Original Research Article

Antimicrobial Activities of the Leaf Extracts of *Ficus sycomorus* Linn. on *Helicobacter pylori* and *Citrobacter freundii*

ABSTRACT

Aims: The aim of this study is to determine the antimicrobial activities of *Ficus sycomorus* leave extract on selected bacteria. Phytochemical screening showed the presence of tannins, saponins, alkaloids and flavonoids, it also revealed the absence of glycoside.
Place and Duration of Study: Microbiology laboratory, Biological Sciences Department, Bingham University Karu, Nasarawa State, Nigeria.
Methodology: Plant extracts were prepared by boiling, soaking and methanol extraction. 96-well plates dilution method for determination of Minimum Inhibition Concentraction (MIC) and Minimum Bactericidal Concentration (MBC) were carried out. The MIC for *Helicobacter pylori* the hot water extract showed antimicrobial activities at 12.5µl/ml, cold water extract at 25µl/ml and methanol extract at 3.125µl/ml which is the least concentration. There was no significant difference (P>0.05) between the various plant extracts on *Helicobacter pylori*. For *Citrobacter freundii* the hot and cold water extract showed antimicrobial activities at 3.125µl/ml each which is the least concentraction, while methanol extract was at 25µl/ml.
Results: There was no significant difference (P>0.05) between the various plant extracts on *Citrobacter freundii*. The MBC for *Helicobacter pylori* and *Citrobacter freundii* showed antimicrobial activities at 400µl each. The plant has exhibited strong antimicrobial activity on *Helicobacter pylori* and *Citrobacter freundii*

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Conclusion: The antimicrobial compounds produced by plant extracts are active against bacteria. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microorganisms.

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Keywords: Medicinal plant, Antimicrobial, *Helicobacter pylori* , *Citrobacter freundii*

1. INTRODUCTION

Medicinal plants have been the basis of treatment of various diseases in African traditional medicine as well as other forms of treatment from diverse cultures of the world. About 80% of the world's population still depends solely on traditional or herbal medicine for treatment of diseases, mostly in Africa and other developing nations. Most of the potent medicinal plants have relatively no toxic or adverse effects when used by humans, while some are very toxic to both humans and animals with the potential of damaging certain organs in the body. This calls for caution in the use of medicinal plants of which the use is presently on the increase due to easy availability, affordability, accessibility, and promising efficacy comparable to the

high cost and adverse effects of standard synthetic drug agents. Several medicinal plants were evaluated for their good toxicological profile using online research. After much screening, only those medicinal plants without serious toxic effects in animals and cell culture experiments were chosen and precisely discussed.[1]

In Nigeria, and other African countries, several roots, fruits, leaves and bark of plants are used for different medicinal purposes; some of which have been discovered in many researches to be rich in secondary metabolites like tannins, alkaloids, flavonoids, saponins, glycosides and volatile oils which are responsible for their therapeutic activities.[2]

Traditional medicine (also known as indigenous or folk medicine) comprises medical aspects of traditional knowledge that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness." Traditional medicine is usually contrasted with scientific medicine .[3]

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus* are mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning.[4] Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia. [5] Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multi factorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors.

This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost of production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs[6]. These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as ‘forgotten plants.’ Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.[7]

Helicobacter pylori (*H. pylori*) infection occurs when *H. pylori* bacteria infect the stomach. This usually happens during childhood. A common cause of peptic ulcers, *H. pylori* infection may be present in more than half the people in the world. Most people don't realize they have *H. pylori* infection, because they never get sick from it. If you develop signs and symptoms of a peptic ulcer, your doctor will probably test you for *H. pylori* infection. If you have *H. pylori* infection, it can be treated with antibiotics .[8,9]

Citrobacter freundii and *Citrobacter koseri* can cause urinary tract infections, and are found in wounds, respiratory organs, meningitis, and sepsis. They can cause healthcare-associated infections, especially in pediatric and immune-compromised patients.[10]

Ficus sycomorus belongs to the family *Moraceae,* it is commonly known as sand paper tree ("Baure" in Hausa) and it is widely spread in West Africa. *Ficus sycomorus* has many traditional medicinal uses in the treatment of snake bites, jaundice, chest pains, dysentery, coughs and throat infection. *Ficus sycomorus* is claimed by Jarawa people of Bauchi State to treat urinary tract infections and ulcer.

2. MATERIAL AND METHODS

Area of Study

The laboratory work was carried out in the Microbiology laboratory, Biological Sciences Department, Bingham University Karu, Nasarawa State. Bingham University is approximately 25km from the Federal Capital Territory (FCT), Abuja. Bingham University is located at longitude 7.6^{o} E and latitude 8.9^{o} N in Karu, Nasarawa State, Nigeria [11] Fresh leaves of *Ficus sycomorus* were obtained at a forest near Babele, Plateau State of Nigeria and were taken to the Botany Department of Federal College of Forestry, Jos, for identification. The fresh leaves of *Ficus sycomorus* were rinsed with distilled water and dried at room temperature for fourteen days. The dried sample was then crushed into powdery form using mortar and pestle and was blended with USHA Mixer Grinder, MG2053N and stored in polythene bags for analysis.

Preparation of Plant Extracts

Three different extracts were prepared - hot water extract according to the local method, cold water extract and methanol extract. Both hot and cold water extraction were made. 200g of the powdered leaves was dissolved into 1000ml of distilled water, and allowed to boil for 15 minutes, it was then left to stand for about 6hours to ensure maximum extraction of nutrients. The mixture was sieved using a sterile sieve. The filtrate was subjected to drying in a hot air oven at 55˚C to obtain the extracts in solid forms and were stored in air tight sterile containers with each fully labeled. Same gram of the powered leaves was weighed into a sterile jar and mixed with 1000ml of cold water, it was left to stand for 24hours at room temperature to allow for maximum extraction. It was filtered using a sterile sieve. The filtrate was subjected to drying in a hot air oven at 55˚C to obtain the extracts in solid forms and were stored in air tight sterile containers with each fully labeled.

Methanol Extraction

A weighed portion of powdered material was extracted by cold maceration in 1000ml of methanol and shaken intermittently for 72 hours then filtered with a sterile sieve and also

through a Whatman Filter paper[12.] The rotary evaporator was used to separate the solvent from the extract. The solid extract was stored in an airtight sterile container until further analysis. The extracts of the plant samples were screened for alkaloids, saponins, tannins, glycosides and flavonoids according to the method of Sofowora [13].

Source of Bacterial Isolates

The pure bacterial isolate of *Helicobacter pylori* and *Citrobacter freundii* were obtained from the bacteriology division of the National Veterinary Research Institute, (NVRI) Vom and Bacteriology Laboratory, Federal College of Veterinary and Medical Laboratory Technology Vom. Purity plates of each of the isolates were obtained by sub-culturing the isolates unto appropriate media and biochemical tests were performed to confirm their identity.

Preparation of Mcfarland Turbidity Standard

Barium sulphate suspension at 1.0% w/v was prepared as follows: One percent (1% w/v) solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 in 99ml of water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of barium chloride in 50ml of distilled water. Barium chloride solution (0.6ml) was added to 99ml of sulphuric acid solution given Mcfarland standard (0.5) an equivalent density of 1×10^8cfu/ml of turbidity.

Preparation of dilution of different extracts

0.8g of the hot, cold and methanolic extracts were dissolved in 8ml of Dimethylsulfoxide (DmSO4) respectively to give a stock solution of 400mg/ml, doubling dilution was then carried out to give concentrations of 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml. The test control was prepared using 500mg amoxicillin. The drug was dissolved in 10ml of distilled water.

Determination of Minimal Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) for plant extract was evaluated according to the method described by the minor modification of employing 96 well microtitre plates.[14] For each 100µl of MHB was placed to each well followed by 100µl of plant extract (which contains 400mg/ml of plant extract) added to the first column of the microtiter plates. This made each well of the first column to have a total volume of 200µl. Starting from the first column serial dilution was conducted up to the 10^{th} column with double folding; the final volume (3.125µl) of the plant extract and the broth were drawn from the 10^{th} column and the last dilution was discarded. In the negative control column, 30µl of 2% DmSO4 was dispensed in the well plates. Bacterial isolate added to normal saline was compared with Mcfarland turbidity standard and dispensed at 50µl into the wells up to 10^{th} column aseptically. 50µl of amoxicillin was added to the positive control. Subsequently, the plates were wrapped with foil paper and incubated for 24hours at 37˚C in the incubator. The minimum inhibitory concentration (MIC) was determined

by adding 30µl (2mg/ml) of 0.02% resazurin dye and incubated at 37˚C for one hour. Resazurin dye was used as an indicator for bacteria growth; bacteria metabolize it and change into pink colour. The wells that had no change in colour after the addition of resazurin dye indicated no growth of the microorganisms and they were taken as MIC values using 96 well microtiter plate method.

Determination of Minimal Bactericidal Concentration (MBC)

The Minimal Bactericidal Concentration (MBC) is defined as the lowest concentration where no bacterial growth is observed. This was determined by aseptically sub-culturing the contents of wells with the lowest inhibition from the MIC results for individual bacterium concentration (MBC) on Mueller Hilton agar. The plates were incubated for 48hours after which it was observed if growth was present. [15]

3. RESULTS AND DISCUSSION

The Analysis of Variance (ANOVA) was used as a statistical method to test the hypothesis

Table 1: Results of Qualitative Phytochemical Analysis of *Ficus sycomorus* Extracts

Phytochemicals	Status	Degree of Occurrence
Alkaloids	Present	++
Tannins	Present	++
Glycosides	Absent	-
Saponins	Present	+++
Flavonoids	Present	+

Key**:**
- = Absent
+ = Present
++ = Highly present
+++ = Excessively present

Phytochemical analysis was conducted on the aqueous and methanol extracts of *Ficus sycomorus* and the following results were obtained and recorded. The phytochemical analysis of *Ficus sycomorus* showed that the extract contains excessively high presence of saponins, high presence of alkaloids and tannins, presence of flavonoids, and absence of glycosides.

Table 2: Results of Minimum Inhibitory Concentrations (MIC) of *Ficus sycomorus* against selected Bacteria

Extract	Concentration of organisms (ul/ml)	
	H. pylori	*C. freundii*
Boiled	12.50	3.125
Soaked	25.00	3.125
Methanol	3.125	25.00

Table 3: Results of Minimum Bactericidal Concentration (MBC) of *Ficus sycomorus* against selected bacteria

Extract	Concentration of organisms (ul/ml)	
	H. pylori	*C. freundii*
Boiled	400	400
Soaked	400	400
Methanol	400	400

DISCUSSION

From Table 1 the qualitative analysis of the phytochemical constituents of the plant extract of *Ficus sycomorus* revealed the presence of saponins, alkaloids, tannins and flavonoids, it also revealed the absence of glycosides. This shows excessively high presence of saponins, alkaloids and tannins were highly present, flavonoids were present. The analysis also revealed the absence of glycosides. The result from the study of antifungal activity and phytochemical analysis of *Ficus sycomorus* leaf extracts on *Malassezia glubosa* showed the excessively high presence of tannins, glycoside and alkaloids while saponins and flavonoid were highly present.[16]These compounds are known to exhibit great antibacterial activities. The presence of these phytochemicals gives the plant its antimicrobial property. These phytochemicals according to Adeshina, [17]are the chemical compounds synthesized by plants to ensure therapeutic effects.

From Table 2 the result observed shows the MIC values of the extracts against *H. pylori* and *C. freundii* which showed antimicrobial activity. The activities recorded were that of *Ficus sycomorus* extracts (boiled, soaked and methanol) against *H. pylori* and *C. freundii*, the MIC values were determined at the lowest concentrations of the plant extracts that showed inhibition against microorganisms. The MIC values of *H. pylori* for soaked extract was 25µl/ml, which showed inhibition at high concentration, followed by the boiled extract 12.5µl and methanol extracts was 3.125µl/ml which is the lowest. For *C. freundii* for methanol extract was 25µl/ml, which showed inhibition at high concentration, soaked and boiled extract were 3.125µl/ml each. This shows that all three plant extracts are effective against the bacteria.

From Table 3 the MBC values of the plant extracts against *H. pylori* and *C. freundii* were 400µl/ml each, for both boiled, soaked and methanol extracts, this shows that higher concentrations of the plant extracts are needed to show antimicrobial activity.

From the ANOVA analysis, we had a P value of P > 0.05. A p-value less than 0.05 indicates that there is a significant difference between MIC and MBC of the different plant extracts used, if it is greater than 0.05, there is no significant difference. However, there was no significant difference observed in the reactions of the bacterial populations examined with respect to method of extraction used for both MIC and MBC. The null hypothesis which states that the plant showed no antimicrobial property against the test organism is rejected and we accept the alternate hypothesis which states that the plant showed antimicrobial property against the test organism.

4. CONCLUSION

Ficus sycomorus has for a very long time been used traditionally as a medicinal treatment of different ailments to replace antibiotics. This research focused on finding the scientific backing for claims of its healing property. The antimicrobial activity has been determined on certain drug resistant pathogenic microorganisms. The plant has shown to exhibit strong antimicrobial activity on *H. pylori* and *C. freundii*. The phytochemicals are also active to prevent bacterial growth without giving cognizance to method of extraction of the extract.

RECOMMENDATIONS

1. Based on the result from this study *Ficus sycomorus* has been proved to contain antimicrobial properties, it is a widely distributed plant which is easily affordable, therefore it should be further exploited as a means of treatment for drug resistant microorganisms.
2. Further research should be done to determine the toxicity and dosage of *Ficus sycomorus* for effective administration which can reduce unwarranted side effects.

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