*Available online at*www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2015, 7 (3):37-42
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Antimicrobial activities of aqueous and ethanolic leaves extracts of *Ficus Platyphylla* Del.

[1]Mohammed Adamu Milala*, [1]Adamu Mohammed Warabe, [1]Ali Abdulrahman Ali and [2]Isa Adamu Gulani

[1]Biochemistry Department, Faculty of Science, University of Maiduguri
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Borno state, Nigeria

---

ABSTRACT

The study investigated the antimicrobial potencies of the aqueous and ethanolic leaves extracts of Ficus platyphylla D. on some selected microorganisms namely Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis and Candida albican. The methods used were paper disc and agar well diffusion. In both methods the aqueous leaves extracts did not show any antimicrobial activity against the tested organisms but the ethanolic extract markedly inhibited the growth of K. pneumoniae, P. aeruginosa and B. subtilis at the concentrations used (100, 200 and 400mg/ml).The minimum inhibitory concentration (MIC) of the ethanolic extract was 12.5mg/ml, 25mg/ml and 100mg/ml for P. aeruginosa, B. subtilis and K. pneumoniae respectively in paper disc method. In Agar well diffusion method, the MIC was 6.25mg/ml, 12.5mg/ml and 50mg/ml against P. aeruginosa, B. subtilis and K. pneumoniae. The minimum bactericidal concentration (MBC) of the extract was 12.5mg/ml 50mg/ml and 100mg/ml in paper disc method and 12.5mg/ml, 25 mg/ml and 100mg/ml in Agar well method against P. aeruginosa, B. subtilis and K. pneumoniae respectively. The result of this study showed that ethanol was a better extractive solvent for the antimicrobial active component(s) of the leaves of the plant and may be a good candidate for drug development which could be a cure for some ailments.

Key words: *Ficus platyphylla*, aqueous, ethanolic, Paper disc and Agar well diffusion.

INTRODUCTION

Plants are used medically in different countries and are sources of many potent and powerful drugs [1]. Most of the developing countries have adopted traditional medical practice as an integral part of their culture [2]. Plants remain the primary source of many important drugs in orthodox medicine today. A Phytopharmaceutical preparation or herbal medicine is any manufactured medicine obtained exclusively from plant (aerial and non-aerial plant juice, resin and oil) either in crude state or as a phytopharmaceutical formulation [3]. The plants that possess therapeutic properties or extract with beneficial pharmacological effects on animal body are generally designated as “medicinal plants”. Although, there are no apparent morphological characteristics in medicinal plant growing with them, yet they possess some special qualities or virtues that make them medicinally important. Herbs and herbal formulations for the treatment of ailments have continued to receive increased attention because of the strong belief that these products are safe since they belong to natural source [4]. New therapeutic agents are of great demand. Many

infectious diseases are known to be treated with herbal medicines throughout the human civilization. Today, plant materials continue to play roles in primary healthcare and higher plants have been shown to be potential source for the new antimicrobial agents [5]. Indigenous plants are reservoirs of various metabolites and provide a limitless source of important chemicals that have diverse biological properties [6]. Many therapeutic attributes of medicinal plants are traced to plant constituents and the medicinal actions of these constituents are unique to particular plant species or family. In particular, the antimicrobial activity of plant extracts has formed the basis of many applications including traditional medicine.

The use of medicinal plants in preventive (prophylactic) or curative (therapeutic) disease condition is not new. In view of this, more plants are being searched for their possible beneficial effects with the aim of bringing hope of adequate and affordable delivery to mankind [7]. It is in line with this that the present study was designed to investigate the potency of *Ficus platyphylla D.* leaves extracts against some selected microorganisms.

MATERIALS AND METHODS

All chemicals and reagents used were of analytical grades.

Plant material and extraction

The leaves of matured *Ficus platyphylla D.* were collected on the campus of the University of Maiduguri, Borno, Nigeria, and were identified by a plant taxonomist of the Department of Biological Sciences, University of Maiduguri. The leaves of the plant were washed with distilled water and thoroughly shade-dried for a week. The dried leaves were then pounded to fine powder using pestle and mortar. The powder weighed 200g, was stored in a clean dry container.

Aqueous extraction

One hundred (100) gram of the powdered leaves stored was dissolved in 500ml of water. The suspended solution was left to stand at room temperature for 5hours. The solution therefore, was filtered using Whatman #1 filter paper and the residue discarded. The dark-green extract yielded was measured [8,9] and stored in an air-tight container for further analysis.

Ethanolic extraction

Ethanolic extraction was carried out by dissolving 100g of the powdered leaves in 500ml of ethanol. The suspended solution was left to stand at room temperature for 5hours. The solution therefore was filtered using Whatman filter paper and the residue discarded. The filtrate was concentrated by direct exposure to air. The dark green extract yielded was weighed [8,9] and stored in an air-tight container for further analysis.

ANTIMICROBIAL SENSITIVITY TESTS

Preparation of inocula

Clinical pure isolates of *Klebsiella pneumoniae, Escherichia coli, Salmonella typhy, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Candida albican* were obtained from the Department of Veterinary Medicine Research Laboratory, University of Maiduguri, Borno State. Each inocula was prepared by inoculating the stock culture that has been suspended in saline solution (0.9%) into a freshly prepared nutrient agar at 37°C for 24hours.

Preparations of discs

Three concentrations of the plant extract (400mg/ml, 200mg/ml, and 100mg/ml) were used. Discs of Whatman filter paper with a diameter of 6.00mm were impregnated with the different concentrations of the extracts. Commercially available antibiotic discs were used as control for comparison.

Disc diffusion

Disc diffusion was determined using the method of [10]. The disc of Whatman #1 filter paper impregnated with known volume and appropriate concentration of the extract were placed on plates of sensitivity test agar uniformly inoculated with the test organisms. Tetracycline was used as standard antibiotic. The media were incubated at 37°C for 24hours and the zones of inhibition were measured.

Agar well diffusion
The antimicrobial sensitivity test was carried out using the method described by [11]. The culture plates with the test organisms were allowed to solidify and punched with a sterile cork borer (6mm diameter) to make open wells. The open wells were filled with 0.05ml of the three different concentrations of the plant extract. The preparations were incubated for 37°C for 24hours. Subsequently, the plates were appropriately measured for zones of inhibition.

Determination of minimum inhibitory concentration (MIC)
The MIC was determined by the method of Ellof [12] as modified by [13]. For each bacterium, five test tubes were used each containing the culture medium, inoculum and extract. The concentrations were 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.3 mg/ml, and 6.2mg/ml. The test tubes were covered and incubated overnight at 37°C for 24hours. Inhibition of bacterial growth was adjusted by turbidity of the medium. The MIC was defined as the lowest concentration at which no visible turbidity was observed.

Determination of minimum bactericidal concentration (MBC)
Determination of the MBC was done as described by [11]. The MBC was defined as the lowest concentration of the lowest of the extract at which 99.9% of the bacterial populations were killed after 24hours of incubation at 37°C. MBC was carried out by sub-culturing the growth in the test tube into another plate and incubated at 37°C for 24hours. From the tube showing no visible sign of growth in MIC determination, 0.1ml was inoculated into a sterile nutrient agar and then incubated at37°C for 24hours. The least concentration that did not show growth of test organism was considered as the MBC.

RESULTS

Table 1 is the result for sensitivity of test organisms to the aqueous leaves extract at different concentrations. From the table all the organisms were resistant to the aqueous leaves extract, using both the paper disc and agar well diffusion methods.

Table 2 and 3 are the results of the antimicrobial sensitivity test of the ethanolic leaves extract of *F. platyphylla* on the test organisms using disc method (table 2) and agar well method (table 3) respectively, which had varying degrees of antimicrobial activity against three organisms: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, while *E. coli*, *Salmonella typhy*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Candida albican* were resistant to the plant extract at all concentrations used. The highest antimicrobial sensitivity was observed against *P. aeruginosa* with diameter zone of inhibition of 25mm and 30mm at 100mg/ml concentration in table 2 and table 3 respectively. The lowest activity was observed in *K. pneumoniae* with the diameter zone of inhibition 8mm and 11mm at 100mg/ml in table 2 and table 3 respectively.

From table 4, the minimum inhibitory concentrations (MIC) in disc diffusion method showed that at concentrations of 12.5mg/ml, 25mg/ml and 100mg/ml can inhibit *P. aeruginosa*, *B. subtilis*, and *K. pneumoniae* respectively, while the MIC in well diffusion method, it showed that at concentrations of 12.5mg/ml, 50mg/ml, and ≤6.3mg/ml the extract can inhibit the growth of *B. subtilis*, *K. pneumoniae*, and *P. aeruginosa* respectively.

From table 5, the minimum bactericidal concentrations (MBC) of 12.5mg/ml, 50mg/ml, and 100mg/ml of the plant extract is bactericidal to *P. aeruginosa*, *B. subtilis*, and *K. pneumoniae* respectively in disc method. While the MBC of concentrations 12.5mg/ml, 50mg/ml and 100mg/ml of the ethanolic leaves extract showed that the extract was bactericidal to *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae* respectively in well diffusion method.

TABLE 1. Sensitivity of test organisms to the aqueous leaves extract at different concentrations using disc and agar well method

PLANT EXTRACT CONCENTRATION (mg/ml)	DIAMETER ZONES OF INHIBITION (mm)							
	ECO	SAT	KLP	PSD	STP	STR	BAS	CAN
400	R	R	R	R	R	R	R	R
200	R	R	R	R	R	R	R	R
100	R	R	R	R	R	R	R	R
Tetracycline (control)	21	23	19	22	18	20	26	20

KEY: R=resistant, ECO= Escherichia coli, SAT= Salmonella typhi, KLP= Klebsiella pneumoniae, PSD= Pseudomonas aeruginosa, STP= Staphylococcus aureus, STR= Streptococcus pyogenes, BAS= Bacillus subtilis, CAN= Candida albican

TABLE 2. Sensitivity of test organisms to the aqueous leaves extract at different concentrations using disc diffusion method

PLANT EXTRACT CONCENTRATION (mg/ml)	DIAMETER ZONES OF INHIBITION (mm)							
	ECO	SAT	KLP	PSD	STP	STR	BAS	CAN
400	R	R	13	35	R	R	14	R
200	R	R	11	29	R	R	12	R
100	R	R	8	25	R	R	10	R
Tetracycline (control)	21	23	19	22	18	20	26	20

Key: R= resistant, ECO= Escherichia coli, SAT= Salmonella typhi, KLP= Klebsiella pneumoniae, PSD= Pseudomonas aeruginosa, STP= Staphylococcus aureus, STR= Streptococcus pyogenes, BAS= Bacillus subtilis, CAN= Candida albican

Table 3. Sensitivity of test organisms to the ethanolic leaves extract at different concentrations using agar well method

PLANT EXTRACT CONCENTRATION (mg/ml)	DIAMETER ZONES OF INHIBITION (mm)							
	ECO	SAT	KLP	PSD	STP	STR	BAS	CAN
400	R	R	17	40	R	R	18	R
200	R	R	14	35	R	R	15	R
100	R	R	11	30	R	R	13	R

Key: R= resistant, ECO= Escherichia coli, SAT= Salmonella typhi, KLP= Klebsiella pneumoniae, PSD= Pseudomonas aeruginosa, STP= Staphylococcus aureus, STR= Streptococcus pyogenes, BAS= Bacillus subtilis, CAN= Candida albican

Table 4. Minimum inhibitory concentrations (MIC) for the test organisms which were sensitive to the plant extract

PLANT EXTRACT CONCENTRATION (mg/ml)	TEST ORGANISMS					
	DISC DIFFUSION			AGAR WELL DIFFUSION		
	KLP	PSD	BAS	KLP	PSD	BAS
100	*	-	-	-	-	-
50	+	-	-	*	-	-
25	+	-	*	+	-	-
12.5	+	*	+	+	-	*
6.25	+	+	+	+	-	+

*Key: += turbid, -= no turbidity, *= MIC, KLP= Klebsiella pneumoniae, PSD= Pseudomonas aeruginosa, , BAS= Bacillus subtilis.*

Table 5. Minimum bactericidal concentration (MBC) for the test organisms which were sensitive to the plant extract.

PLANT EXTRACT CONCENTRATION (mg/ml)	TEST ORGANISMS					
	DISC DIFFUSION			AGAR WELL DIFFUSION		
	KLP	PSD	BAS	KLP	PSD	BAS
100	*	-	-	-	-	-
50	+	-	*	*	-	-
25	+	-	+	+	*	-
12.5	+	*	+	+	+	*
6.25	+	+	+	+	+	+

*Key: += growth, -= no growth, *= MBC, Klebsiella pneumoniae, PSD= Pseudomonas aeruginosa, BAS= Bacillus subtilis.*

DISCUSSION

In this study, the antimicrobial activities of the aqueous and ethanolic leaves extract of ficus platyphylla, the results obtained from the aqueous extract sensitivity test (table 1) showed that all the organisms were resistant. Tables 2 and 3 showed that some of the test organisms: *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* were sensitive to the extract, but the rest of the organisms were resistant. This shows that ethanol was a better solvent for extracting antimicrobial substance(s) from the leaves of *F. platyphylla*. The result obtained in this study partially agrees with the results obtained by [14], who reported that the tested organisms developed resistance against aqueous extract of F. *platyphylla* leaves. According to [15], there is need to employ a broad range of extractive solvents in the extraction of possible phytochemicals of medicinal plants.

Tables 2 and 3 showed that the sensitivity of microorganisms increases with increase in concentration of the plant extract. Using the paper disc method, the sensitivity of the plant extract against *Klebsiella pneumonia* at concentrations of 100mg/ml, 200mg/ml, and 400mg/ml produced 8mm, 11mm, and 13mm diameter zones of inhibition respectively. The same thing applied to *Pseudomonas aeruginosa* and *Bacillus subtilis* in both the disc and agar well diffusion methods.

At concentration of 100mg/ml, the zone of inhibition was 11mm, 30mm and 13mm of the ethanolic leaves extract, in well diffusion method, against *K. pneumoniae*, *P. aeruginosa* and *B. subtilis* respectively, as against 8mm, 25mm, and 10mm for the paper disc diffusion method against the respective organisms. According to [16], agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms. Paper disc may act as a barrier between the extract and the organisms thus; preventing total diffusion of active compounds absorbed by the discs into the medium and may be responsible for the observed differences.

In paper disc method of sensitivity test (table 2), the lowest concentration of the plant extract (100mg/ml) was observed to have higher zone of inhibition (25mm) then the standard control (22mm). The result showed that the plant extract had more antimicrobial sensitivity against *P. aeruginosa* than the control used. However, certain studies have indicated that certain bioflavonoids have inhibitory activities against human pathogens [17] and other components that act in similar way as control. This may be the reason for some fractions having more inhibitory effect than the control. [14] reported the presence of saponins, tannins, volatile oils and phenols in phytochemical screening of aqueous and ethanolic leaves extract of *F. platyphylla*. The phytochemical analysis of *F. platyphylla* leaves revealed the presence of secondary metabolites such as tannins, anthraquinones, flavonoids, saponins, and alkaloids [18], which have been previously reported for their antimicrobial activities [19]. Mixtures of such chemicals show a broad spectrum of biological effects and pharmacological properties. To a large extent, the morphological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and method of extraction are possible sources of variation of chemical composition, toxicity and bioactivity of the extract [20].

The minimum inhibitory concentration (MIC) showed that low concentrations of 12.5mg/ml and 25mg/mll of the plant extract, in disc method, can inhibit the growth of *P. aeruginosa* and *B. subtilis* respectively as described by Ellof, (1998) and (NCCLS, 2001), and at as high concentration as 100mg/ml, *K. pneumoniae* can be inhibited by the plant extract. The MIC showed that the concentration of ≤6.3mg/ml and 12.5mg/ml of the extract, in well diffusion method, can inhibit the growth of *P. aeruginosa* and *B. subtilis* respectively, but *K. pneomoniae* can be inhibited at concentrations of 50mg/ml.

The minimum bactericidal concentration (MBC) of concentrations of 12.5 mg/ml, 50 mg/ml, and 100mg/ml of the plant extract showed that the plant extract, in disc method, can destroy (is bactericidal to) *K. pneumoniae*, *P. aeruginosa*, and *B. subtilis* at the respective concentrations, while the MBC of 12.5, 25, and 50mg/ml of the plant extract, in well diffusion method, can destroy *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae* at the respective concentrations.

CONCLUSION

As the current study revealed that all the tested organisms were resistant to the aqueous leaves extract, while some organisms were sensitive to the ethanolic leaves extract. Therefore, ethanolic leaves extract of *Ficus platyphylla* may contain certain antimicrobial agent(s) and could be a candidate for drugs development and validate the folkloric claim, as a cure for some of human ailments.

REFERENCES

[1] Strivastava, J., Lambert, J., and Vietmeter, N, *World Bank Technical paper*, **1996,** No. 320
[2] Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarta, D.D., and Eno, Z., *WHO Bulletin*, **1985**, 63(6): 965-981
[3] Ayoola, P.E. and Adeyeye A., *IJRRS*, **2010**, 5; 325-328
[4] Farnsworth, N.R., and Soejarto, D.D., Global importance of medicinal Plants, **1991**. In: Akerele, O., Heywood, V., Synge, H., (Eds.), The Conservation of Medicinal Plants, Cambridge Press, Cambridge, pp. 25-52.
[5] Mitscher, L.A., Drake, S., Gollapudi, S.R., and Okwute, S.K., *J. Nat. Prod.* **1988**, 50, 1025-1035
[6] Cowan, M.M., *Clinical Microbiol. Rev.*, **1999**, 12(4): 564-582
[7] World Health Organization, The Selection and the Use of Traditional Remedies in Primary Health Care, Geneva, **1986**, *WHO Document* No. TRN/86.1
[8] Brain, K.R. and Turner, T.D.Extraction Procedures in Practical Evaluation of Phytochemicals, *Weight Scentechnical*, Bristol, UK., **1975**, Pp 98
[9] Sofowora, A., Standardization of Herbal Medicine: Medicinal Plants and Traditional Medicine in Africa, *Spectrum Books Limited*, Lagos, Nigeria, **1993,** Pp 55-61

[10] Murray, P.K., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., Manual of Clinical Microbiology, *ASM*, Washington DC**, 1995**, 6th edition.
[11] Adeshina, G.O., Okeke, C.E., Osuagwu, N.O., and Ehinmidu, J.O., *African Journal of Microbiology*, **2010**, 4(8): 598-601
[12] Ellof, J.N., *Plant Medica*, **1998,** 64: 711-713
[13] NCCLS, Performance Standard for Antimicrobial Susceptibility Testing, Eleventh International Support, Weyne, PA, USA, **2001,** Document M100-S11.
[14] Kumbarawa, D., Khan M.E., Punah, A.M., and Hassan, M., *Pacific Journal of Science and Technology*, **2009**, 10(1): 382-386
[15] Takazawa, H., Tijima, F. and Miyashifa, C., *Yakugaku Zassi (Japanese)*, **1982**, 102: 489-491
[16] Omenka, C.A. and Osuoha, J.O.. *Nigerian Journal of Microbiology*, **2000,** 14(2): 39-42
[17] Linn, Y.M., Flavin, M.T., Cassidy, C.S., Mar, A., and Chen, F.C., *Bioorg. Med. Chem Lett*., **2001,** 11(16): 2101-4
[18] Adeshina, G.O., Okeke, C.E., Osuagwu, N.O., and Ehinmidu, J. O., *International Journal of Boil. Chem. Sci*., **2010**, 3(5): 1013-1020
[19] Amos, S., Binda, L., Chindo, B., Akah, P., Abdulrahman, M., Danmallam, H.U., Wambebe, C., and Gamaniel, K., *Indian Journal of Experimental Biology*, **2001**, 39(1): 63-67
[20] Felix M.T. Medical Microbiology. *Churchill Livingstone Publishers*, London, UK, **1982**, pp 445-459