PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF THE ROOT AND STEM BARK EXTRACT OF *FICUS SYCOMORUS* ON SOME SELECTED MICRO-ORGANISMS

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.

Comment [sp1]: Sentence correction

Key words: *Ficus sycomorus, Escherichia coli, Staphylococcus aureus, Shigella dysentrae,* and *Salmonella typhi, phytochemical screening and 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 (Cragg and Newman, 2001). At least 12000 of such compounds have been isolated so far, a number estimated to be less than 10% of the total (Tapsell, 2006, Lai and, Roy, 2004). 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 (Tapsell, 2006, Lai and Roy 2004) .

Plant lives longer than every other living things due to their ability to synthesized 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).

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 (Orwa *et al*, *2009*). The sycamore fig is sensitive to frost but can withstand some cold. The root and stem-bark of *Ficus sycomorus are sai*d to be used as herb in Northern Nigeria for treatment of diseases like diarrhea, dysentery, cough, soar throat, chest diseases, and infertility and as antidote for snake. *

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

Comment [sp2]: other

Comment [sp3]: adjust space

Comment [sp4]: Such comments need proof with published reference. If no research proof is available, comment must be deleted.

Comment [sp5]: DELETE SUCH REFERENCES

Comment [sp6]: Delete one .

Comment [sp7]: Wrong statement. It is native to Africa and surrounding area.

Comment [sp8]: DELETE SUCH REFERENCES

Comment [sp9]: Correct grammar

Comment [sp10]: At which form these are used in traditional practices? Oral feeding/ poultice/ ….. state it.

Comment [sp11]: The leaves and latex are used in many important diseases. These should be included with references.

some pathogenic microorganisms (Hassan, 2005; Oyeleke *et al*., 2008; Sandabe *et al*., 2006; Solomon-Wisdom *et al.*, 2011 Stary, 1998; Udobi *et al*., 2008).

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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. Sampling was carried out in the month of May from the tree.

Sample preparation

The root or 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 extraction

For the aqueous extraction, decoction procedure was used. Two hundred grams (200 g) each of the root and stem bark powder (separately) were weighed and soaked in 1000mLs of distilled water in a beaker and heated to boil. It was left to cool and then filtered using sterile filter paper (Whatman No 1) into a clean conical flask. The filtrate was concentrated on a water bath at a temperature of sixty degree Celsius (60^{o}C).The final yield of the extracts was then stored in a refrigerator until the onset of the experiments as per Fatofe et al., 1993.

Ethanol Extraction

Maceration method of extraction as described by Fatofe et al., (1993) was adopted in this study. Two hundred grams (200 g) for each of the root and stem bark powdered material was weighed and soaked in 1000 mL of 70% ethanol and left for 24 hours .Thereafter, it was decanted. The procedure was repeated with another 1000 mL to ensure complete extraction of the active ingredient .The extracts was filtered and evaporated to dryness on a water bath at a temperature

Comment [sp12]: For good journal, try to limit the number of references for one comment within three.

Comment [sp13]: As per your statement, the antimicrobial screening and identification of phytochemicals of that plant were performed by several researchers previously. Then why you have performed the study?
You have to state clearly the objective of your study. The new angle and parameters you are studying for the first time, you have to state. Otherwise that article CAN NOT BE RECOMMENDED FOR PUBLICATION.

Comment [sp14]: Add year also.

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Comment [sp21]: Generally, it is kept under continuous movement inside shaking incubator etc.for better extraction.

Comment [sp22]: Generally, Vacuum evaporator is used for this purpose. At high temperature, many phytochemicals may be lost or evaporated.

of sixty degree Celsius (60^{o}C).The dried extract was then weighed and stored in tightly closed bottles in a refrigerator until required.

Qualitative Phytochemical analysis.

The qualitative phytochemical screening of the samples was carried out as described by Harborne (1973), Nweze *et al.*, (2004) and Senthilkumar and Reetha, (2009) with slight modification. The root or stem bark extracts was screened for carbohydrates, alkaloids, flavonoids, steroids, phenols and tannins, saponin, glycosides, and proteins.

Test for Tannins

To 1 mL of plant extract, 2 mL of 5 % ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

To 1 mL of plant extract, 5-10 mL of distilled water was added and shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of Saponins.

Test for Terpenoids

To 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 will carefully be added to the mixture to form a layer. An interface with a reddish brown coloration will indicate that terpenoids constituent is present.

Test for Flavonoids

i) To 2 mL of plant extract 1 mL of 1N aqueous NaOH solution was added and observed for the formation of yellow-orange colouration.

ii) 2 mL of plant extract was treated with 4 drops of concentrated sulphuric acid and observed for the formation of orange colour.

Test for Alkaloids

To 2 mL of plant extract, 2 mL of concentrated hydrochloric acid was added. Then 3 drops of Mayer's reagent was added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for glycosides

To 2 mL of plant extract, 1 mL of glacial acetic acid and 5% ferric chloride was added. To these 3 drops of concentrated sulphuric acid was added. Presence of greenish blue colour indicates the presence of glycosides.

Test for Steroids

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

Test for phenols

To 1 mL of the extract, 2 mL of distilled water followed by 5 drops of 10% ferric chloride was added. Formation of blue or green colour indicates 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

Antimicrobial test; *E.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.

Preparation of inoculants and inoculation (using Agar Well Diffusion method)

The method described in the National committee for Clinical Laboratory Standard (1997) was used. This was determined by dispensing 10 mL of nutrient agar into a sterile Petri dish and shaken for evenly distribution and allowed to solidify.

Five wells, 4mm each in diameter were created using cork borer and a wire loop was used to pick the microorganism from the culture plate and smeared into the Petri dish containing the solidified nutrient agar and the well was filled with the drops of the extract and a standard drug (tetracycline capsule 100 µg/mL) to compare the activity of the extracts with various concentration of 100, 50, 25 and 12.5 mg/mL .

Comment [sp23]: How the capsular drug was diluted?

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Agar plate preparation – incubation for checking sterility-addition of broth culture of bacteria-creation of wells- addition of samples and control in the wells- incubation etc.

A candle light was used to sterilize the wire loop after picking the microorganisms from the plate to avoid contamination of the medium during the process of inoculation of the organisms. Then it was allowed to stand for 1hr diffusion at room temperature and was then incubated for 24-48 hrs at room temperature 37 ^{0}C. The zones of inhibitions developed were measured using a transparent ruler in (mm) and the zone of inhibition for each concentration were measured and recorded.

Media preparation

Thirty eight gram (38g) of Mueller Hinton agar was weighed using weighing balance and was dissolved in 1000 mL of distilled water in a conical flask by swirling. The flask was covered with cotton wool and the flask content was autoclaved at 121^{o}C for 15 minutes in an autoclave. It was holed down to about 45^{o}C after which it was dispensed into Petri dishes aseptically and was then allowed to gelled.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was evaluated by the method described by Greenwood (1989).

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 bacterial 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.

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Comment [sp25]: Re write whole description correctly.

Comment [sp26]: It was kept standing? How?

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Comment [sp30]: Zones of inhibition in broth culture? Or it is the absence of change of visibility of broth media even after incubation?

Comment [sp31]: WHAT DO YOU WANT TO SAY?
If broth culture was added, then the concentration of the bacteria must be standardized before.
You may check the following article after downloading PDF:
Pattanayak S, Pal S, Mandal TK, Debnath PK, Bandyopadhyay SK (2014) A comparative study of extract of succulent leaves of living plant with methanolic and aqueous extract of *Berleria lupulina* Lindl. against pathogenic microbes by disc diffusion and spectrophotometry. Explor Anim Med Res 4(2): 148-157.

Comment [sp32]: NOT DESCRIBED PROPERLY.

Comment [sp33]: What number/ quantity of bacteria was added through nutrient broth cultured bacteria to overlay the nutrient agar of the petridishes? You have to write it in detail.

RESULTS AND DISCUSSION

Table 1: Percentage yield of the root and stem extracts

Extract	**Initial weight**	**YIELD(g)**	**%**
ERE	150.00g	11.00g	7.33
ESBE	150.00g	16.00g	10.67
ARE	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

Comment [sp34]: Write: No inhibition

Comment [sp35]: All the plant materials capable of showing some zone of inhibition can not be considered as effective against the test bacteria. You may get such effect in plant extracts of many plants. Many bacteria are already resistant against penicillin. You can just compare the data.

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

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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

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

Comment [sp42]: delete

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*

	100	50	25	12.5
Staphylococcus aureus	-	-	+	+
Escherichia coli	-	-	+	+

Microorganism **MIC (mg/mL)**

Salmonella spp	-	-	+	+
Shigella spp	-	-	+	+

+ = Growth ; - = No growth

	100	50	25	12.5
Staphylococcus aureus	-	+	+	+
Escherichia coli	-	+	+	+
Salmonella spp	-	+	+	+
Shigella spp	-	+	+	+

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

Comment [sp43]: Table 7 and table 8 should be given before the tables of antimicrobial efficacy testing, as these were performed earlier than the antimicrobial tests.

Microorganism	**MBC (mg/mL)**

+ = Growth ; - = No growth

DISCUSSIONS

Comment [sp44]: Modify after modifying the materials and methods.

Tables 3 – 6 above, is the result of the zones of inhibition of the different extracts (ARBE, ASBE, ESBE and ERBE) against the tested pathogens, 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 dysentraiae 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 (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 , Abdullahi, 2014,Adeshina et al., (2010) and Bello et al., (2013) who asserted that many plants have been reported for therapeutic purposes because of the chemical compounds synthesized in these plants. 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.

Tables 7 and 8, shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentartion (MBC) of the extracts. 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 (Abdullahi, 2014) 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. This result therefore suggests that the extracts are more of bacteriostatic.

Conclusions

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 inhibitory 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.

Comment [sp45]: It can not be concluded. In oral use, many phytochemicals are metabolized/ changed to other constituent to act. The extracts may have some toxicity. Dose of use of the plant part is also very important..

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Comment [sp46]: After correction/ modification of the text, cross check all the references with the text and then from the text with the reference table. Write references as per style of the journal.

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