mL of Wagner's reagent was added to each filtrate. The appearance of a creamy white precipitate indicated the presence of alkaloids.

2.5.1.2 Test for Tannins

In separate test tubes, 2 mL of each extract was boiled gently for 20 minutes and then allowed to cool. Three drops of ferric chloride solution were added to each cooled extract. A green coloration indicated the presence of tannins.

2.5.1.3 Test for Glycosides

To 1 mL of aqueous extract in a test tube, 10 mL of 50% sulfuric acid (H_2SO_4) was added and the mixture was heated for 15 minutes. After cooling, 10 mL of Fehling's solution A was added, and the mixture was boiled again for 15 minutes. The formation of a brick-red precipitate confirmed the presence of glycosides.

2.5.1.4 Test for Flavonoids

Three milliliters (3 mL) of each extract were pipetted into separate conical flasks. To each, 10 mL of distilled water was added, and the mixture was shaken thoroughly. Then, 1 mL of 10% sodium hydroxide (NaOH) solution was added. The development of a yellow coloration indicated the presence of flavonoids [26].

2.5.1.5 Test for Steroids
Five drops of concentrated sulfuric acid (H_2SO_4) were added to 1 mL of each extract in separate test tubes. The appearance of a reddish coloration was taken as a positive result for the presence of steroids [26].

2.6 Preparation and Sterilization of Materials
All glassware used in this study was initially soaked in a detergent solution for 35 minutes, thoroughly washed, rinsed with clean water, and allowed to air dry. The dried glassware was subsequently sterilized using a hot air oven.

2.7 Preparation of Culture Media
The antimicrobial activity was evaluated using Nutrient Agar (NA), which was prepared according to the manufacturer's specifications. Specifically, 28 g of Nutrient Agar powder was dissolved in 1000 cm^3 of distilled water. The medium was dispensed into 15 cm^3 aliquots and sterilized in an autoclave at 121 °C for 15 minutes. Seeded agar plates were prepared by pouring 15 cm^3 of molten sterilized Nutrient Agar into sterile Petri dishes. After the agar solidified, 0.1 cm^3 of the test microorganism suspension was added to each plate.

2.8 Preparation of Standard Drugs
Standard drugs were prepared using the same solvent as for the plant extract stock solutions, though at different concentrations. Amoxicillin was used as the standard antibiotic for bacterial testing, and fluconazole was used as the antifungal agent.

For **Amoxicillin**: One 500 mg capsule was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to obtain a concentration of 500 mg/mL. A further dilution was carried out to achieve a working concentration of 1000 µg/mL.

For **Fluconazole**: One capsule containing 150 mg of fluconazole was dissolved in 1 mL of DMSO to yield a stock solution of 150 mg/mL. This was further diluted to obtain a final concentration of 1000 µg/mL.

2.9 Preparation of Stock Solution of the Extract
Stock solutions of each plant extract were prepared by accurately weighing 0.2 g of the dried extract and transferring it into sterilized test tubes. To each tube, 2 cm^3 of dimethyl sulfoxide (DMSO) was added. The mixtures were thoroughly dissolved to obtain a homogenous stock solution.

2.10 Sensitivity Test
The antimicrobial sensitivity test was conducted following the method described by Vincent [27]. Agar plates seeded with the respective test microorganisms were prepared as previously described. Using a sterile cork borer, three wells were carefully bored into each agar plate.
Three drops (approximately 0.1 cm^3) of each plant extract stock solution were added to their respective wells. Similarly, three drops of the standard drug stock solution were added to designated wells as the positive control. Distilled water was used as the negative control. The plates were allowed to stand for 30 minutes at room temperature to facilitate diffusion of the extracts and drugs into the agar. Subsequently, the plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured to assess antimicrobial activity.

2.11 Response of the Test Organisms
After the incubation period, the zones of inhibition around each well were measured using a graduated ruler. The measurements were recorded in millimeters (mm) to evaluate the antimicrobial activity of the extracts and standard drugs against the test organisms.

2.12 Determination of Minimum Inhibitory Concentration (MIC)
The MIC of the extracts was determined using the broth dilution method as described by [28]. Nutrient broth was prepared according to the manufacturer's instructions. Five milliliters (5 ml) of nutrient broth was dispensed into a series of sterilized test tubes, each labeled for the following extract concentrations: 500, 250, 125, and 62.5 mg/ml.
One milliliter (1 ml) of the plant extract was added to the test tube labeled 500 mg/ml and mixed thoroughly. From this tube, 2 ml of the solution was serially transferred to the subsequent tubes to achieve the desired concentration gradients. The tubes were allowed to stand for 30 minutes at room temperature to facilitate interaction before incubation. After incubation at 37 °C for 24 hours, the tubes were examined for turbidity. The lowest concentration of the extract that showed no visible turbidity, indicating inhibition of microbial growth, was recorded as the MIC. Control tubes containing only nutrient broth and microorganisms were used to validate the results.

3.0 Result

3.1 TABLE 1: Phyiscal appearance of various crude extracts of Ficus sycamores leaf extracts

Extracts	Appearance
Aqueous	Light green liquid
Ethanol	Dark green liquid
Methanol	Dark green liquid
Hexane	Light green liquid
Petroleum ether	Light green liquid

Qualitative Phytochemical Screening
The results of the qualitative phytochemical screening for the methanol, ethanol, water, n-hexane, and petroleum ether extracts of *Ficus sycamorus* are summarized in Table 2. Alkaloids were detected in all the extracts. Glycosides were absent in all extracts except for the aqueous (distilled water) extract, where they were present. Steroids were found in the ethanol, methanol, and aqueous extracts but were absent in the n-hexane and petroleum ether extracts. Flavonoids were present in all extracts. Tannins were detected in all extracts except the aqueous extract.

3.2. Table 2: Result for phytochemical analysis

Test	Ethanol	Methanol	n-hexane	Petroleum ether	Distilled water
Steroid	++	++	--	--	++
Flavonoid	+	++	++	++	++
Tannin	++	++	++	++	--
Alkaloid	++	++	++	++	++
Glycoside	--	--	--	--	++

Keys: +++: Abundantly present, ++;Moderately present,+;Present,- Absent

The analysis of the phytochemical screening reveals the following results as shown above

3.3 Table 3: Morphological Characteristics of Organisms Isolated

Gram reaction	Organism
Gram negative	E.*coil*
Gram negative	*Pseudomonasaeruginosa*
Gram positive	*Staphylococcusaureus*

3.4.1 Table 4: Zone of inhibation produced using methanol extracts

Organisms	Concentration of extract(mg/ml)					
	500	250	125	62.5	Amoxicillin 500mg/ml	Flucomazole 150mg/ml
Staph-aerus	15.2	9.0	8.0	7.8	19.1	-
E.coil	20.0	181	12.2	10.0	20.0	-
P. aerusginosa	16.1	14.2	12.1	9.0	18.0	-
P.chrysogenum	22.1	18.1	15.0	8.0	-	24.2
A .fumigatus	24.0	20.2	18.1	12.2	-	29.5

Key: 0 = no inhibition, 0-10 = moderate sensitivity, 10-20 = sensitive 20 and above =very sensitive.

3.4.2 Table 5: Zone of inhibation produced using ethanol extracts

Organisms	Concentration of extract(mg/ml)					
	500	250	125	62.5	Amoxicillin 500mg/ml	Flucomazole 150mg/ml
Staph-aerus(mm)	20.2	18.0	15.1	11.1	22.1	-
E.coil(mm)	24.1	15.0	12.0	10.0	20.0	-
P. aerusginosa(mm)	19.1	15.2	13.1	12.0	18.0	-
P.chrysogenum(mm	21.0	20.1	15.0	12.2	-	22.2
A fumigatus (mm)	20.0	19.1	15.1	12.0	-	20.0

Key: 0 = no inhibition, 0-10 = moderate sensitivity, 10-20 = sensitive 20 and above =very sensitive.

3.4.3 Table 6: Zone of inhibation produced using n-haxane extracts

Organisms	Concentration of extract(mg/ml)					
	500	250	125	62.5	Amoxicillin 500mg/ml	Flucomazole 150mg/ml
Staph-aerus	20.2	15.0	12.1	11.0	22.1	-
E.coil	24.1	12.1	10.1	9.0	25.0	-
P. aerusginosa (mm)	20.0	15.2	13.1	11.1	22.0	-
P.chrysogenum(mm)	21.0	20.1	15.0	13.2	-	25.2
A .fumigatus (mm)	24.1	19.1	15.1	11.0	-	22.1

Key: 0 = no inhibition, 0-10 = moderate sensitivity, 10-20 = sensitive 20 and above =very sensitive.

3.4.4 Table 7: Zone of inhibation produced using petroleum ether extracts

Organisms	Concentration of extract(mg/ml)					
	500	250	125	62.5	Amoxicillin 500mg/ml	Flucomazole 150mg/ml
Staph-aerus(mm)	15.2	13.0	11.0	9.1	19.1	-
E.coil(mm)	20.0	18.1	15.2	10.0	22.0	-
P.aerusginosa (mm)	18.1	15.2	13.1	11.0	18.0	-
P.chrysogenum(mm)	24.1	20.5	18.0	15.4	-	25.2
A. fumigates	18.0	10.1	9.0	7.1	-	20.1

Key: 0 = no inhibition, 0-10 = moderate sensitivity, 10-20 = sensitive 20 and above =very sensitive

3.4.5 Table 7: Zone of inhibation produced using distilled water extracts

Organisms	Concentration of extract(mg/ml)					
	500	250	125	62.5	Amoxicillin 500mg/ml	Flucomazole 150mg/ml
Staph-aerus	29.1	25.0	23.0	18.2	27.1	-
E.coil	20.0	18.1	15.2	11.0	22.0	-
P. aerusginosa(mm)	18.1	15.2	13.1	10.0	15.0	-
P.chrysogenum(mmm)	21.0	19.0	17.5	16.1	-	18.1
A. fumigatus(mm)	20.1	19.0	15.0	13.1	-	18.0

Key: 0 = no inhibition, 0-10 = moderate sensitivity, 10-20 = sensitive 20 and above =very sensitive.

3.5. Table 8: Minimum inhibatory concentration of all extracts of ficus sycamorus

Microorganism	Turbidity at various concentration of the extracts (mg/ml)				
	500	250	125	62.5	
Staphylococcus- auerus	-	-	+	+	Methanol
	-	-	-	++	Ethanol
	-	+	+	+++	Aqueous
	-	+	++	++	n-hexane
	-	-	+	++	Petroleum ether
E. coil	-	-	-	+	Methanol
	-	-	+	++	Ethanol
	-	-	+	++	Aqueous
	-	+	++	+++	n-hexane
	-	+	+	++	Petroleum ether
P. aerusginosa	-	-	+	+	Methanol
	-	-	-	+	Ethanol
	-	+	++	+++	Aqueous
	-	++	+	+++	n-hexane
	-	+	+	++	Petroleum ether
P. chrysogenum	+	+	+++	++	Methanol
	+	+	++	+++	Ethanol
	-	++	++	+++	Aqueous
	-	-	++	++	n-hexane
	+	++	++	+++	Petroleum ether
A . fumigatus					Methanol
					Ethanol
					Aqueous
					n-hexane
					Petroleum ether

Key -: no growth, +: slight turbidity, ++: moderate turbidity, +++: very turbid.

4.1 Discussions

The results obtained from the phytochemical analysis of *Ficus sycamorus* (Table 3) revealed the presence of various secondary metabolites in different solvent extracts, namely methanol, water (aqueous), n-hexane, ethanol, and petroleum ether. The phytochemicals detected included alkaloids, flavonoids, steroids, tannins, and glycosides.

Among the solvents, ethanol extract demonstrated the presence of all the listed phytochemicals except tannins, while methanol, aqueous, n-hexane, and petroleum ether extracts showed varying presence of these compounds. Previous studies on *Ficus sycomorus* leaf extracts using methanol and ethanol also reported the presence of flavonoids, glycosides, reducing sugars, resins, tannins, and saponins. Additionally, the stem bark of this plant has been traditionally used to treat diarrhea, dysentery, and wound infections, highlighting its therapeutic potential [29].

In rural communities, leaf extracts of *Ficus sycomorus* are traditionally used to treat snake bites, jaundice, and are also applied as latex for chest diseases, colds, and dysentery. The stem bark is employed in remedies for cough, throat infections, and chest pains. This ethnomedicinal usage supports the need to scientifically screen these plant parts against pathogenic organisms responsible for such ailments. To integrate medicinal plants effectively into modern healthcare, there is a need to train researchers and practitioners in both traditional and modern medical practices to utilize plant chemical compounds efficiently [30]. Phytochemicals such as alkaloids, flavonoids, steroids, tannins, and glycosides are known to contribute to the therapeutic effects of plants.

Our qualitative phytochemical analysis confirmed the presence of alkaloids, tannins, steroids, and flavonoids in all extracts except tannins, which were absent in the aqueous extract, and steroids, which were absent in petroleum ether and n-hexane extracts. Glycosides were found only in the aqueous extract. The antibacterial activity of the crude extracts was evaluated by measuring the inhibition zone diameters against selected members of the Enterobacteriaceae family, with results presented in Tables 4 to 8. All tested organisms were susceptible to *Ficus sycomorus* extracts to varying degrees, reflecting species-dependent susceptibility patterns.

Both Gram-positive and Gram-negative bacteria were included in the study. Gram-negative bacteria are generally more resistant to many antimicrobial agents, including plant extracts, due to the presence of an outer membrane that restricts entry of compounds. In contrast, Gram-positive bacteria lack this outer membrane but have a thick peptidoglycan layer that retains crystal violet dye.

Minimum inhibitory concentrations (MIC) of the extracts (methanol, ethanol, n-hexane, petroleum ether, and aqueous) were determined using broth dilution methods at concentrations ranging from 62.5 to 500 mg/ml.

The lowest MIC (62.5 mg/ml) was observed for aqueous extract against *Staphylococcus aureus*, while ethanol extract showed an MIC of 125 mg/ml against *Pseudomonas aeruginosa*. Methanol and aqueous extracts exhibited MIC values of 500 mg/ml against *Aspergillus fumigatus*, and ethanol extract showed the same MIC value against *Penicillium chrysogenum*.

4.2 Conclusion

The research carried out on *Ficus sycamorus* demonstrates that this plant is a valuable medicinal resource due to the presence of various important phytochemicals identified through phytochemical analysis. These compounds include alkaloids, which are nitrogen-containing bases often used as precursors in drug synthesis; tannins, which aid in the breakdown of sugars in the body; glycosides, which contribute to fat metabolism; flavonoids, known for their antioxidant properties that help

remove toxic substances from the body; and steroids, which play roles in blood cell formation. The antimicrobial studies confirmed that the extracts from *Ficus sycamorus* possess inhibitory effects against several microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, and *Penicillium chrysogenum*. The minimum inhibitory concentration (MIC) results suggest that with proper dosing, the extracts have the potential to effectively inhibit or kill these pathogenic microorganisms. *Ficus sycamorus* shows promising medicinal properties that warrant further investigation and possible development as a source of natural antimicrobial agents.

4.3 Recommendation

Ficus sycamorus was found to contain valuable bioactive compounds exhibiting significant antibacterial and antifungal activities. Therefore, further research is strongly recommended to carry out detailed phytochemical and pharmacological studies. This should focus on isolating the active constituents and evaluating their antimicrobial effects against a broader spectrum of microbial pathogens. Such studies will help in the development of potential therapeutic agents from this plant.

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