British Journal of Pharmaceutical Research
11(6): 1-10, 2016, Article no.BJPR.25608
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Activity and Phytochemical Profile of Leaf Extracts of *Ficus abutilifolia*

F. O. Taiwo[1*], A. A. Fidelis[1] and O. Oyedeji[2]

[1]Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors FOT and OO designed the study. Author FOT wrote the protocol and wrote the first draft of the manuscript. Authors FOT, OO and AAF carried out the research in the laboratory. Authors OO and AAF managed the literature searches and analyses of the study. Author AAF performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/25608
Editor(s):
(1) Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, Hunan, China.
Reviewers:
(1) James Adams, University of Southern California, USA.
(2) Carmen Lizette Del Toro Sanchez, Universidad De Sonora, Mexico.
(3) Anonymous, Finland.
Complete Peer review History: http://sciencedomain.org/review-history/14713

Original Research Article

Received 11th March 2016
Accepted 7th April 2016
Published 21st May 2016

ABSTRACT

Aims: To determine the antibacterial activities of leaf extract of *Ficus abutilifolia* against selected clinical bacterial isolates. Also, to analyse the extract for the constituent phytochemical compounds.
Study Design: *In vitro* antimicrobial assay of solvent fractions of plant leaf extract against selected clinical bacterial isolates.
Place and Duration of Study: Chemistry and Microbiology Laboratories, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria, between February 2014 and February, 2015.
Methodology: 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.

**Corresponding author: E-mail: oftaiwo@yahoo.co.uk;*

Results: *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.

Conclusion: The significant antibacterial activities exhibited by the ethyl acetate and other fractions suggest the presence of bioactive compounds in the leaf extract of *F. abutilifolia.* The plant could be a good source of antibacterial agents which can be developed as a pharmaceutical product. This study also supports the traditional use of the plant in the treatment of several infectious ailments.

Keywords: Ficus abutilifolia; antibacterial activity; medicinal plants; bacterial resistance; phytochemical.

1. INTRODUCTION

Medicinal plants have been used by man for the treatment of varieties of diseases, including microbial infections of several organs of the body [1-3]. The use of extracts from various parts of medicinal plants has therefore significantly supported primary health care among and within human communities [4,5]. Infections caused by microorganisms such as bacteria, fungi and viruses have been the major cause of diseases throughout the history of human population. Along the course of human history, antibiotics were discovered and used to combat infectious diseases caused by bacteria. Antibiotic therapeutic failure as a result of the emergence of resistant bacterial strains is however a current global health problem [6,7]. For instance, *Staphylococcus aureus*, implicated in several infectious diseases, began the development of penicillin-resistant strains not long after the introduction of penicillin use [6]. The menace of infectious pathogenic microorganisms, which were thought to have been mitigated by antibiotics has led to a re-emergence of more virulent microorganisms in new form of resistant strains [8]. Increase in antibiotic resistance is attributable to a combination of microbial characteristics, the selective pressure of antibiotics use and technical changes that enhance transmission of resistant organisms [9]. To combat this worrisome development, scientists have been engaged in the study of varieties of plants as potential sources of more effective, natural antimicrobial agents [10-12]. Plants contain several secondary metabolites which exert biological effects against external agents, especially microorganisms [1]. These natural compounds exert their effect by mechanisms such as disintegration of cytoplasmic membrane, inhibition of cell wall synthesis, substrate deprivation, enzyme inhibition and metal ion complexation [1].

Ficus species, which belong to the family Moraceae, is a genus of about eight hundred species of woody trees, shrubs and vines occurring throughout the tropical and sub-tropical regions of the world [13,14]. *Ficus abutilifolia* is a small to medium-sized tree which seldom exceeds 5 m in height and is generally encountered along streams [15]. It is widely distributed on the African continent [15,16]. Several species of the genus *Ficus* have been reported to exhibit antimicrobial as well as pharmacological effects. The sedative and anti-convulsant activities of *Ficus sycomorus* have been reported [17]. Extract of *F. platyphylla* was reported to possess analgesic [18] as well as anti-inflammatory and anti-conceptive [19] activities. The antibacterial activities of *F. sycomorus* L and *F. platyphylla* Del. were reported [20] while ethanolic root extract of *F. benghalensis* and *F. racemosa* produced moderate antibacterial activities against *S. aureus*, *P. aeruginosa* and *K. pneumoniae* [21]. Also, the acetone leaf extract of *F. tsiela* produced inhibitory activity against *S. aureus*, *P. aeruginosa* and *K. pneumoniae* [22]. Extract from the leaves of *F. abutilifolia* are used traditionally to treat various ailments such as typhoid fever, chronic dysentery, sexually transmitted infections, malaria and infertility by indigenous communities especially in South eastern and Northern parts of Nigeria [23]. To our knowledge, there is paucity of information regarding the

antibacterial activity of *F. abutilifolia*, hence this study was carried out to investigate the antibacterial activities of leaf extract of the plant against selected clinical bacterial isolates. Analysis of the extract for the constituent phytochemical compounds was also carried out.

2. MATERIALS AND METHODS

2.1 Plant Materials

Ficus abitilifolia leaves were collected from several places in the Obafemi Awolowo University, Ile-Ife, Nigeria campus in February, 2014 and authenticated taxonomically by Dr. M. Oziegbe at the Ife herbarium in the University. The leaves were air-dried for four weeks and then blended into powder.

2.2 Preparation of Plant Extracts

Extractions were performed by maceration in which 1700 g of the powdered *Ficus abitulifolia* leaves were soaked in 50% aqueous-methanol (5 L) at room temperature for 72 h. The extracts were then filtered using Whatman filter paper no. 2 and concentrated in vacuo at 40℃ on a rotary evaporator (Heldolph, Germany) to about one-third of its original volume. Concentrated crude extract of the plant was in turn dissolved in distilled water and partitioned with n-hexane (2.5×1 L), dichloromethane (2.5×1 L), ethyl acetate (2.5×1 L) and n-butanol (2.5×0.7 L). The partitioned fractions were concentrated to dryness *in vacuo* to afford five different fractions: hexane, dichloromethane, ethyl acetate, n-butanol and aqueous fractions with compounds of appropriate polarity. The fractional solvent extracts obtained were concentrated to dryness on a rotatory evaporator and then screened for their antibacterial activities.

2.3 Preliminary Phytochemical Analysis of *Ficus abitulifolia* Extracts

The phytochemical screening of crude extracts from the plant material was carried out to detect the presence of active, plant secondary metabolites. The plant extracts were screened for the presence of carbohydrates, alkaloids, saponnin, tannins, flavonoids, anthraquinones, phlobatannin and terpenoids according to established procedures [24,25].

2.4 Test for Alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. One millilitre of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

2.5 Test for Tannins

About 1 g of the extract was dissolved in 20 ml of distilled water and filtered, 2 to 3 drops of 10% of $FeCl_3$ was added to 2 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins. To another, 2 ml of the filtrate was added 1 ml of bromine water. A precipitate was taken as positive for the tannins.

2.6 Test for Flavonoids

A 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated hydrochloric acid. The occurrence of a red or orange colouration was indicative of the flavonoids.

2.7 Test for Saponins

Freshly prepared 7% blood agar medium was used and wells were made in it. The extract in methanol was applied with distilled water and methanol used as negative control while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35℃ for 6 h. Complete haemolysis of the blood around the extract was indicative of saponins.

2.8 Test for Phlobatannins

About 1.0 g of the plant extract was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitates was taken as indication of the presence of phlobatannins.

2.9 Test for Anthraquinones

About 1.0 g of the plant extract was dissolved in petroleum ether and filtered. Aqueous ammonia was then added to the filtrate, formation of pink colouration was taken as indication of the presence of anthraquinones in the plant extract.

2.10 Test for Cardiac Glycosides

About 1.0 g of the plant extract was dissolved in pyridine and few drops of 2% sodium

nitroprusside solution with few drops 2% NaOH solution were added. A deep red colour which fade to brownish yellow was taken as indication of the presence of cardenolides.

2.11 Test for Carbohydrates

About 1.0 g of the plant extract was dissolved in distilled water and filtered. Few drops of Molisch's reagent and concentrated H_2SO_4 were added and formation of red or dull violet colouration at the interface of two layers was taken as indication of the presence of carbohydrates in the plant extract.

2.12 Microorganisms and Growth Conditions

Pure clinical isolates of *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Clostridium sporogenes* (Gram positive bacteria) and *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella typhi* (Gram negative bacteria), collected from the stock cultures of Medical and Parasitology Laboratory of the Obafemi Awolowo University Teaching Hospitals, Ile-Ife, Nigeria, were used for the experiment. The bacterial strains were maintained at 4℃ on agar slant and incubated at 35℃ for 24 h on nutrient agar before use.

2.13 Antimicrobial Assays

2.13.1 Disc diffusion assay

Antimicrobial activities were determined by the disc diffusion method [26], with modifications. Two hundred microliter suspensions of standardized microbial cells (10^6 CFU/ml) were inoculated into 20 ml of Mueller Hinton agar and poured into sterile Petri dishes. Dried solvent fractions of *F. abutilifolia* leaf extract were reconstituted in 10% dimethylsulfoxide (DMSO) solution to give a final concentration of 1000 µg/ml. Sterile paper discs (6 mm) were impregnated with 10 µl sterile solutions of the solvent fractions and placed, aseptically, on the agar surface. Standard discs (6 mm) containing the broad spectrum antibiotic, streptomycin (10 µg/disc) (Oxoid, UK), were used as positive controls. The plates were incubated at 37℃ for 18-24 h after which they were examined for zones of inhibition. The effects of the fractions on the test isolates were compared with that of the standard antibiotic, streptomycin. The experiments were carried out in triplicates and zones of growth inhibition were recorded in millimetres. Statistical analysis was performed using SPSS software.

2.13.2 Determination of the minimum inhibitory concentrations (MICs)

Broth micro dilution method was used to determine the minimum inhibitory concentrations (MIC) of the solvent fractions [27]. The dried solvent fractions were dissolved in 10% DMSO solution to give a concentration of 400 µg/ml. Serial 2-fold dilutions were then made in concentrations ranging from 12.5 to 400.0 µg/ml. The 96-well microtitre plates were prepared by dispensing into each well 100 µl of Mueller Hinton broth and each of the concentration dilution of solvent fraction. Inoculum (10 µl) of test bacteria suspensions and 50 µl (0.2 mg/ml) of p-iodonitrotetrazolium (INT) chloride were then added into each well. The plates were covered with parafilm, shaken to mix the contents and then incubated at 37℃ for 24 h. Each experiment was carried out in triplicates. The MIC was defined as the lowest concentration at which no visible growth was observed. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-colored formazan product by biologically active organisms [28]. Where bacterial growth was inhibited, the solution in the well remained clear after incubation with INT.

2.13.3 Determination of minimum bactericidal concentrations (MBC)

Minimum bactericidal concentrations (MBC) of the solvent fractions were determined by removing 100 µl of the test bacteria suspension from cultures demonstrating no visible growth in the MIC assay and inoculating them on freshly prepared Mueller Hinton agar plates. Plates were incubated at 37℃ for 48 h with experiments carried out in triplicates. The MBC was taken as the concentration of the solvent fraction that did not show any growth on the new set of agar plates.

2.13.4 Determination of rate of killing

Rate of killing studies on representative of each Gram-positive and Gram-negative bacterial isolates were carried [29]. *Staphylococcus aureus* was chosen for Gram positive while *Pseudomonas aeruginosa* represented the Gram-negative bacterial strains. Standardized

inocula (10^6 CFU ml^{-1}) of test organisms (0.5 ml) were mixed with 4.5 ml of MBC of ethyl acetate fraction. The preparations were allowed to stand at room temperature and the rate of killing was determined over 2 h. At each 15 min interval, 0.1 ml of mixture was taken and transferred to 4.5 ml of brain heart infusion broth recovery medium containing 3% "Tween 80" to neutralize the effects of antimicrobial extract carry overs from the test organisms. The suspension was then serially diluted 10-fold with sterile normal saline and plated out on sterile Mueller Hinton agar in triplicates. The plates were incubated at 37℃ for 24 h. Control plates containing organism suspension without solvent fraction were also set up. The number of surviving colonies were counted and recorded against time.

3. RESULTS

Exactly 350 g of crude extract was obtained from 1700 g of the powdered leaves of *Ficus abutilifolia.* The extract was dark brown in colour.

Investigations on the leaf extract of *F. abutilifolia* for its phytochemical constituents revealed the presence of all the phytochemical groups screened for such as alkaloids, anthraquinones, saponnins, flavonoids, tannins, cardiac glycosides, carbohydrate and phlobatannins (Table 1). Five fractions were obtained from the crude extract of the plant material which were the aqueous, n-hexane, dichloromethane, ethyl acetate and n-butane fractions. All the fractions, with the exemption of the aqueous fraction, exhibited antibacterial activities against all test clinical bacterial isolates with zones of inhibition which ranged between 9.33±0.58 and 31.67±0.58 mm. All the test bacterial isolates were found to be susceptible to the four active fractions of the leaf extract (Table 2). The reference antibiotic, streptomycin exhibited antibacterial activities with zones of inhibition ranging between 10.67±1.15 and 25.0±1.00 mm, against the test isolates. The ethyl acetate fraction exhibited the highest antibacterial activity against all the test bacterial isolates with mean zones of inhibition ranging between 27.67±1.15 mm and 31.67±0.58 mm (Table 2). The minimum inhibitory concentrations (MIC) exhibited by the ethyl acetate fraction against the test bacterial isolates ranged between 0.0313 µg/ml and 0.0625 µg/ml while the MIC exhibited by n-butanol, hexane and dichloromethane fractions against the test isolates ranged between 0.125 µg/ml and 0.25 µg/ml (Table 3). The MIC exhibited by streptomycin against the test isolates ranged between 0.125 µg/ml and 0.25 µg/ml. The minimum bactericidal concentration (MBC) values ranged between 0.0625 µg/ml and 0.25 µg/ml for the ethyl acetate fraction and between 0.25 µ/ml and 0.50 µg/ml for all other active fractions and reference antibiotic streptomycin (Table 4). The killing rate test was carried out to determine the bactericidal effects of the 1 x MIC and 2 x MIC of the ethyl acetate fraction of *Ficus abutilifolia* leaf extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. At a concentration of 1 x MIC, the percentage of *Staphylococcus aureus* cells killed after 15 min exposure time of the cells to the ethyl acetate fraction was 20.5±0.71 %. The percentage of cells killed rose to 55.0±1.41 % after 60 min exposure time. When the contact time was increased to 120 min, 88.0±1.41 % of the *Staphylococcus aureus* cells had been killed (Fig. 1). The concentration of the fraction was increased to 2 x MIC and the test isolates were subjected to the effect of the fraction. At 15 min exposure time, 38.5±0.71% of the *Staphylococcus aureus* cells were killed and this rose to 100% at the end of 120 min exposure time. A linear relationship was also observed between the percentage of *Pseudomonas aeruginosa* cells killed and increase in MIC concentration and exposure time (Fig. 2).

Table 1. Phytochemical compounds detected in the leaf extract of *Ficus abutilifolia*

Phytochemical compound	Test	Observation	Indication
Tannins	Ferric chloride	Blue-green colour	Positive
Anthraquinones	Borntrager	Bright pink colour	Positive
Saponins	Frothing test	Frothing formation	Positive
Flavonoids	HCl	Red or orange colour	Positive
Alkaloids	Meyer	Absence of turbidity	positive
Cardiac glycoside	H_2SO_4	Pink colour	Positive
Carbohydrate (ketonic sugar)	HCl	Cherry red colour	Positive
Phlobatannins	HCl	Cloudy red colour	Positive

Table 2. Antibacterial activities of solvent fractions of *Ficus abutilifolia* leaf extract against selected clinical bacterial isolates

Test bacteria	Fraction/ Mean zones of inhibition in (mm*)					
	AQS (1000 µg/ml)	HEX (1000 µg/ml)	DCM (1000 µg/ml)	ETAC (1000 µg/ml)	BUT (1000 µg/ml)	STR (10 µg/disc)
S. aureaus	0.0	10.33±0.58	20.33±0.58	29.33±1.53	22.33±1.15	14 .67±1.15
E. faecali	0.0	20.33±0.58	20.67±1.15	30.67±0.58	15.00±1.00	24. 67±1.15
B. subtilis	0.0	18.33±0.58	23.33±1.15	30.67±1.15	18.33±0.58	24 .33±0.58
K. pneumonia	0.0	10.67±1.15	10.33±1.15	29.67±0.58	20.33±0.58	21 .67±1.15
E. coli	0.0	17.67±1.15	20.67±1.15	31.67±0.58	24.33±0.58	18 .33±0.58
C. sporogenes	0.0	9.67±1.15	16.33±0.58	30.67±1.15	9.33±0.58	25. 00±1.00
S. typhi	0.0	9.67±1.15	20.67±1.15	30.67±1.15	20.33±1.53	10. 67±1.15
P. aeruginosa	0.0	17.67±1.15	20.67±0.58	27.67±1.15	24.67±1.15	24 .67±1.15

mm - Mean of three replicates±standard deviation, AQS – aqueous, HEX – hexane, DCM – dichloromethane, ETAC – ethyl acetate, BUT – butane, STR - streptomycin*

Table 3. Minimum inhibitory concentrations (MIC) of fractions of *Ficus abutilifolia* leaf extract against selected clinical bacterial isolates

Test bacteria	Fraction/ average MIC (µg/ml)				
	Hexane	Dichloromethane	Ethyl acetate	Butanol	Streptomycin
S. aureus	0.125	0.125	0.0625	0.125	0.125
E. faecalis	0.125	0.125	0.0313	0.250	0.125
B. subtilis	0.125	0.250	0.0625	0.125	0.125
K. pneumonia	0.125	0.125	0.0313	0.250	0.125
E. coli	0.250	0.125	0.0625	0.125	0.250
C. sporogenes	0.125	0.125	0.0625	0.125	0.125
S. typhi	0.125	0.125	0.0625	0.250	0.125
P. aeruginosa	0.125	0.125	0.0625	0.250	0.125

Table 4. Minimum bactericidal concentrations (MBC) of fractions of *Ficus abutilifolia* leaf extract against selected clinical bacterial isolates

Test bacteria	Fraction/ average MBC (µg/ml)				
	Hexane	Dichloromethane	Ethyl acetate	Butanol	Streptomycin
S. aureaus	0.25	0.25	0.125	0.25	0.25
E. faecalis	0.25	0.25	0.0625	0.50	0.25
B. subtilis	0.25	0.50	0.125	0.50	0.25
K. pneumonia	0.25	0.25	0.0625	0.50	0.25
E. coli	0.50	0.25	0.125	0.25	0.50
C. sporogenes	0.25	0.25	0.125	0.25	0.25
S. typhi	0.25	0.25	0.125	0.50	0.25
P. aeruginosa	0.25	0.25	0.25	050	0.25

4. DISCUSSION

Medicinal plants have been used by several human communities to treat diverse diseases, including infections. They could therefore constitute a potential source for the production of new medicines which may complement or enhance the effects of the conventional antimicrobials. Moreover, the search for effective antibacterial agents from natural sources such as plants has become necessary in order to overcome the problem of bacterial resistance, especially in clinical practice, where treatment failure due to the problem is a global challenge. All the fractions obtained from the crude extract of *Ficus abutilifolia* except the aqueous, exhibited high degree of antibacterial activities against all the test clinical bacterial isolates used in this study. The effects of the fractions also compared favourably with that of standard antibiotic streptomycin used as positive control. The broad spectrum and high activities exhibited by *Ficus abutilifolia* extracts against the tested bacterial

isolates in this study showed that the plant could be a good source of antibacterial drug of natural origin, for the treatment of infectious diseases in human. Several other species of the genus *Ficus* have been reported to exhibit significant antibacterial activities such as *F. capensis* [30,31], *F. carica* [32] *F. sycomoros* and *F. platyphylla* [20]. All the organic solvent fractions exhibited antibacterial activities whereas the aqueous fraction showed no activity against the test isolates. This may be due to the fact that antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent media [33]. Some phytochemical compounds were detected in *Ficus abutilifolia* leaf extract which included tannins, anthraquinones, saponnins, flavonoids, alkaloids, reducing sugar, cardiac glycosides, carbohydrates and phlobatannis. Some of these compounds have been reported to be present in other *Ficus species* [34,35]. Medicinal values of plants lie in the bioactive phytochemicals present in them and the significant antibacterial activities exhibited by *F. abutilifolia* leaf extract in this study, could be due to the presence of these different bioactive compounds. Tannins exert antimicrobial effects through mechanisms such as membrane disruption, binding to proteins, enzyme inhibition, substrate deprivation and metal ion complexation [36]. Flavonoids have been reported to display strong antibacterial activity by mechanisms such as formation of complexes with extracellular proteins and bacterial cell wall [37,38]. Alkaloids interfere with processes such as deoxyribonucleic acid replication and ribonucleic acid transcription, which are important to microbial functioning [36]. These phytochemical compounds present in *Ficus abutilifolia*, may also be exploited for the development of drugs for the treatment of diseases caused by the bacterial isolates used in this study.

The fractions obtained from *Ficus abitulifolia* exhibited low minimum inhibitory and minimum bactericidal concentrations. The ethyl acetate fraction exhibited the lowest MIC (range 0.0313 to 0.0625 µg/ml) and MBC (range 0.0625 µg/ml to 0.125 µg/ml), of all the fractions. The range of MIC and MBC values exhibited by the ethyl acetate fraction showed that it was more effective against the test bacteria when compared with the reference antibiotic streptomycin. All other fractions also compared favourably with that of the reference antibiotic. The low values of the MIC of these fractions indicated a better antibacterial activity [39]. Hence, this plant could serve as a good source from which antibacterial agents could be developed. Such antibacterial agents could go a long way in health care delivery for the treatment of infections caused by bacteria.

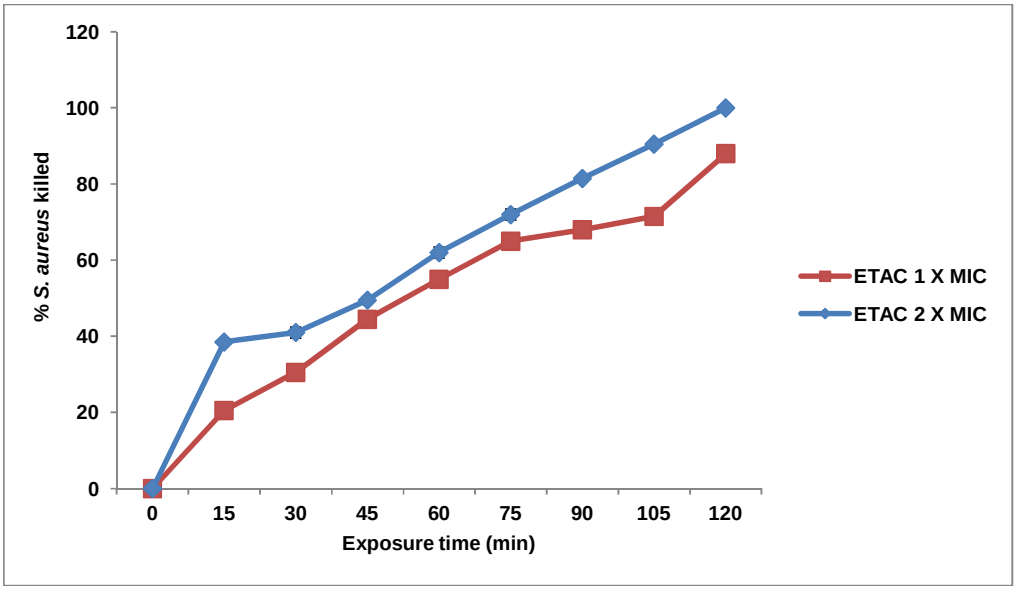

Fig. 1. Rate of killing of *S. aureus* by the minimum inhibitory concentrations of ethyl acetate fraction of *A. abutilifolia* leaf extract

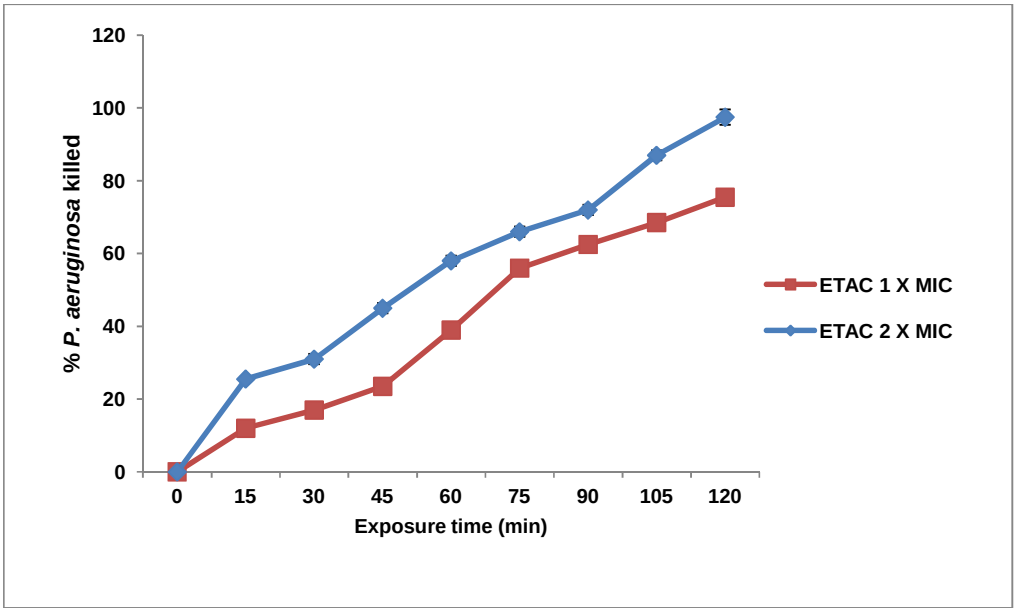

Fig. 2. Rate of killing of *P. aeruginosa* by the minimum inhibitory concentrations of ethyl acetate fraction of *A. abutilifolia* leaf extract

The biocidal effects of extract of *Ficus abutilifolia* on the test isolates were studied based on the rate of killing of the test isolates. The ethyl acetate fraction exhibited appreciable killing rate against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. As the concentrations and exposure time increased, there was increase in population of the test isolates killed. This was an indication of monophasic effect exhibited by the extract in killing the microorganisms.

5. CONCLUSION

The potency exhibited by *Ficus abutilifolia* leaf extract against the clinical test isolates used in this study, at low concentrations and minimal contact time, has shown that drugs formulated from this plant for clinical trials will go a long way in health care delivery. The present study supports the claimed uses of *Ficus abutilifolia* in the traditional system to treat various infectious diseases caused by pathogenic microbes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999;12:564–582.
2. Nostro A, Germano MP, D'Angelo A, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl.Microbiol. 2000;30(5):379-385.
3. Okigbo RN, Mbajiuka CS, Njoku CO. Antimicrobial potentials of (Uda) *Xylopia aethiopica* and *Ocimum gratissimum* on some pathogens of man. Int. J. Mol. Med. Adv. 2005;1(4):392-397.
4. Hostettmann K, Marston A, Ndjoko K, Wolfender JL. The potentials of african medicinal plants as a source of drugs. Curr. Org. Chem. 2000;4:973-1010.
5. Maciel MAM, Pinto AC, Veiga Jr. VF, Grynberg NF, Echevarria A. Medicinal Plants: The need for multidisciplinary scientific studies Quim Nova. 2002;25(3):429-438.
6. National Institute of Allergy and Infectious Diseases (NIAID). The problem ofantimicrobial resistance (overview).

National Institute of Health, Bethesda. MD 20892. U. S. Department of Health and Human Services. 2006;2.

7. Okeke IN, Abiodun OA, Byarugaba DK, Ojo KK, Opintan JA. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerging Infect. Dis. 2007; 13(11):1640-1646.
8. Levy SB, Marshall B. Antibacterial resistance worldwide: Causes, challenges and responses. Nat. Med. 2004;10(12 Suppl.):S122-S129.
9. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioral factors leading to acquired bacterial resistances to antibiotics in developing countries. Emerging Infect. Dis. 1999;5:18-27.
10. Anowi CF, Cardinal NC, Mbah CJ, Onyekaba TC. Antimicrobial properties of the methanolic extract of the stem bark of *Nauclea lantifolia*. IJPTS J Pharma Herb Form. 2012;2:10-21.
11. Josephs GC, Ching FP, Nnabuife AC. Investigation of the antimicrobial potentials ofsome phytochemical extracts of leaf and stem bark of *Berlinia grandiflora* (Leguminoceae) Caesalpinioidae against pathogenic bacteria. Afr. J. Pharmacol. Ther. 2012;1:92-96.
12. Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RMV, Martins A, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J. Appl. Microbiol. 2013;115:346-357.
13. Zerega NJC, Clement WL, Datwley SL, Weiblen GD. Biogroegraphy and divergence times in the mulberry family moraceae. Mol. Phylogenetics Evol. 2005; 37(2):402-416.
14. Hamed MA. Beneficial effect of *Ficus religiosa* linn on high fat-induced hypocholesterolemia in rats. Food Chem. 2011;129:162-170.
15. Burrows J, Burrows S. Figs of Southern and South-Central Africa. Umdaus Press, Hartfield; 2003.
16. Coates Palgrave M. Keith coates palgrave trees of Southern Africa, edn 3. Struik, Cape Town, South Africa. 2002.
17. Sandabe UK, Onyelili PA, Chibuzo GA. Sedative and anticonvulsant effects of aqueous extract of *Ficus sycomorus* L. (Moraceae) stem bark in rats. Veterinarski Arhiv. 2003;73(2):103-110.
18. Wakeel FE, Aziba PI, Ashorobi RB, Umukoro S, Aderibigbe AO, Awe EO. Neuropharmacological activities of *Ficus platyphylla* stem bark in mice. Afr. J. Biomed. Res. 2004;7(2):75-78.
19. Amos S, Chindo B, Edmond I, Akah P, Wambebe C, Gamaniel K. Anti-inflammatory and anti-ninoceptive effects of *Ficus platyphylla* extracts in mice and rats. J. Evol. Biol. 2002;18(5):1234-1238.
20. Adeshina GO, Okeke CE, Osuagwu NO, Ehinmidu JO. Preliminary in-vitro antibacterial activities of ethanolic extracts of *Ficus sycomoros* Linn and *Ficus platyphylla* del. (Moraceae). Afr. J. Microbiol. Res. 2010;4(8):598-606.
21. Murti K, Kumar U. Antimicrobial activity of *Ficus benghalensis* and *Ficus racemosa* roots L. Am J Microbiol. 2011;2:21-24.
22. Shamila IMR, Jeeva S, Sheela DJ, Brindha JR, Lekshmi NCJP. Antimicrobial spectrum and phytochemical study of *Ficus tsiela* L. (Moreceae). Drug Invention Today. 2012;4:337-339.
23. Ukwulibe CA. Phytochemical screening and physical constant evaluation of *Ficus abutilifolia* Miq. (Moraceae) leaves, stem barks and roots for quality control. Scientific Journal of Crop Science. 2014;3(9):98-108.
24. Trease GE, Evans WC. Pharmacognosy, 13th ed. ELBS/Bailliere Tindall, London. 1989;345-346:772-773.
25. Sofowora AE. Medicinal Plants and traditional medicines in Africa. 2nd Ed. Spectrum Books, Ibadan, Nigeria. 1993;289.
26. Mothana RA, Lindequist U. Antimicrobial activity of some medicinal plants of the Island of Soqotra. J. Ethnopharmacol. 2005;96:177-181.
27. Clinical Laboratory Standards Institute (CLSI). Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard, 8th ed. Wayne, PA: M07-A8. CLSI; 2009.
28. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64:711-713.
29. Akinpelu DA, Aiyegoro AO, Okoh AI. Studies of on the biocidal and membrane-disruption potentials of stem bark extracts of *Afzelia africana* (Smith). Biological Res. 2009;42:339-349.
30. Oyeleke SB, Dauda BEN, Boye OA. Antibacterial activity of *Ficus capensis* Afr. J. Biotechnol. 2008;7(10):1414-1417.

31. Adebayo-Tayo BC, Odeniyi AO. Phytochemical screening and microbial inhibitory activities of *Ficus capensis*. Afr. J. Biomed. Res. 2012;15:35-40.
32. Ahmad J, Khan I, Khan S, Iqbal D. Evaluation of Antioxidant and antimicrobial activity of *Ficus carica* Leaves: An *In vitro* approach. J. Plant Pathol. Microbiol. 2013;4:157-160.
33. Mohanasundari C, Natarajan D, Srinavasan K, Umamaheswari SA, Ramachandran A. Antibacterial properties of *Passiflora foetida* L. – a common exotic medicinal plant. Afr. J. Biotechnol. 2007;6(23):2650-2653.
34. Hassan SW. Antimicrobial screening, phytochemical analysis and toxicological studies on some medicinal plants. PhD dissertation, Usmanu Danfodiyo University, Sokoto, Nigeria; 2005.
35. Sandabe UK, Onyelili PA, Chibuzo GA. Phytochemical screening and effects of aqueous extract of *Ficus sycomorus* L. (Moraceae) stem bark on muscular activity in laboratory animals. J. Ethnopharmacol. 2006;104:283-285.
36. Rodriguez-Fragoso L, Reyes-Esparza J, Burchiel S, Herrera-Ruiz D, Torres E. Risksand benefits of commonly used herbal medicines in Mexico. Toxicol. Appl. Pharmacol. 2008;227(1):125-135.
37. Cushnie TP, Lambie AJ. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents. 2005;26(5):343-356.
38. Özçelik B, Deliorman OD, Özgen S, Ergun F. Antimicrobial activity of flavonoids against extended-spectrum ß-Lactamase (ESßL)-producing *Klebsiella pneumoniae.* Trop. J. Pharm. Res. 2008;7(4):1151-1157.
39. Achinto S, Munirudin A. The analgestic and anti-inflammatory activities of the extract of *Albizia zygia* in animal model. Pakistan J. Pharm. Sci. 2009;22:74-77.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14713