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Antimicrobial Activity of Leaf Extracts and Fractions of *Ficus vogelii* and *Ficus mucuso* on Urinary Tract Isolates

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The increase in the worldwide prevalence and resistance of organisms causing urinary tract infections (UTIs) to the conventional antibiotics used in treatment has led to sub-therapeutic outcome, hence, the need to discover novel antibiotics from natural products. *Ficus mucuso* Welw. and *Ficus vogelii* Miq. have been used in ethnomedicine for the treatment of diseases of microbial origin.

Objective: To investigate the antimicrobial activity of *Ficus vogelii* and *Ficus mucuso* leaf extracts and fractions against UTI clinical isolates.

Materials and Method: The pulverized leaves of plants were screened for secondary metabolites. Crude extracts were obtained by maceration with methanol and partitioned into methanol, ethyl acetate and n- Hexane. Antibiogram profile of clinical isolates; *Escherichia coli*, *Proteus vulgaris*, *Serratia marcescens*, *Salmonella* Typhi , *Pseudomonas aeruginosa*, *Providencia stuartii*, *Klebsiella pneumoniae* and *Staphylococcus aureus* was determined using disc diffusion method. Antimicrobial activities of the leaf extracts and fractions of both plants were screened by agar-well diffusion. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by micro-dilution broth assay.

Results: The phytochemical screening revealed the presence of terpenoids, steroids, anthraquinones, alkaloids, tannins, flavonoids, saponin. The antibiogram profile showed that 87.5% of the isolates were multidrug resistant. Extracts and fractions showed appreciable inhibitory zones on most of the test organisms. MIC ranged from 6.25 - 25 mg/mL while MBC ranged from 50 - >100 mg/mL with ethyl acetate fraction of *Ficus mucuso* having the highest bactericidal activity against the isolates.

Conclusions: Extracts of *Ficus mucuso and Ficus vogelii* possess antimicrobial constituents which could be useful in the treatment of UTIs.

Keywords: *Ficus mucuso, Ficus vogelii,* Urinary tract infection, Minimum inhibitory concentration, Minimum bactericidal concentration

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in the world. They are mainly caused by Gram negative organisms. UTIs occur mostly in females due to the anatomical structure of their reproductive organs (Vasudevan, 2014). The infections can be treated with use of various antibiotics. However, in recent times, the organisms causing UTI have developed high degree of resistance to these antibiotics and this has led to increase in prevalence of UTI. The high resistance rate makes it difficult to control and treat UTIs effectively therefore necessitating the need to explore

medicinal plants to obtain novel drugs which are efficacious for the effective treatment of UTI (Sudeshna, *et al*., 2017).

Medicinal plants are plants whose organs contain active ingredients that have beneficial pharmacological effects in the body. They are used to cure or prevent diseases. The active ingredients present in medicinal plants which serve as therapeutic agents are used in the manufacture of drugs or pharmaceutical agents (Kutuma, *et al.,* 2018).

The plant *Ficus vogelii* family Moraceae is commonly known as West African Rubber tree. It is also known as *Ficus lutea*. It belongs to the fig family. It is located mostly in the Guinea Savannah vegetation belt of west and central Africa (Igile, et al., 2015). *Ficus vogelii* is traditionally used in treatment of malaria, seizure, boils, sores, rabies, and mental disorders (Awad, *et.al.,* 2015; Uchewa, *et.al.,* 2017,). The leaf extracts are used in the treatment of anorexia, anaemia, diabetes, and cardiovascular disease such as hypertension (Igile, *et al.,* 2015).

Ficus mucuso family Moraceae is also a member of the fig family. It occurs mainly in mountain areas and the tropical rain forests. It is found in China, Uganda, Zimbabwe, Cameroon, India, Nigeria etc. (Atasi, *et.al.,* 2019). It has copious seeds and voluminous trees. It is a semi evergreen spreading savannah tree with greenish flowers. *Ficus mucuso* is eaten as food by apes, and humans due to its high nutritive content (Ayoka, *et.al.,* 2014). It is used traditionally to treat mental illness, diarrhea, generalized edema, leprosy and also used as an antimicrobial agent especially in respiratory diseases and urinary infections. The bark and leaf decoction is used to treat dysmenorrheal (Oguntoye, *et al*., 2016). In Fongo Tongo, Cameroon, *Ficus mucuso* mixed with palm oil is used in treatment of epilepsy. Also, the decoction of the stem bark is used in thetreatment of jaundice (Bankeu, *et al*., 2010).

From published literature, there is no report on the antimicrobial activity of *Ficus vogelii* on uropathogens and none on fractions of both plants on uropathogens. Therefore, this study aimed at investigating the antimicrobial activities of *Ficus mucuso* and *Ficus vogelii* against clinical isolates from UTI, thus validating its use in ethnomedicine.

METHODOLOGY

Plant Collection and Preparation

The fresh leaves *Ficus mucuso* and *Ficus vogelii* were collected at the Botanical Garden, University of Ibadan in June, 2018. The plants were identified and authenticated at Department of Botany, University of Ibadan and Forestry Research Institute of Nigeria (FRIN) respectively where the plant samples were deposited at herbarium with voucher numbers UIH-22830 for *Ficus mucuso* and FHI-112110 for *Ficus vogelii*. The plant samples were air-dried, pulverized, weighed and stored appropriately for analysis.

Phytochemical analysis

Qualitative phytochemical screening was carried out using standard chemical procedures (Sofowora, 2012). The dried pulverized plant was used to screen for secondary metabolites.

Plant extraction and fractionation

Cold maceration method was used in the extraction of the powdered plants. To obtain crude methanol extract, 1.3 g and 1 g of the powdered plants of *Ficus mucuso and Ficus vogelii* were transferred into glass containers respectively. Five litres (5L) of methanol was transferred into each glass container. The mixture was stirred at intervals of 2 hours and allowed to stay for 72 hours after which the solvents containing the extracts were collected using muslin bag. The above process was repeated by adding pure methanol solvent into the shaft collected. The extract obtained from each plant was further filtered using Whatmans filter paper (1 mm). The filtrate was concentrated using rotary evaporator set at 40°C. The crude methanol extract was further concentrated using a vacuum oven set at 40°C with a pressure of 700 mmHg. To obtain the methanol, ethyl acetate and hexane fractions of the crude extracts, 10.133 g and 5.485 g of the crude methanol extract of *Ficus mucuso and Ficus vogelii* were mixed with 100 mL of methanol each and stirred thoroughly.

Extraction by partitioning was carried out using the method described by Abu *et al*., (2017) with slight modification in the solvents, using methanol, hexane and ethyl acetate.

The fractions were concentrated using rotary evaporator set at 40°C. Further concentration was achieved using a vacuum oven set at 40°C with a

pressure of 700 mmHg. The fractions were weighed and the percentage yield calculated.

Microorganisms

Fifteen strains of clinical bacterial isolates from patients with UTI were obtained from Medical Microbiology Department, University College Hospital, Ibadan. The organisms which include *Escherichia coli* (3), *Pseudomonas aeruginosa* (3), *Providencia stuartii* (3), *Klebsiella pneumoniae* (2), *Proteus vulgaris* (1), *Serratia marcescens* (1), *Salmonella* Typhi (1), *Staphylococcus aureus* (1) were used for the study. The isolates were screened for purity and further confirmed by biochemical tests. The isolates were inoculated on nutrient agar slants and stored at 4°C prior to use.

ANTIMICROBIAL SCREENING

Determination of antibiogram profile of microbial isolates

The disc diffusion technique (CLSI 2018) was used to determine the susceptibility of the isolates to standard antibiotics. Overnight broth cultures of test isolates were inoculated into nutrient broth and dilutions corresponding to McFarland standards were used to inoculate the surface of sterile Mueller Hinton agar plates using sterile cotton tipped applicators. The seeded plates were allowed to dry for about 30 minutes after which the antibiotic multi-disc (Abtek Biologics Ltd) was firmly placed on the surface of the inoculated agar using a pair of sterile forceps. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured and the interpretation was done according to the Clinical Laboratory Standards Institute (CLSI) 2018 edition.

The antibiotic disc for Gram negative organisms contained Ciprofloxacin 5 µg, Ofloxacin 5 µg, Nitrofurantoin 300 µg, Cefuroxime 30 µg, Gentamicin 10 µg, Augmentin 30 µg, Ceftazidine 30 µg, Cefixime 5 µg while the disc for Gram positive organisms contained Ceftazidine 30 µg, Cefuroxime 30 µg, Gentamicin 10 µg, Ceftriaxone 30 µg, Erythromycin 5 µg, Cloxacillin 5 µg, Ofloxacin 5 µg and Augmentin 30 µg.

Antimicroial screening of plant extracts and fractions.

The organisms (0.5 McFarland standard equivalent suspensions) were inoculated into the agar, equidistant holes were bored using standard sterile 8mm cork borer and 0.1mL of different concentrations of the plant extracts and fractions were placed into the respective wells. Ciprofloxacin (5 µg/mL) was used as the standard drug control while 10% Dimethyl sulphoxide (DMSO) was used as negative control. The plates were left for about an hour to allow for the diffusion of plant extracts into the agar medium after which the plates were incubated at 37°C for 24 hours. The experiment was done in duplicates.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts

Micro-dilution broth method as described by Kuete *et al.* (2008) was used to determine the MIC of the extracts and fractions. The crude extracts and fractions were dissolved in DMSO and a stock concentration of 100 mg/mL was prepared. A serial two-fold dilution was made to obtain concentrations which ranged from 3.125 mg/mL to 100 mg/mL. Subsequently, 100 µL of each concentration was put into the wells of the microtitre plates which contained 95 µL of Mueller Hinton Broth (MHB) and 5 µL of inoculum (McFarland standard equivalent suspensions). Wells which contained 195 µL of MHB and 5 µL of inoculum (served as the negative control while ciprofloxacin (5 µg/mL) served as the positive drug control.

The plates were covered with a sterile plate sealer and the contents of the wells agitated with a shaker for even mixture and the plates incubated at 37°C for 24 hours. The MIC was obtained by the addition of 40 µL of 0.2 mg/mL of *p*-iodonitrotetrazoliumchloride to contents of the wells and incubated at 37°C for 30 minutes. A color change to pink indicated the presence of viable bacteria cells. The lowest concentration in which there was no growth was taken as the MIC.

A loop full of the inoculum from the wells showing no visible growth was inoculated on Mueller Hinton Agar and incubated at 37°C for 24 hours. The lowest concentration showing no growth was taken as MBC.

RESULTS AND DISCUSSION

The phytochemical screening (Table 1) confirmed the presence of secondary metabolites such as terpenoids, steroids, anthraquinones, alkaloids, tannins, flavonoids, saponin in *Ficus mucuso* which is similar to the results gotten by Oguntoye, *et al.*, (2016). *Ficus vogelii* contained terpenoids, steroids, anthraquinones, alkaloids, tannins and flavonoids which is also similar to the research carried out by Uchewa, *et al.,* (2017). Secondary metabolites such as alkaloids, triterpenes, sterols, flavonoids and saponins have been reported to have antimicrobial activities (Tkachenko, *et al.*, 2017).

The antimicrobial activity of tannins could be as a result of its ability to inhibit protein transfer enzymes (transferase) which is present in the cell membrane , or direct action of tannins on the microorganism metabolism, or through inhibition of oxidative phosphorylation, inhibition of enzyme activity by complexation with substrates of bacteria, a mechanism involving the complexion of tannins with metabolic ions, decreasing the availability of essential ions to the metabolism of the microorganisms (Wafa, *et al.*, 2016).

Table 1: Phytochemical screening of the powdered leaves of *Ficus mucuso* and *Ficus vogelii*

Metabolites tested	Observation	
	Ficus mucuso	*Ficus vogelii*
Terpenoids	+	+
Steroids	+	+
Anthraquinones	+	++
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Saponin	+	-
Cardiac glycosides	-	-

KEY:++ = abundance of metabolites; + = metabolites is sparingly present or requires warming; - = absence of metabolite

Saponins possess detergent like properties that might increase the permeability of the bacterial cells without destroying them. This action might facilitate the entry of antimicrobial agents into the bacterial cells and enhance antimicrobial activity of the agents (Arabski, *et al.,* 2012).Therefore, it can be inferred that secondary metabolites presentin these plants could be responsible for the antimicrobial activities discovered in the course of the study.

The difference between the phytochemical screening results of both plants is that presence of anthraquinones was more abundant in *Ficus vogelii* than in *Ficus mucuso.* Also saponin was absent in *Ficus vogelii* but present in *Ficus mucuso* and this could account for the higher antimicrobial effects of *Ficus mucuso* crude extract and fractions against the clinical isolates compared to *Ficus vogelii* crude extract and fractions. Phytochemicals have been reported in various *Ficus* species (Dangarembizi *et al*., 2013; Coker and Oaikhena, 2020).

The antibiogram profile (Table 2) of the clinical isolates showed that all of the clinical isolates except *Salmonella typhi* are multidrug resistant organisms because the isolates were resistant to three or more classes of antibiotics. However, majority of the organisms were susceptible to ciprofloxacin (control drug) except *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae*. The only Gram positive organism (*Staphylococcus aureus*) used for the study was resistant to all the antibiotics except ofloxacin which is in the same class as ciprofloxacin.

Table 2: Antibiogram profile of Gram negative isolates

Isolates	CPR	NIT	GEN	CRX	OFL	CAZ	CXM	AUG
E.c1	R	S	R	R	R	R	R	R
E.c2	R	S	R	R	R	R	R	R
E.c3	R	R	R	R	R	R	R	R
P.a1	S	R	S	R	S	R	R	R
P.a2	S	R	R	R	S	R	R	R
P.a3	S	R	S	R	S	R	R	R
P.s1	S	R	R	R	S	R	R	R
P.s2	I	R	S	R	S	R	R	R
P.s3	S	R	R	S	S	R	R	R
K.p1	R	S	R	R	R	R	R	R
K.p2	R	S	R	R	I	R	R	R
P.v	R	S	R	R	R	R	R	R
S.m	S	R	S	R	S	R	R	R
S.t	S	S	S	R	S	R	R	R

CAZ – Ceftazidime, CRX – Cefuroxime, GEN – Gentamicin, CXM – Cefixime, OFL – Ofloxacin, AUG – Augmentin, NIT – Nitrofurantoin, CPR – Ciprofloxacin, *E.c = Escherichia coli, P.v =Proteus vulgaris, S.m =Serratia marcescens, S.t =Salmonella* Typhi, *P.a = Pseudomonas aeruginosa, P.s =Providencia stuartii, K.p =Klebsiella pneumoniae*, R = Resistant, S = Susceptible, I = Intermediate

From the results of the antimicrobial screening of the crude extracts and fractions of the plants against uropathogens (Tables 3 and 4), it was observed that the various extracts and fractions had varying antibacterial activity on the clinical isolates with methanol fractions of both plants showing the highest antibacterial activity with the highest zones of inhibition. This is similar to the research carried out by Oguntoye, *et al.,* (2016) on crude extracts of *Ficus mucuso*. Hexane fractions of *Ficus mucuso* and *Ficus vogelii* showed the least activity and this could be due to inadequate penetration of the extracts into the agar inoculated with organisms. Also, *Proteus vulgaris* was not susceptible to the control drug, ciprofloxacin during the study but it was susceptible to the plant extracts. This result was confirmed by the MIC and MBC results.

Table 3: Antimicrobial screening of crude methanol extract and fractions of *Ficus vogelii*

Isolate	Methanol extract (mg/mL)			Hexane fraction (mg/mL)			Ethyl Acetate fraction (mg/mL)			Methanol fraction (mg/mL)			CIP (µg/mL)
	100	50	25	100	50	25	100	50	25	100	50	25	5
	Zones of Inhibition (mm)												
E.c1	12	12	10	12	12	11	14	12	12	16	14	12	12
E.c2	14	12	10	11	NZ	NZ	10	10	10	14	13	13	12
E.c3	12	12	12	11	10	09	11	11	10	16	14	10	15
P.a1	14	12	12	12	10	10	10	10	NZ	14	12	12	14
P.a2	14	10	NZ	11	10	10	11	11	10	12	10	10	28
P.a3	12	10	NZ	11	11	10	14	12	11	15	14	12	25
P.s1	14	12	NZ	12	12	11	NZ	NZ	NZ	12	12	10	17
P.s2	14	12	10	11	11	11	12	11	11	13	12	11	22
P.s3	12	10	10	12	11	11	11	11	10	15	14	13	17
K.p1	NZ	NZ	NZ	13	12	11	12	11	NZ	16	14	12	14
K.p2	NZ	NZ	NZ	12	12	11	14	14	14	14	13	12	12
P.v	14	12	12	14	11	11	11	11	10	15	13	11	NZ
S.m	12	10	10	11	11	10	11	11	10	15	14	10	20
S.t	NZ	NZ	NZ	NZ	NZ	NZ	12	11	11	14	12	12	20
S.a	NZ	NZ	NZ	NZ	NZ	NZ	12	NZ	NZ	15	14	13	27

Key: *E.c = Escherichia coli, P.v =Proteus vulgaris, S.m =Serratia marcescens S.t =Salmonella* Typhi , *P.a = Pseudomonas aeruginosa* , *S.a = Staphylococcus aureus* , *P.s =Providencia stuartii, K.p =Klebsiella pneumoniae,* NZ= No zone of growth inhibition, CIP=Ciprofloxacin

Table 4: Antimicrobial screening of crude methanol extract and fractions of *Ficus mucuso*

Isolate	Methanol extract (mg/mL)			Hexane Fraction (mg/mL)			Ethyl Acetate Fraction (mg/mL)			Methanol Fraction (mg/mL)			CIP (µg/mL)
	100	50	25	100	50	25	100	50	25	100	50	25	5
	Zone of Inhibition (mm)												
E.c1	12	12	10	11	10	NZ	14	12	10	15	13	12	12
E.c2	13	12	10	13	12	11	15	14	10	16	15	13	12
E.c3	12	12	10	15	14	12	15	12	11	15	13	10	15
P.a1	12	12	10	14	14	13	12	12	11	14	12	10	14
P.a2	12	10	10	NZ	NZ	NZ	10	10	NZ	16	12	12	28
P.a3	14	12	11	12	10	10	12	11	NZ	15	14	12	25
P.s1	12	12	10	11	11	10	10	NZ	NZ	16	14	14	17
P.s2	14	12	10	11	11	10	10	10	09	14	12	12	22
P.s3	13	12	10	11	10	10	NZ	NZ	NZ	15	14	10	17
K.p1	NZ	NZ	NZ	11	NZ	NZ	12	12	11	13	12	10	14
K.p2	12	10	10	10	10	10	14	12	11	14	14	12	12
P.v	12	10	10	12	12	10	14	11	10	15	13	13	NZ
S.m	14	12	10	11	11	10	12	11	10	16	14	12	20
S.t	12	12	10	11	10	09	NZ	NZ	NZ	14	12	12	20
S.a	14	14	12	11	NZ	NZ	12	12	11	14	12	10	27

Key: *E.c = Escherichia coli, P.v =Proteus vulgaris, S.m =Serratia marcescens S.t =Salmonella* Typhi , *P.a = Pseudomonas aeruginosa , S.a = Staphylococcus aureus , P.s =Providencia stuartii, K.p =Klebsiella, pneumoniae,* NZ= no zone of growth inhibition, CIP=Ciprofloxacin

The MIC values (Table 5) of both plants ranged between 6.25 - 25 mg/mL with hexane fraction of *Ficus mucuso* having the highest inhibitory activity against the test isolates. *Serratia marscencens* and *Salmonella* Typhi were most susceptible to the crude plant extract with MIC value of 6.25 mg/mL. The MBC values (Table 5) of both plants were between the ranges of 50 mg/mL - >100 mg/mL with ethyl acetate fraction of *Ficus mucuso* having the highest bactericidal activity against the clinical isolates.

Table 5: MIC and MBC of *Ficus mucuso and Ficus vogelii.*

Isolate	**Hexane Fraction**				**Ethyl Acetate Fraction**				**Methanol Fraction**			
	FV		FM		FV		FM		FV		FM	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
E.c	25	>100	12.5	100	25	100	25	100	25	>100	25	>100
P.a	25	>100	25	>100	25	100	25	50	25	100	25	100
P.s	25	>100	25	>100	25	100	25	50	25	50	25	>100
K.b	25	>100	25	100	25	100	25	50	25	>100	25	>100
P.v	25	>100	25	>100	25	100	25	50	25	50	25	>100
S.m	25	>100	12.5	100	25	100	25	100	25	100	25	100
S.t	25	>100	6.25	100	25	100	25	50	25	50	25	>100
S.a	25	>100	6.25	100	25	100	25	100	25	100	25	100

Key: *E.c = Escherichia coli , P.v =Proteus vulgaris, S.m =Serratia marcescens S.t =Salmonella* Typhi , *P.a = Pseudomonas aeruginosa , S.a = Staphylococcus aureus , P.s =Providencia stuartii, K.p =Klebsiella, pneumonia, F.v = Ficus mucuso, F.m = Ficus vogelii*, NZI= No zone of growth inhibition

CONCLUSION

In conclusion, the results of the extracts and fractions of *Ficus mucuso and Ficus vogelii* leaves provide the evidence that these plants have antibacterial activities against MDR isolates from UTI and therefore could be useful alternatives to currently existing drugs, which are used in the treatment of urinary tract

infections. Further studies should be carried out to isolate and characterize pure antimicrobial compounds from the plants.

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