**PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF**

**FICUS SYCOMORUS LEAVES**

**BY**

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**U14/NAS/ICH/015**

**DEPARTMENT OF INDUSTRIAL CHEMISTRY, FACULTY OF**

**NATURAL AND APPLIED SCIENCE, GODFREY OKOYE UNIVERSITY,**

**UGWUOMU-NIKE, ENUGU STATE.**

**JULY 2018**

**TITLE PAGE**

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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE**

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**NATURAL AND APPLIED SCIENCE, GODFREY OKOYE UNIVERSITY,**

**UGWUOMU-NIKE, ENUGU STATE.**

**SUPERVISOR: MR AYUK E.L.**

**JULY 2018**

**CERTIFICATION**

**I certify that this work carried out by Nwochi Samson Prince in the department of industrial chemistry, with the registration of U14/NAS/ICH/015, Godfery Okoye University, Enugu state, under my supervision.**

**………………………… ……………………….**

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**Supervisor**

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**Mr. Ayuk, Eugene L Date**

**HOD of Department**

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**EXTERNAL SUPERVISOR Date**

**DEDICATION**

**I dedicated this work to God almighty and to my family and friends and also to the entire world.**

**ACKNOWLEDGEMENT**

**My gratitude goes to God almighty, the bringer of life, and the fountain of all existence and the reason for the very air that I breathe.**

**I wish to express my humble gratitude to an epitome of excellence, my able and visionary supervisor, Mr. Ayuk Eugene for his unrelenting guidance, resourceful ability, fatherly love, and rigid criticism during the period of my project research.**

**Who is also Head of Department Mr. Ayuk Eugene, all lecturers of chemical sciences department who has lit up a burning candle in me?**

**My love also goes mostly to my beloved family whose moral and financial input has made this work a success. Also to my beloved brother and Sisters who laid good footsteps for me to follow and gave me a resilient spirit during my moments of drawbacks.**

**To my parents: HRM Igwe Donald N O Nwochi and his Lolo, especially to my mother, I say thank you all for all your financial support, prayer and encouragement. You shall live to enjoy the fruit of your labor.**

**To my friends, Chukwu Nnadozie Orie, Jessica (Jessy berry), Miracle, my trouble mate (Anastasia) and to David, and Patrick, Ebere, ifyanyi and bravery, I say thank you all for your active support, may God bless you all**

**I pray that the almighty whom we all serve reward you all abundantly.**

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**ABSTRACTS**

**Antibiotic resistance has become one of the major problems facing humanity. The need for new antimicrobials has been increased dramatically. Plants are considered as a major sources of new antibiotics due to the presence of phytochemicals. In Nigeria and other developing countries medicine plant materials have been used to treat various diseases since ancient times. The goal of our research is to evaluate the phytochemical and antimicrobial efficiency of Ficus sycamorus leaf extracts. The leave of Ficus sycamorus were screened for phytochemicals, antimicrobial activity and minimum inhibition concentration (MIC). The ethanol, methanol, n-hexane, petroleum ether and aqueous extracts of this plants were subjected to phytochemical and antimicrobial analysis as well as minimum inhibitory concentration (MIC) analysis. The result of the phytochemical analysis revealed the presence of tannins, alkaloids, steroid, glycosides and flavonoids. The crude extracts showed various zones of inhibition against the following microorganisms namely; Staphy aerus, Escherichia coli, Pseudomonas aeruginosa, Penicillin chrysogenum and Aspergillus fumigatuss chrysogenum and Aspergillus fumigatuss. The minimum inhibitory concentrations (MIC) for bacteria. and fungi Staphylococcus aerus for ethanol is 125(-), methanol is 250(-) n-hexane is 500(-), aqueous is 500(-) and petroleum ether is 250(-) and also every other and fungi present and this showed that this plant is a very medicinal plant.**

**CHAPTER ONE**

**INTRODUCTION**

**1.1 BACKGROUND OF THE STUDY**

Plants have been a good source of valuable of medicine. Plants are rich in several secondary metabolites and are a major source of chemical resource, they are a potential source of new drugs for man whose use to control diseases is an old practice. Among the known plant species on earth estimated about 250,000–500,000, only a small fraction have been investigated for the presence of antimicrobial compounds and only 1–10% of these plants are used by humans (Borris, 1996). Plant species are estimated to be around 250–500 thousands (Cowan, 1999). However, only a small part of them have been investigated for antimicrobial activity (Savoia, 2012); (Petrosyan, et al, 2015) ;( Borris, 1996,). People started to use plant materials to treat infectious diseases since ancient times even without any knowledge on their causative agents (Ríos et at, 2005). Nowadays, herbs are continually used in traditional medicine to heal various infectious conditions in many countries, including Nigeria. Moreover, in the last decades this tendency has increased (Rojas, et al 2006).

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, 1996), antimicrobial drugs (Phillipson et al, 1996), According to World Health Organization (WHO), medicinal plants are the best sources to obtain variety of drugs. About 80% of individuals from developing countries use traditional medicines, which have compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Arunkumar et al, 2009).

Plants have been known to be used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating of cancer and harmful diseases (Naczk, et al 2006). Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Suffredini, et al 2004). Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Boots, et al 2008).

Nowadays, herbal medicines are still widely used in conventional as well as alternative medical practices in developed and developing countries as a complementary medicine (Calixto, et al 2003). Plants have been used as a source of inspiration in the development of novel drug. Phytochemicals that occur naturally in plants are responsible for color and organoleptic properties and knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information could be of value in disclosing new resources of such chemical substances. (Simpson et al, 1999).

**1.2AIM OF THE STUDY**

The aim of this research is to carry out the phytochemicals and the antimicrobial analyses of sycamore (*Ficus sycamores*) leaves extracts.

 **OBJECTIVES**

1. To extrac and identify the phytochemicals present in the leaves extracts of sycamore *(Ficus sycamores)* leaves extracts using the following solvents; ethanol, methanol, n-hexane, petroleum ether and distilled water.

2. To determine the antimicrobial activity of the extracts in (1) above against the following microorganisms; *Staphylococcus areues, E. coli, Pseudomonas aeruginosa, Aspergillus fumigatus and Pencilluim chrysogenum,* by;

(i) Noting the inhibition zones (ii) determing the minimum inhibition concentration of the extracts

**1.3 STATE OF THE PROBLEM (RESEARCH QUESTION)**

1. The percentage composition of phytochemicals in medicinal plants differs.

2. Many microbial have become resistant to antibiotics.

3. Over or under dosage of antibiotics is challenging to traditional medicine.

**1.4. JUSTIFICATION OF THE STUDY**

The result of this work will be of great significance to the scientific community because it would provide evidence based information on the importance of this medicinal plant as well as provide a guide to pharmacist on the minimum concentration of the extracts that maybe used in drug formulation. The result would also provide useful information to pharmacist to elucidate the medicinal potentials of the vegetable. The result if positive would create awareness to the general public on the use of the plant as remedy to different health challenges.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1. HISTORY OF THE USE OF MEDICINAL PLANTS**

Medicinal plants besides therapeutic agents are also a big source of information due to a variety of chemical constituents which could be developed as drugs with precise selectivity. They are reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design (Vijyalakshmi et al, 2012). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss et al, 2009). Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well (Pandey et al, 2013).

Medicinal plants are presently in demand and their acceptance is increasing progressively. Undoubtedly, plants play an important role by providing essential services in ecosystems. Without plants, humans and other living organisms cannot survive. In fact, the use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings started cooking for a tool in their environment to recover from a disease, the use of plants was their only choice of treatment (Halberstein et al, 2005).

***2.2. FICUS SYCAMORUS***

*F. sycomorus* is a tree that belongs to the family of *Moraceae* which is native to Ethiopia (Orwa et al 2009). It is available in Amhara national regional State of Ethiopia.

*F. sycomorus* has been identified as feed for cattle, goat and sheep (Teferi et al, 2008). *F. sycomorus* leaves and petioles are well accepted by West African Dwarf lambs and led to higher levels of apparent digestibility than the other tree species (Anugwa, et al 1987). Fruit of the plant are round from 2.8-5 cm in diameter with conspicuous opening that may break at the one end and with various colours.

*F. sycomorus* is also used for decorations purposes and shades to provide shatter on roadsides and other places such as market centers in rural areas. It also serves as soil erosion controller and sand-dune fixation and riverbank stabilization (Orwa, et al, 2009). The Shaded leaves improve the nutrient status, infiltration rate and water-holding capacity of the soil. (Lemlem et al, 2006).

**2.3. DISTRIBUTION OF FICUS SYCOMORUS:**

*F. sycomorus* belongs to the family *Moraceae*. It is native to Middle East, South West Africa, Egypt, Ethiopia, Israel, and Kenya. It grows well in the area, which receives mean annual rainfall ranging from 500-1800 (max. 2200) mm per year with deep, well-drained loamy to clay soil types or sandy soils with a shallow ground water (Orwa et al, 2009). The best sites for the plant includes drainage lines, streams, rivers, springs or dams. The plant grows in altitude, which ranges from 0-2000 m. and a mean annual temperature range from 0-40°C.

The plant grows to 20m tall and 6 m wide with a dense round crown of spreader branches while leaves are heart-shaped, deep green with round apex of about 14 cm long by 10 cm wide (Kubmarawa et al, 2008). The tree can bear several crops of fruit a year and growth rate is fairly fast at 1-1.5 m/year in frost-free areas (Orwa, et al, 2009) At high altitudes in India, fruit yields of F. sycomorus12 kg/tree/year were reported (Purohit,, 1989). The fruits contain up to several hundred to thousand seeds and seeds are delicious.

**2.4. SOCIO-ECONOMIC SIGNIFICANCE OF FICUS SYCOMORUS:**

The economic significance of *F. sycomorus* trees can be determined from the fact that they are hardy and can provide year-round fodder to be used as a supplement in lean periods. With proper management and propagation techniques, this fodder can be a viable feed resource to supplement small ruminants for landless farmers*.* The fruits and forage serves as feed for livestock in Ethiopia highlands (Bayafers, et al, 2002). The leaves are a much-sought fodder with fairly high nutritive value; they are valuable fodder in overstocked semi-arid areas where the trees occur

. Its fruit are available all the year round especially in Africa, fruiting 3-5 times per year and used as keystone or staple food of early living (Kinnaird 1992). In the hills of Nepal, numerous *Ficus spp*. show potential for bridging the gap between the amount of feed needed by existing livestock populations and the availability from present feed resources (Gatenby et al, 1989). *F. sycomorus* plays a significant role in nutrition of ruminants’ livestock in tropical region.

**2.5. PREVIOUS WORKS CARRIEDOUT BY DIFFERENT AUTHORS ON PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS.**

Bello et al (2013) worked on comparative studies of phytochemical screening of Ficus sycomorus linn stem bark extract and Piliostigma thonningii roots extract. The author reported that the aqueous, chloroform, ethyl acetate, methanol and n-hexane extracts of stem bark of Ficus sycomorus and extract of roots of Piliostigma thonningii contain alkaloids, flavonoids, glycosides, reducing sugars, resins, saponnins and tannins.

Okoronkwo et al (2014) worked on nutritional and phytochemical composition of Utu (Icacina Senegalensis) and Sycamore (Ficus Sycomorus) Seeds. In their research they discovered that the moisture content of sycamore (9.65 0.10%) is lower than 12.89 0.26% of Utu seeds respectively. therefore, sycamore will have more storage advantage than the Utu seeds. The crude fat value of both seeds are 28.62% and 31.34% for Utu and sycamore respectively. This means that both seeds can serve as energy supplier in food. The mineral composition of both seeds were analyzed. The results obtained revealed that copper, sodium and zinc have the least value of minerals in the seeds. Phosphorus, magnesium and calcium content of sycamore seeds are 380.24 0.031, 300.67 0.021, 390.77 0.012mg/100g respectively, they are higher than the results of the utu which are 119.14 0.040, 138.15 0.040, 309.71 0.023% mg/100g. Phytochemical screening of the two samples show small amount of antinutrient like saponin, phytate and flavonoid etc. The tannin content of Utu is 5.84 0.012% which is higher than 4.03 0.015% of Sycamore. The alkaloid content is higher in sycamore (5.65 0.021%) than that of utu (3.92 0.025%). These values are below the toxic levels, which means that they will not be harmful when consumed.

Mohamed et al (2015) worked on the antibacterial effect of some medicinal plant extracts and their synergistic effect with antibiotics. The author reported on the aqueous and alcohol extracts using agar disc diffusion method. The minimal inhibitory concentration (MIC) of the plant extracts against selected bacteria were assessed using micro dilution method. In their research they discovered that the ethanol extracts used against E. coli, S. aureus and P. aeruginosa showed better antimicrobial effect

Abubakar et al (2015) worked on phytochemical and antimicrobial screening of methanol root bark extract of Ficus sycomorus linn.(moraceae). The author reported that the methanol extract of Ficus sycomorus Linn. (Moraceae) revealed the presence of tannins, saponins, carbohydrate, alkaloids, flavonoids, steroids, terpenoids and cardiac glycosides. They also discovered that the in vitro antimicrobial screening of the extracts against microorganisms like (Enterococcus faecalis, Escherichia coli, Salmonella typhi, Shigella dysenteries and Candida albicans) was positive

Festus et al (2016) worked on antibacterial Activity and phytochemical profile of leaf extracts of Ficus abutilifolia. The author reported that the disc diffusion method was used to determine the susceptibility of clinical bacterial isolates to fractions of leaf extract of Ficus abutilifolia. The minimum inhibitory concentrations (MIC) were determined by the microdilution assay. The rate of killing of representative isolates as well as phytochemical profile of plant leaf extract were studied using standard methods. In their research they discovered that F. abutilifolia exhibited broad spectrum antibacterial activity against all the tested bacterial isolates with mean zone diameter of inhibition ranging from 9.33±0.58 to 31.67±0.58 mm. The ethyl acetate fraction exhibited the highest antibacterial activity with mean zone diameter of inhibition against the tested bacterial isolates being 27.67±1.15 to 31.67±0.58 mm. The MIC of the fractions ranged from 0.0313 to 0.250 µg/ml which compared favourably with that of the reference drug, streptomycin with mean MIC of 0.125 to 0.250 µg/ml. The ethyl acetate fraction was the most potent fraction with mean MIC of 0.0313 to 0.0625 µg/ml. Phytochemical assay of leaf extract revealed the presence of tannins, anthraquinones, saponnins, flavonoids, alkaloids, reducing sugar, cardiac glycosides, carbohydrates and phlobatannins.

Mikayel Ginovyan,et al (2017) worked on the antimicrobial activity of some plants materials used in Armenian traditional medicine. The author reported that the crude extracts were obtained by maceration technique using five solvents namely: distilled water, methanol, chloroform, acetone, and hexane. Agar well diffusion assay was used for initial evaluation of antimicrobial properties of plant materials against five bacterial and two yeast strains. Minimum inhibitory concentrations of the most active plant parts were determined by broth micro dilution method. In their research they discovered that the crude extracts of all five tested plants expressed antimicrobial activity against at least four test strains at 500 μg ml−1 concentration. Minimum inhibitory and bactericidal/fungicidal concentrations of selected plant parts were determined. Crude acetone and hexane extracts of *Hypericum alpestre* and acetone extract of *Sanguisorba officinalis* inhibited the growth of *P. aeruginosa* even at 64 μg ml concentration. Chloroform and acetone extracts of *Sanguisorba officinalis* exhibited cidal activity against *P. aeruginosa* at 256 μg ml. Acetone was the most effective solvent for solubilizing antimicrobial compounds for almost all tested plant materials.

M. A. Song (2017) work on in vitro antimycobacterial screening of *Ficus sycomorus* extracts on susceptible strain of mycobacterium tuberculosis. The author reported that the anti-mycobacterial activity of *Ficus sycomorus* (stem bark, root bark, leaves and fruits) was studied in vitro using standard assay techniques against susceptible strain of mycobacterium tuberculosis. Phytochemical analysis of the n-hexane fruit extract was done using standard test methods. Partial fractionation of the n-hexane fruit extract was done using thin layer and column chromatography. In their research they discovered: n-hexane fruit extract showed activity against the tested mycobacterium tuberculosis strain. This activity was observed between 100-400 µg/mL against the susceptible strain to standard TB drugs. The crude n-hexane, leaves, root bark and stem bark extracts lacked activity against the susceptible M-Tb strain. The methanol and aqueous extracts of fruit, leaves, stem bark and root bark also lacked activity against the tested susceptible M-Tb strain.

Fateh et al (2017) worked on phytochemical and antimicrobial screening of stem bark and leaves extracts from ficus sycomorus. The author reported that the ethanol extracts prepared from stem bark and leaves of *ficus sycomorus* as well as the bioactive compounds screened from these crude extracts, were tested for their antimicrobial activity against some gram –ve bacteria *(Pseudomonas aeruginosa, Escherichia coli)* , and gram +ve bacterial *(Bacillus subtitis, Staphyloccus aureus)* and two fungal species *(Aspergillus nigor, Candida albicans)* using agar diffusion method. In their research they discovered that the crude extract was active against all pathogens tested and having broad spectrum of activity against bacteria and fungi.

**CHAPTER THREE**

**MATERIAL AND METHODS**

**3.1. COLLECTION OF PLANT MATERIAL**

Fresh leaves of ficus sycamorus were collected from the sycamore tree from Akama-Oye in Eziagu Local Government area of Enugu state.

**3.2. APPARATUS AND MATERIAL USED**

Spatula

Conical flask

Beaker

Muffler furnace

Platinum crucible

Filter paper

Measuring cylinder

Micro pipette

Test tube

Thermostat oven

Hot plate

Water bath

Desiccator

Ph. meter

Electrical blender

Glass rod

Auto clave

Marker pen

Petri dish

Freezer

Tripod stand

Measuring ruler

Electronic weighing balance.

**3.2.1 REAGENTS**

All the reagent used for my analysis was obtain Godfrey okoye University Chemistry and Microbiology laboratories, They include;

Ethanol

Methanol

Sulphuric acid [H2SO4]

5% Ammonia

Distilled water [H2O]

Petroleum ether

Chloroform

Nutrient broth

Wagers solution

Sodium Hydroxide [NAOH]

Hydrochloric acid [HCL]

Ammonia [NH3]

**3.2.2 MICROORGANISMS USED**

Bacteria used includes:

*Escherichia coli*

*Pseudomonas aeruginosa*

*Staphylococcus aureus*

Fungi used includes:

*Aspergillus fumigatus*

*Penicillium chrysogenum*.

**3.3.1 IDENTIFICATION OF PLANT MATERIAL**

The plant was identified and authenticated in Chemical Science Department

**PREPARATION OF PLANT SAMPLE**

Sycamore leaves were washed with distilled water and air dried at room temperature. The dried sycamore leaves were blended using an electric blender which had been sterilized with ethanol (70%).

**3.4. EXTRACTION METHOD**

The crude extracts were obtained using cooled extraction method. 400g of dry blended leaves of *Ficus sycamorus* were packed into a conical flasks of 800 ml and extracted with 500 ml of the following solvent methanol, ethanol, H2O, n-hexane and petroleum ether respectively. The crude extracts were obtained and concentrated using rotory evaporator to obtain the crude extracts. These were put into sterile sample bottles and kept for phytochemical, and antimicrobial analyses

**3.5. ANALYSIS**

The crude extract obtained from the plants were subjected to the following analyses.

Phytochemical analysis

Antimicrobial analysis

**3.6. PHYTOCHEMICAL ANALYSIS;**

**3.6.1 Qualitative test**

Qualitative phytochemical analysis was used to ascertain the secondary metabolites contained in each of the solvent extracts. The standard screening procedures were followed as descried by Tease and Evans (2012) and sofowora (1993) and the phytochemical analysis of all the alkaloids, flavonoid glycosides, steroid and tansies. But not all the extracts showed all these phytochemicals.

One gram of each the crude extracts each was dissolved in 100ml of each of the following solvent; methanol, ethanol, n-hexane, petroleum ether and distilled water to obtain a stock solution which was subjected to qualitative screening as discussed below.

**3.6.1.1 Test for Alkaloids:**

A solution of 3ml of each of the crude extracts respectively were pipetted into six different test tubes and 1ml of HCl was added. The mixtures were heated for 20 minutes in a water bath and shaken while heating. After heating, the extracts were allowed to cool and filtered with filter paper into six new test tubes. Thereafter 1ml of Wagner’s reagent was added to the filtrates. A creamy white coloration indicated the presence of alkaloids.

**3.6.1.2. Test for Tannins**

In separate test tubes, 2ml of the extracts were boiled gently for 20 minutes and allowed to cool. Thereafter, 3 drops of ferric chloride solution were added to each test tube. A green color produced indicated the presence of tannins

**3.6.1.3. Test for Glycosides**

In the test tubes 1ml of aqueous extracts and 10ml of 50% H2SO4 was added, also heated for 15 minutes and allowed to cool. Then, 10ml of Fehling solution A was added and boiled again for the same minutes.A brick red precipitate formed showed the presence of glycosides

 **3.6.1.4 Test for flavonoids:**

Pipette 3ml of extracts with different solvents into different conical flasks, 10ml of distilled water was added into each and shake very well. Also 1ml of 10% NaOH solution was added to the mixture (Okaka, 2006). A yellow coloration was produced which showed the presence flavonoids.

 **3.6.1.5 Test for Steroids:**

5 drops of concentrated H2SO4 was added to 1ml of the extracts in separate test tubes.

Observation: Reddish color indicates that steroids is present (Okaka, 2006).

**3.7 MICRO ASSAY**

**3.7.1. Microorganism’s collection**

The chemical isolates were collected from the stock organisms from the department of Biological science laboratory Godfery Okoye University. The microorganism used are; Bacteria Escherichia *coli, Staphylococcus aureus and Pseudomonas aeruginosa*. Fungi: *Aspergillus fumigatus and penicillium chrysogenum.*

**3.7.2 Preparation and sterilization of materials**

All glass wear used were first soaked in detergent solution for 35 minute, washed and rinsed with clean water and allowed to dry. All these were sterilized using hot air oven.

**3.7.2.1. Preparation of the culture media**

The antimicrobial activity was carried out using nutrient Agar which was prepared according to the manufacturer’s recommendation. 28g of nutrient agar was dissolved in 1000cm3 of distilled water. The nutrient agar prepared was distributed in 15cm3 portioned each and was sterilized in an autoclave at 121oc for 15minuties. The seeded agar plates were prepared by pouring 15cm3 of the molten nutrient agar into sterile petri-dish which 0.1cm3 of the test microorganism was added.

**3.7.2.2 Preparation of the standard drugs used**

In the preparation of the standard drugs, the materials used were the same as those of the stock solution of the extracts. But the concentration of the standard drugs was not the same as the crude extract. The standard drug for the bacteria was Amoxicillin and that of the fungi was fluconazole. One capsule of Amoxicillin was dissolved in 1ml of DMSO to give 500mg/m. 1mg/ml of the Amoxicillin was dissolved in 1ml of DMSO to give 1000ug/ml concentration. For the fluconazole, one capsule contains 150mg and was dissolve in 1ml DMSO to give 150mg/ml and 1mg/ml of the fluconazole was dissolved in 1ml of DMSO to give 1000ug/ml concentration.

**3.7.2.3. Preparation of stock solution of the extract**

The preparation of stock solution was carried out by using 0.2g of each extract which was carefully weighed and transferred into the sterilized test-tube. 2cm3 of DMSO was added to each of the test-tube containing the extract and was dissolved completely to get the stock.

**3.7.3 Sensitivity Test**

The method used in this work decried by Vincent (2005). The sealed agar plates of various test organisms were prepared as discussed above. Well were made at the respective plates using the Cork-borer. Each plates contains three wells. Three drop of each of the extract were transferred into their respective well, and three drops of stock solution of the drug were transferred to it well respectively and distilled water served as the negative control and the drugs as the positive control. The extracts and drug were allowed to diffuse for 30miunties; this was then incubated at 370C for 24 hours.

**3.7.3.1 Response of the test organisms.**

The zone of inhibition were then taken after the incubation period using a graduated ruler and then recorded.

**3.7.3.2. DETERMINATION OF MINIMUM INHIBATION CONCENTRATION (MIC)**

Minimum inhibition concentration was determined using dilution method.(Williams and Wilkins, 2007) the nutrient broth was prepared according to the manufacturer instruction and 5ml of the nutrient broth was dispensed into separate test-tube well labelled according the concentration 500, 250, 125, and 62.5mg/ml then 1ml of the extract was transferred into the test-tube of different concentration containing the nutrient broth from these concentrations, 2ml of each concentration was transferred to its corresponding test-tube serially. It was allowed to stand for 30 minutes before incubation.

After incubation, the lowest concentration which showed no turbidity in the test-tube was recorded as the MIC. The extracts the microorganisms served as control

**CHAPTER FOUR**

**4.0 RESULT AND ANALYSIS**

**4.1 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:**

Result of the qualitative phytochemical screening of the methanol, ethanol, water, n-hexane and petroleum ether of *Ficus sycamorus* is given in the Table 2. Alkaloid is present only is present in all the extracts, glycosides was observed to be absent in all extracts expect distilled water, steroid was observed to be present in three extracts (ethanol, methanol and aqueous) and absent in two (n-hexane and petroleum ether), flavonoid was observed to be present in all extracts, tannin was observed to be present in all extracts expect distilled water.

**4.2. TABLE 2: RESULT FOR PHYTOCHEMICAL ANALYSIS**

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

The analysis of the phytochemical screening reveals the following results as shown below.

Keys **: +++:** Abundantly present, **++;**Moderately present,**+**;Present,**- A**bsent

 **4.3 TABLE 3: MORPHOLOGICAL CHARATERISTICS OF ORGANISMS ISOLATED**

|  |  |
| --- | --- |
| Gram reaction | Organism |
| Gram negative  | E.coil  |
| Gram negative  | Pseudomonas aeruginosa |
| Gram positive | Staphylococcus aureus |

 **4.4 ANTIMICROBIAL ANALYSIS RESULTS OF *FICUS SYCAMORUS***

**4.4.1 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.

**4.4.2 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.

**4.4.3 TABLE 6: ZONE OF INHIBATION PRODUCED USING N-HAXANE EXTRACTS**

|  |  |
| --- | --- |
| **Organisms** | **Concentration of extract(mg/ml)** |
|  | 500 | 250 | 125 | 62.5 | Amoxicillin500mg/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.

**4.4.4 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. fumigatus* | 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 sensitiv

**4.4.5 TABLE 7: ZONE OF INHIBATION PRODUCED USING DISTILLED WATER EXTRACTS**

|  |  |
| --- | --- |
| **Organisms** | **Concentration of extract(mg/ml)** |
|  | 500 | 250 | 125 | 62.5 | Amoxicillin500mg/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.

**4.5. TABLE 8: MINIMUM INHIBATORY CONCENTRATION OF ALL EXTRACTS OF *FICUS SYCAMORUS***

|  |  |
| --- | --- |
| **Micro Organism**  | **Turbidity at various concentration of the extracts (mg/ml)** |
|  | 500 | 250 | 125 | 62.5 |  |
|  |  |  |  |  |  |
| *Staph- aerus* | ----- | --++- | +-++++ | ++++++++++ | MethanolEthanolAqueousn-hexanePetroleum ether |
| *E. coil* | ----- | ---++ | -+++++ | ++++++++++ | MethanolEthanolAqueousn-hexanePetroleum ether |
| *P. aerusginosa* | ----- | --++++ | +-++++ | ++++++++++ | MethanolEthanolAqueousn-hexanePetroleum ether |
| *P. chrysogenum* | ++--+ | ++++-++ | +++++++++++ | +++++++++++++ | MethanolEthanolAqueousn-hexanePetroleum ether |
| *A . fumigatus* |  |  |  |  | MethanolEthanolAqueousn-hexanePetroleum ether |

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

**4.6. DICUSSIONS**

The result obtain from the phytochemical analysis of Ficus sycamorus, in table 3, shows different solvent used for the extractions of the sample and they are methanol, H2O, n-hexane, ethanol and petroleum ether. Different phytochemical determined the presence of alkaloids, flavonoids, steroids, tannins and glycosides. The ethanol solvent used was able to show all phytonutrients listed above expect tannins. While methanol, aqueous, n-hexane and petroleum ether showed some. It has been reported that the phytochemical screening of methanol and ethanol extracts for leaves of Ficus sycomorous revealed the presence of flavonoids, glycosides, reducing sugar, resins, tannins and saponins. The stem bark of this plant had been reported to be used against diarrhea, dysentery and wound infections. It was therefore imperative to screen the said part of the plant against some pathogenic organisms responsible for such diseases. In the rural areas, the leaves extracts of the plant are used in the treatment of snakes bite, jaundice and also they are used as latex to effect for chest diseases, cold and dysentery. The stem barks of plant are used for the remedies treatment of cough, throat injection and chest pains. (Ahmad et al, 2016)

This chemical are used for herbal medicine and it plays a golden role not only as traditional medicine but also as trade commodities, meeting the demand of distant markets for the development of new drugs. To realize the effective integration of plants into a medical system, researchers and practitioners should be trained in both modern and traditional medicine in the using of plants chemical compounds. (Fatemeh, et al 2018) Phytochemical in plants includes alkaloids, flavonoids, steroids, tannins, glycosides, etc. the qualitative phytochemical analysis of methanol, H2O, n-hexane, ethanol and petroleum ether extracts of Ficus sycamorus revealed the presence of alkaloid, tannins, steroid and flavonoid in all extracts, but tannins was absent only in distilled water extract and steroid was also absent in petroleum ether and n-hexane but glycoside was absent in all expect aqueous.

Antibacterial activity of the crude extracts were evaluated by measuring the diameter of the growth inhibition on some member on some members of enterobacreiaceac and the result are presented as showed in (table 4-8) respectively. All the test organisms were susceptible to Ficus sycamorus extract though to varying degrees. This is because, the susceptibility of the bacteria to plant extract on the basis of inhibition zone diameters varied according to it species. The bacteria used in this study were gram- positive and gram – negative.

Gram-negative bacterial are known to be resistant to the action of most antimicrobial agents including plant based extracts. The gram – positive bacterial do not have outer cell membrane found in the gram – negative bacteria. The cell wall of gram-positive bacteria is high in peptidoglycan which is responsible for retaining the crystal violet dye. The methanol, ethanol, n-hexane, petroleum ether and distilled water of Ficus sycamorus, minimum inhibitory concentration (MIC) was also determined .for bacteria, in various concentration of methanol, ethanol, n-hexane petroleum ether(62.5-500mg/ml)was used to inhibit the test organisms using broth dilution methods

The lowest MIC (62.5) was found in aqueous staphylococcus aerus and the same concentration (125mg/ml) was recorded against Pseudomonas aerginosa exhibit by ethanol extracts.

5.2 FOR FUNGI

The lowest concentration (500mg/ml) was exhibited by methanol and aqueous extracts against A.fumigatus respectively. 500mg/ml was recorded against P . chrysogenum exhibited by ethanol extracts

**CHAPTER FIVE**

**5.1 CONCLUSION**

From the research carried out it can be observed that this plant (Ficus sycamorus) is a very good medicinal plant since different phytochemicals were present from the phytochemical analysis. These phytochemical e.g. alkaloids are nitrogen basic compounds are used as starting material for drug production, tannins help in sugar breakdown in the body, glycosides help in burning of fats while flavonoids are antioxidants which removes toxic substances from the body, steroids are blood cell builders and also in the antimicrobial studies it can be observed the this extracts worked against this microorganisms, it was able to inhibit (*Staphylococcus areues, E.coli, Pseudomonas aeruginosa, Aspergillus fumigatus and Penicillium chrysogenumand*) and from the minimum inhibitory concentration it was observed that if proper dosage of this plant is used their kill microorganisms.

**5.2 RECOMMENDATION**

Ficus sycamorus was found to contain some good bioactive compounds with pronounced antibacterial activities, antifungal activities

Further research is highly recommended for the phytochemical and pharmacological studies to isolate the active constituent and evaluate the antimicrobial activities against a wide range of microbial pathogen.

**APPENDIX**

**MATERIALS AND THEIR USES IN THE LAB**

Petri-dish used for culturing

Test tube used for culturing of liquid

Beaker used for mixing of samples

Conical flask used for the mixing of sample

Swap stick used for striking of organisms

Weighing balance used for measuring

Blender used for grinding the dry leaves

Core borer for creating well on agar

DMSO used to dissolve the extracts

Ethanol used for extraction

N-hexane used for extraction

Methanol used for extraction

Aqueous used for extraction

Petroleum ether used for extraction

Dropper pipette used to dispense the leave extract

Wangers reagents used for test for alkaloids



**Different extracts color for phytochemicals**

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