PRELIMINARY PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIMICROBIAL STUDY OF THE ROOT AND STEM BARK EXTRACTS OF *FICUS SYCOMORUS LINN*.

Abstract

This study was conducted to carryout preliminary phytochemical analysis and *in vitro* antimicrobial activities of aqueous and ethanolic root and stem bark extracts of *Ficus sycomorus.* Qualitative phytochemical analysis for tannins, saponin, terpenoids, flavonoids, alkaloids, glycosides, steroids, phenols, and reducing sugar was done using standard methods. The antimicrobial activities of the extracts were tested against four micro- organisms; *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysentrae*, and *Salmonella typhi*. Agar well diffusion method was used for the antimicrobial studies. Phytochemical screening of both root and stem bark aqueous extracts showed the presence of tannin, saponin, terpeniod, flavonoid , alkaloids, glycoside, steroid, reducing sugar, and phenol. Glycoside was not detected in both the aqueous and ethanolic extracts of the root bark. The result of the antimicrobial studies showed that the aqueous root extract have higher antimicrobial activity ranging from (2-12 mm) on the tested microorganisms than aqueous stem bark extract (3-9 mm), while for ethanol extract both stem and root bark extract has almost the same effect or antimicrobial activity on the tested pathogens ranging from (2-15 mm) which is having higher activity compared to the aqueous extracts. The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of both the extracts were found to be 50 mg/mL and 100 mg/mL respectively. From this study, it can therefore be concluded that, the root and stem bark extract is a potential antimicrobial agents which support the claim of the traditional users of this plant in herbal medicine for the treatment of diseases that are of microbial origin.

Key words: *Ficus sycomorus, Escherichia coli, Staphylococcus aureus, Shigella dysentrae,* and *Salmonella typhi, phytochemical screening, antimicrobial activity.*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources [1]. At least 12000 of such

compounds have been isolated so far, a number estimated to be less than 10% of the total[2,3]. Chemical compounds in plants mediates their effects on human body through processes identical to those we already understood for their chemical compound in conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as pharmaceutical drugs to cause side effects [2, 3].

Plant lives longer than every other living things due to their ability to synthesize phytochemicals in their cells which serve as strong antifungal, antibacterial and antimicrobial agents, as a result of this, their susceptibility to diseases attack to some extent is low as compared to other living things (personal contact,15th,January 2016).

In Nigeria like many African countries, several plants are still being used for the treatment of various ailments. Nigeria is naturally blessed with both savannah and tropical rainforests vegetation and these offer a wide distribution of plants believed to possesses secondary metabolites which are responsible for treating or curing various diseases [4]. Quite a number of plants are used as medicines virtually in all cultures of the world. A good number of these medicinal plants are in common use in African traditional medicine. Most of the plants grow near houses and are easily overlooked, especially by urban dwellers [5].

This research work was carried out to study the phytochemical screening and *in vitro* anti-microbial activities of root and stem bark extract of *Ficus sycomorus* on some selected micro organisms. *Ficus sycomorus* is a common savannah tree that grows or can be found almost everywhere. It is called in English Language as “Wild fig” “sycamore fig”, or common cluster fig. Spanish call it “sicomoro”. The Sukur people call it “Dashakwai”, Tiv people called it “Tur’’, in Hausa it is known as “Baure”, Kilba and Marghi people called it “Kamda” , in Fali Language is called “Boduven” and Gude call it “Bodeva” (personal contact, 15th, January 2016). It grows in high water table areas, it can be found along water courses such as streams, rocky places, swamps and water holes [6]. The sycamore fig is sensitive to frost but can withstand some cold. The relevance of this plant in traditional medicine is as a result of the secondary metabolites such as glycosides, reducing sugar, phenols, saponins, steroids, tannins, alkaloids, terpenoids and flavonoids which they have been screened to contain. Also referred to as phytochemicals, they are reported to possess inhibitory activities against the growth and disease inducing activities of some pathogenic microorganisms [7,8,9,10,11].

The root and stem-bark of *Ficus sycomorus* are said to be used as herb in Northern Nigeria for treatment of diseases like diarrhea, dysentery, cough, sore throat, chest diseases, and infertility and as antidote for snake. Therefore, this study was conducted to carry out the phytochemical screening and to evaluate antimicrobial activity of root and stem-bark of *Ficus sycomorus* in order to validate the claims of the traditional users of this plant.

MATERIALS AND METHODS

Sample Collection and identification of plant material.

Plant roots and stem-barks of the plant *Ficus Sycomorus* were collected from Sukur Kingdom in Madagali Local Government Area, Adamawa State, Nigeria. It was identified and authenticated by a Botanist from the Department of Biological Sciences, Adamawa State University, Mubi. A voucher number AD170023 was assigned. Sampling was carried out in the month of May from the tree.

Sample preparation

The root and Stem-barks (cut into small pieces) washed with water and rinsed with distilled water and then dried in the shade for two weeks. The dried samples was grinded by wooden mortar and pestle and sieve using clean Kitchen sieve to obtain a fine powder and was stored in a tight container until required for use.

Extraction

Aqueous Extract

For the water extraction was done by cold maceration method according to the procedure described by Fatope *et al* [12] and Nguta *et al* [13] with little modification. Two hundred grams (200 g) of each of the stem and root barks powder was weighed and soaked in 1000 mL of distilled water in a beaker for 48 h to obtain aqueous extracts. The aqueous extracts were filtered using sterile filter paper (Whatman No.1) into a clean conical flask. The filtrate was concentrated with a rotary evaporator. The extracts were then stored in a refrigerator.

Percentage yield was calculated as: weight of extract/ weight of dried powdered sample × 100

Preparation of ethanol extracts

Maceration method of extraction as described by Fatope *et al* [12] and Nguta *et al* [13] was adopted in this study. Two hundred grams (200g) each of the root and stem bark powdered material was weighed and soaked in 1000 mL of 70% ethanol and left for 24 h .Thereafter, it was

decanted. The procedure was repeated with another 1000 mL to ensure complete extraction of the active ingredient .The extract was filtered and evaporated to dryness wit rotary evaporator. The dried extract was then weighed and stored in tightly closed bottle in a refrigerator until required.

Percentage yield was calculated as: weight of extract/ weight of dried powdered sample × 100

Qualitative Phytochemical analysis.

The qualitative phytochemical screening of the samples was carried out as described by Trease and Evans [14], Nweze *et al* [15], Senthilkumar and Reetha [16] with slight modification. The root or stem bark extracts was screened for carbohydrates, alkaloids, flavonoids, steroids, phenols and tannins, saponin, glycosides, and proteins.

Preparation of stock solution

Two grams (2g) each of root or stem bark extracts were dissolved in 10 mL of water or ethanol to make a concentration of 200 mg/mL

Test for Tannins

One milliliter (1 mL) of the extracts was taken in a test tube and 2 mL of 5 % ferric chloride was added. Formation of blue –black, green or blue – green precipitate was taken as evidence for the presence of tannins.

Test for Saponins

One milliliter (1 mL) of the extracts was shaken with 5 mL of distilled water in a test tube for 5 min. Frothing which persists on warming was taken as evidence for the presence of Saponins.

Test for Terpenoids

Five milliliters (5mL) of aqueous extract of each plant sample was mixed with 2mL of $CHCl_3$ in a test tube and then 3mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was considered as indication for the presence of terpenoids.

Test for Flavonoids

A little amount of magnesium powder and a few drops of concentrated hydrochloric acid were added to 3 mL of the extracts. A red or intense coloration indicated the presence of flavonoids.

Test for Alkaloids

To 2 mL of plant extracts, 2 mL of concentrated hydrochloric acid was added. The mixture was filtered and then 3 drops of Mayer's reagent was added. Presence of green colour or white precipitate indicated the presence of alkaloids.

Test for glycosides

Two milliliter (2 mL) of the extracts was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Presence of red precipitate indicates the presence of glycosides.

Test for Steroids (Salkowski's test)

To 1 mL of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid was carefully added to form a lower layer. Formation of brown ring indicates the presence of steroids.

Test for phenols

Five drops of 10% ferric chloride was added to 1 mL of the extracts in a test tube. Formation of green or dirty green precipitate indicated the presence of phenols.

Test for reducing Sugar

To 2 mL of plant extract, 1 mL of Molisch reagent and 4 drops of concentrated sulphuric acid was added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Antimicrobial Analysis

Escherichia coli, Shigella dysentrae, Salmonella typhi and Staphylococcus aureus were used in this study. The microorganisms were obtained at the Microbiology Laboratory of Modibbo Adama University of Technology, (MAUTECH) Yola, Nigeria.

Standardization of Isolates:

Test organisms were sub-cultured onto fresh plates of MacConkey agar and incubated aerobically at 37°C for 24 h. Colonies from these plates were suspended in Mueller- Hinton broth to a turbidity matching 0.5 McFarland standard (108cfu/ml). Mueller-Hinton agar was then used for antimicrobial assay. All the broth cultures were incubated at 37°C.

Preparation of the Extract for Antimicrobial Study

Two grams (2g) each of aqueous and ethanol root or stem bark extracts were separately dissolved in 10 mL of dimethylsulfoxide (DMSO) to obtain a concentration of 200mg/mL. This was the initial concentration of each of the extracts used.

Antimicrobial Test:

The method described by the National committee for Clinical Laboratory Standard [17] was used.

Suspensions of the bacteria obtained contained approximately 1 x 108cfu/mL. Each labeled plate was uniformly seeded with a test organism by means of sterile swab stick rolled in the culture medium. Five wells, 4mm each in diameter were created using cork borer. Aliquots were dropped in each well to fullness at various concentrations of 100, 50, 25 and 12.5 mg/mL for both the root and stem bark extracts on different plates. Each plate was kept in the refrigerator for 1 hour to allow the extracts to diffuse into the culture medium while the immediate growth of the organism was stopped from taking place. These plates were then incubated at 37°C for 24 h. The zones of inhibition around the wells were measured in millimeter (mm). Control antibiotic (tetracycline capsule 100 µg/mL) was placed in a well on each plate along with the test extracts as control.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was evaluated by the method described by Ibekwe *et al* [18].

The extract concentration were serially diluted with distilled water to various concentrations of 100, 50, 25and 12.5mg/mL. The extract and the nutrient agar broth were mixed in the sterile test tube; the cultured medium was added to each test tube and incubated for 24hrs at 37^{o}C .The lowest zones of inhibition for all the tested organisms showing no visible growth of bacteria was taken as the MIC.

Minimum Bactericidal Concentration (MBC).

The minimum bactericidal concentration (MBC) was determined after the minimum inhibitory concentration (MIC) was obtained. This was carried out by selecting the test tube that shows no growth during the MIC determination. A loopful from the test tube containing the media and the extract were inoculated into a sterile nutrient broth media. This was further incubated for another 24-48 hrs at 37^{o}C for bacteria, after which was examined for bacteria for any microbial growth. The lowest concentration at which no growth was observed on the plate was taken as the MBC [18].

RESULTS AND DISCUSSION

This study was undertaken to investigate the antimicrobial activity and phytochemical screening the aqueous and ethanolic root and stem bark extracts of *Ficus sycomorus* Linn. Due to the side effects of the current drugs and the resistance that pathogenic microorganisms build against antibiotics, much attention has led to the study of biologically active compounds isolated from plant species used in herbal medicine [19]. Different scientific studies provided evidence that medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains [20].

The yield of the plant extracts is presented in Table 2. It was observed that Ethanol stem bark extract (ESBE) gave the highest yield 16.00g (8.0%) followed by Ethanol root bark extract (ERBE) 14.14 g (7.07 %) then Aqueous root bark extract (ARBE) 12.23 g (6.12%) and the lowest is Aqueous stem bark extract (ASBE) 11.16 g (5.58 %). In general the solvent, ethanol gave higher yield irrespective of the plant part than the aqueous solvent.

The result of this study shows the presence of phytochemicals considered as active medicinal chemical constituents as shown in table 2. Phytochemicals such as tannins, saponin, terpenoids, flavonoids, alkaloids, glycosides, steroids, phenols and reducing sugars were all found to be present in both the ethanol extracts of roots and stem bark of *Ficus sycomorus.* However, glycosides was the only constituent not detected in Aqueous extracts of the root and stem bark. The result is contrary to the findings of [21] who reported the presence glycoside in the methanolic stem bark extract of *Ficus sycomorus* obtained from Zaria city of Kaduna State, Nigeria. This difference could be attributed to geographical location of the samples.

The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. These secondary metabolites exert antimicrobial activity through different mechanisms. Plant phenolic compounds especially flavonoids are currently of growing interest owning to their supposed properties in promoting health (anti-oxidants) [22]. Flavonoids have been demonstrated to have antiinflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages[23].Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they

form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent antioxidants [23, 24]. They act as binders and for treatment of diarrhea and dysentery [25] Tannins also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and is also used as diuretic [23]

The results of the zones of inhibition of the different extracts (ARBE, ASBE, ESBE and ERBE) against the tested pathogens are exhibited in Tables 3 – 6. It showed that the extracts have dose dependent antimicrobial activities against the pathogens at various concentrations used in this study. It was noticed that the extract was more effective at concentration of 100 mg/mL, but the effectiveness increases as the concentration increases. The highest activity was shown by the ESBE and ERBE at 100 mg/mL (15mm) against *E. coli*. Although most of the extracts at the various concentrations used showed activity against the pathogens, it was observed on the general that the extracts are more effective at 100 mg/mL on *E. coli*, which showed similar activity with the standard drug (Tetracycline at 100µg/mL) used. At lower concentrations, the extracts seem to show more activity against Shigella dysenteriae as seen in tables 3 - 6.

From table 3, it is revealed that the zones of inhibitions of the extract (ARBE) against the tested pathogens showed that the extract has antimicrobial activities against the pathogens at various concentrations respectively. It was noticed that the extract was very effective at a concentration of 100 mg/mL, the effectiveness increases as the concentration increases. The control was more effective on *E. coli* with zone of inhibition up to 20 mm. Table 4 shows the zones of inhibitions of the aqueous stem bark extract (ASBE) on the microorganisms. The result shows that the extract was effective at different concentrations with various zones of inhibitions as the concentration increases. However, *E. coli* was resistant against the extract at higher concentration of 100 mg/mL and 50 mg/mL but effective at lower concentration 25 mg/mL and also the control which has the highest zone of inhibition (11mm) on *E. coli*. From table 5, the ethanol stem bark extract (ESBE) also showed considerable antimicrobial activities on the tested clinical isolates at various concentrations used. The result shows that at a higher concentration the extract was active against the clinical isolates or pathogens but more effective on *Shigella* at lower concentration (25 mg/mL) with zone of inhibition 10 mm, also the control was more effective with the highest zone of inhibition 16 mm. This extract show more activity against *E. coli* than the control drug at 100 mg/mL with 15 mm zone of inhibition. From table 6 the results of ethanol root extract (ERBE) against the pathogens

also shows that the antimicrobial potential of the extract increases considerably as the concentration increases.

The result of the antimicrobial activity of root and stem bark extracts in this study is similar to that of [26,27,28] who asserted that many plants have been reported for therapeutic purposes because of the chemical compounds synthesized in these plants. The antibacterial activities of the ethanolic extracts of the leaves and stem bark of *F. sycomorus* have been previously reported [27]. The present study suggests that *F. sycomorus* may serve as a potential source of antibacterial and/or antimicrobial agents of plants origin. Hence, the observed antimicrobial activity of the root and stem bark extracts against the test organisms in this study may be due to the presence of phytochemical components. The findings demonstrated that the stem and root bark extract were sensitive to all the tested organisms and thus showed that the extract contained potential antimicrobial agents such as tannin, saponin, alkaloid, glycosides as secondary metabolite responsible for curing various sicknesses .The presence of tannin in all the extract could be probably responsible for the observed antimicrobial activity. The claim of literature that *F. sycomorus* has antimicrobial activity is hereby verified. The anti-microbial activity of the extracts, both the ethanol and aqueous of root and stem have shown a reasonable zone of inhibition to the concentration from 12.5 – 100 mg/mL and the control drug (Tetracycline) at 100 µg/mL concentration. However, the ASBE extracts of *F. sycomorus* was observed to be less potent against the tested clinical isolate respectively.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts are shown in Tables 7 and 8. The result has shown that the MIC for all extracts of root and stem bark was 50 mg/mL. At this concentration, the extract was able to inhibit the growth of microorganisms. The result also revealed that the MBC was at 100 mg/mL these means that at this concentration the extract was able to kill the bacteria completely. This result is similar to the work of [26] who reported that the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the aqueous and ethanolic root and stem bark extracts of *Ficus sycomorus* extracts ranged from 3.125 mg/mL to 100 mg/mL. [21]. also reorted that the minimum inhibitory concentration (MIC) of methanol root bark extract of *F. sycomorus* was observed within the range of 2.5– 5.0mg/ml against *E. faecalis, E. coli, S. typhi, S. dysenteriae and C. albicans.* This result therefore suggests that the extracts are more of bacteriostatic.

Table 1: Percentage yield of the root and stem extracts

Extract	Initial weight	YIELD(g)	%
ERBE	200.00g	14.14g	7.07
ESBE	200.00g	16.00g	8.00
ARBE	200.00g	12.23g	6.12
ASBE	200.00g	11.16g	5.58

Key:

ERBE------Ethanol Root Extract

ESBE-----Ethanol Stem Bark Extract

ARBE------Aqueous Root Extract

ASBE-----Aqueous Stem Bark Extract

Table 2: Qualitative Phytochemical analysis of the root and stem bark extract of *Ficus sycomorus*

	Aqueous extract		Ethanol extract	
TEST	**Root**	**Stem bark**	**Root**	**Stem bark**
Tannins	+	+	+	+
Saponin	+	+	+	+
Terpenoid	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Glycosides	_	_	+	+
Steroids	+	+	+	+
Phenols	+	+	+	+
Reducing sugar	+	+	+	+

+ = Present **- = Absent**

Table 3: Zone of Inhibition in (mm) Aqueous Root bark Extract (ARBE) Against Opportunistic Pathogens.

S/No.	Name of Organism	Concentration mg/mL				
		100	50	25	12.5	Tetracyline(Control)
	S. aureus	7	6	5	2	13
	Escherichia coli	10	8	7	4	20
	Salmonella spp	12	7	5	R	13
	Shigella spp	10	9	7	4	13

Key:

Resistant---- R
Aqueous Root bark Extract------ ARBE

Table 4: Zone of inhibition in (mm) of Aqueous stem bark extract (ASBE) against Opportunistic Pathogen

Name of Organism	Concentration mg/mL				
	100	50	25	12.5	Tetracycline(Control)
S. aureus	9	6	4	3	7
Escherichia coli	R	R	10	4	11
Salmonella spp	7	5	4	3	8
Shigella spp	9	6	5	4	10

Key: Resistant-------- R Aqueous stem bark extract ----ASBE

Table 5: Zone of Inhibition in (mm) of Ethanol stem bark extract (ESBE) against Opportunistic Pathogens

Name of Organism	Concentration mg/mL				
	100	50	25	12.5	Tetracycline(Control)
S. aureus	6	5	4	2	10
Escherichia coli	15	9	3	2	12
Salmonella spp	10	6	5	3	11
Shigella spp	5	4	10	5	16

Key: Ethanol stems bark extract-------- ESBE

Table 6: Zone of Inhibition (mm) of Ethanol root bark Extract (ERBE) against Opportunistic Pathogens.

S/No.	Name of Organism	Concentration mg/Ml				
		100	50	25	12.5	Tetracycline(Control)
	S. aureus	6	5	4	2	10
	Escherichia coli	15	9	3	2	12
	Salmonella spp	10	5	6	3	11
	Shigella spp	10	5	5	4	16

Key: Ethanolic root bark extract------ ERBE

Table 7: The Result of Minimum Inhibitory Concentration (MIC) of both aqueous and ethanol extracts of root and stem bark of *Ficus sycomorus*

Microorganism	MIC (mg/mL)			
	100	50	25	12.5
Staphylococcus aureus	-	-	+	+
Escherichia coli	-	-	+	+
Salmonella spp	-	-	+	+
Shigella spp	-	-	+	+

+ = Growth ; - = No growth

Table 8: The Result of Minimum Bactericidal Concentration (MBC) of both aqueous and ethanol extracts of root and stem bark of *Ficus sycomorus*

Microorganism	MBC (mg/mL)			
	100	50	25	12.5
Staphylococcus aureus	-	+	+	+
Escherichia coli	-	+	+	+
Salmonella spp	-	+	+	+
Shigella spp	-	+	+	+

+ = Growth ; - = No growth

CONCLUSION

Phytochemicals such as tannins, saponin, terpenoids, flavonoids, alkaloids, glycosides, steroids, phenols and reducing sugars were all found to be present in both the aqueous extracts of roots and stem bark of *Ficus sycomorus.*

From the studies of the antimicrobial activities, the research revealed that, for aqueous stem and root bark, ARBE had more antimicrobial potentials against the selected pathogens than the ASBE, but for ethanol stem and root bark both have almost the same inhibitory activities on the tested pathogens.

From the research, it was noticed that both the root and stem bark have antimicrobial potentials on the tested pathogens. This validates the claim of the traditional users who used it to treat diseases of microbial origin. Therefore, it can be used for therapeutic purposes.

ACKNOWLEDGEMENT

The authors are grateful to Laboratory Technologists, in person of Mr. Umar, Mallam Sani, Esther Dauda, all from Microbiology Department, Modibbo Adama University of Technology Yola, for their contributions for the success of the research.

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