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The Combination Effect of the Active Fractions of *Ficus mucuso* Welw. Ex Ficalho and Standard Antibiotics against Selected Uropathogens

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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: Urinary tract infections (UTIs) are a global health challenge, causing high mortality rates annually. With rising antimicrobial resistance, the search for effective alternatives has intensified. Medicinal plants serve as substitutes. *Ficus mucuso* Welw. Ex Fichalo has been purportedly used in ethnomedicine for the treatment of UTIs.
Objective: To evaluate the antibacterial effects of *Ficus mucuso* extract and fractions on UTI isolates and assess synergy with first-line antibiotics.
Methods: Pulverized leaves of *Ficus mucuso* were extracted with methanol by cold maceration. The crude extract was partitioned into n- hexane, ethyl acetate and methanol. Qualitative and quantitative phytochemical screening was carried out. Antioxidant activity of the plant was assessed. Antibiogram of clinical uropathogenic isolates and standard strains was determined using disc-diffusion method. The antimicrobial efficacy of plant extract was tested using agar-well diffusion. Bioassay-guided fractionation of the active fractions was by vacuum liquid chromatography. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined via microbroth dilution assay. Combination antimicrobial testing with ciprofloxacin and amoxicillin-clavulanate was performed using checkerboard assay. Brine-shrimp assay was used to assess cytotoxicity of the plant.
Results: The secondary metabolites detected; flavonoids (98.41mg/g), phenols (79.17mg/g) and tannins (27.26mg/g) were more abundant in the ethyl acetate fraction. The plant had appreciable antioxidant activity. Plant extracts and fractions had appreciable inhibitory activities against MDR bacteria with MIC and MBC ranges of 1.5625 to 12.5mg/mL 3.125 and 25mg/mL respectively. Combination testing indicated a synergistic effect with ciprofloxacin. Cytotoxicity assay yielded LC_{50} value of 31.30 µg/mL.
Conclusion: Leaf extracts of *Ficus mucuso* have antimicrobial potentials which demonstrated enhanced synergistic activity against UTI organisms when combined with standard chemotherapeutics.

Keywords: *Ficus mucuso*, Uropathogens, Cytotoxicity, Synergistic activity

INTRODUCTION

Urinary tract infections (UTIs) are among the most frequent infectious disorders worldwide, impacting 150 million people annually and carrying a high risk of morbidity and high medical costs. These infections can arise in the urethra (urethritis), bladder (cystitis), or kidneys (pyelonephritis). UTIs are inflammatory illnesses of the urinary system caused by aberrant pathogen development, are classified as either uncomplicated (uUTIs) or complicated (cUTIs) (Odoki *et al.*, 2019).
Some common risk factors for UTIs include the presence of a foreign body, such as a urinary catheter, disruption of normal urine flow due to blockage or retention and urethral manipulation. Bacterial entry

into the urinary system varies widely, with a few species responsible for most infections. The infection route is usually ascending from the urethra. (Walsh and Collyns, 2017).

Women are more vulnerable than men because their urethras are shorter, and pathogenic germs can spread from the rectum and perineum to the peri-urethral region. Very few simple UTIs are caused by blood-borne bacteria. Most UTIs are caused by *Escherichia coli*, then *Klebsiella pneumoniae,* however *Proteus, Enterobacter*, and *Enterococcus* are all significant species. (Yamaji *et al.,* 2018).

Historically, plants used for medicine have served as remedies to uphold human health and vitality (Palanichamy *et al.,* 2018) and many of these plants are crucial repositories of bioactive compounds utilized in the synthesis of pharmaceuticals for therapeutic applications (Chen *et al.,* 2016).They harbor numerous compounds of therapeutic and preventive significance, making them a traditional cornerstone in managing and preventing various ailments, from mild to severe medical conditions (Hussein and El-Anssary, 2019). These plants are often geographically limited and intertwined with localized indigenous knowledge regarding their applications. Notably, China and India stand out as the leading nations in terms of medicinal plant diversity, followed by Colombia, the United States, and South Africa (Chen *et al.,* 2016). Increasingly, people in developing countries rely on medicinal plants for primary healthcare (Jain *et al.,* 2019).

The *Ficus* genus is known to have phytochemical components, traditional applications, and modern pharmacological activities such as; antioxidant, cytotoxic, antibacterial, anti-inflammatory, antidiabetic, anti-ulcer, and anticonvulsant activities. *Ficus* species are known for their presence of a wide array of phytochemical constituents. For example; several monoterpenes were identified in *F. exasperata* by Oladosu *et al.,* 2009. It was reported that a diterpene (E)-phytol was isolated from *F. ulmifolia* Lam. Steroids, furocoumarins, pigments and alkaloids like piperine, methyl piperate, and piperloguminine were identified in *F. religiosa* (Salehi *et al.,* 2020)

Ficus mucuso commonly known as Wild fig in English and Odan afomo in Yoruba, belongs to the family Moraceae with about 150 species worldwide. It is a big tree that can get up to heights of 60 feet, and its trunk up to 18 inches in diameter. The stem is usually unbranched and has a smooth, grey bark. The edges of the leaves are entire while the flowers are small, in clusters and are either white or greenish-white in color without petals. Its fruit is fleshy, orange-red colour, and it is a drupe that is about an inch in diameter. (Atasi, *et al.,* 2019, Coker and Adeniyi-Aogo, 2021). Some species are used as foods and medicines in Cameroon and China because it has leaves and fruits which has a pleasing aroma.

The leaf and stem bark are known to be responsible for the ethnomedicinal activities of the plant including: the treatment of generalized edemas, leprosy, diarrhea and dysmenorrheal, urinary tract diseases and insanity (Oguntoye *et al.,* 2016).

Antibiotics are crucial in combating bacterial infections, but their efficacy is increasingly threatened due to the rise of antibiotic-resistant bacteria. This resistance has become a leading cause of treatment failure (Xie *et al.,* 2015). Despite over 10 classes of antibiotics targeting different bacterial functions, none have completely avoided resistance (Garvey *et al.,* 2011), leading to the emergence of multi-drug-resistant (MDR) pathogens.

A promising strategy to combat antibacterial resistance is combining antibiotics with medicinal plant extracts, which have intrinsic antibacterial properties. This combination can enhance or restore the effectiveness of antibiotics, especially against resistant strains. For instance, pairing β-lactams with plant-derived compounds like α-mangostin or quercetin significantly improves treatment efficacy (Sakagami *et al.,* 2005). Such synergistic effects can broaden the antimicrobial spectrum, reduce toxicity, and prevent the emergence of resistant bacteria.

Research increasingly focuses on medicinal plants containing active antimicrobial compounds, as these offer the potential to reduce antibiotic dosages and mitigate adverse effects. Synergy often results from targeting multiple bacterial mechanisms, complicating resistance development. Experimental methods like the checkerboard and isobologram techniques are commonly used to assess the combined effects of antibiotics and plant extracts. However, comprehensive evaluations of these methodologies, particularly in combination, remain limited (Ilanko and Cock, 2019).

This research was carried out to determine antibacterial activities of the crude extracts and fractions of *Ficus mucuso* on clinical isolates of urinary tract infection, and to determine the synergy between the most active fraction and some first-line antibiotics used in the treatment of urinary tract infections.

METHODOLOGY

Collection and preparation of plant samples

The leaves of *Ficus mucuso* was obtained from the Botanical Garden, University of Ibadan and authenticated at Botany Department, University of Ibadan, Ibadan with voucher number UIH-22830. The leaves were air-dried, pulverized and weighed.

Plant extraction

The plant was extracted with methanol, using the cold maceration method. This was done in a glass jar containing plant samples and distilled methanol which was occasionally agitated every two hours for 72 hours and filtered. Successive extraction was done using fresh methanol until all the constituents of the leaves were extracted. The combined filtrate was concentrated using a rotary evaporator at 40°C and air dried.

The concentrated crude methanolic extract was reconstituted into a solution by adding equal volume (200 mL) of methanol and distilled water. It was successively partitioned using solvents of varying polarity; hexane and ethyl acetate. The fractions, including; Hexane, ethyl acetate and aqueous methanol fractions were collected in containers and labeled. The partitioned extracts were concentrated using the rotary evaporator and air dried. The extracts were weighed and then stored in the refrigerator at 4°C for subsequent use.

Qualitative and quantitative phytochemical analysis

Qualitative screening for secondary metabolites of *F. mucuso* was done using standard procedures (Vinoth *et al*., 2012). Quantitative phytochemical analysis to determine total alkaloid, flavonoid, tannin, phenol and saponin contents was carried out using the method described by Krishnaiah *et al*, 2009.

Antioxidant Assay

The change in optical density of diphenyl1picrylhydrazyl (DPPH) radicals was monitored in order to obtain the antioxidant activity of the crude extracts (Adegbolagun *et al.,* 2018). The crude extracts of the plants were prepared in varying concentrations of 25, 50, 100, 200 and 400 µg/mL. One ml of the different concentrations were transferred into test tubes containing 1 mL 2,2-diphenyl1picrylhydrazyl (DPPH) solution (0.3 mM methanolic solution). It was further kept at room temperature for 30 minutes and the absorbance was determined at 517 nm. The process was repeated for methanol and ascorbic acid which served as the negative and positive controls respectively. The percentage of the DPPH radical scavenging was calculated using the given formula below:

% Inhibition of DPPH radical = ({Abr – Aar}/Abr) x 100

Where Abr = Absorbance before reaction i.e blank

Aar = Absorbance after reaction

Cytotoxicity assay

The test was carried out using hatched brine shrimps in natural sea water. The assay was done by adding 10 brine shrimps larvae into four labeled test tubes of varying concentrations of plant extract (1000 ppm, 100 ppm, 10 ppm and 1 ppm) and sea water to make up a total volume of 5 mL. The number of surviving larvae was counted after 24 hours. Potassium dichromate was used as positive control at 1 mg/mL, while the negative control was 5% Dimethyl sulfoxide (DMSO).

Microorganisms used

Different clinical strains of bacteria obtained from urine of patients were collected from the Medical Microbiology Department, University College Hospital (UCH) Ibadan, Nigeria. The isolates were sub-cultured on nutrient agar slants. Further biochemical tests were carried out to confirm the identity of the organisms prior to use for the antimicrobial assay. Organisms used for the study were; *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 700603.

Antibiotic susceptibility testing of microbial isolates

Dilutions corresponding to 0.5 McFarland equivalent standard of each isolate were prepared from overnight cultures of the clinical isolates. Sterile cotton tipped applicator was used to spread inoculum from each isolate on the surface of a separate Mueller Hinton agar plate. A pair of sterile forcep was used to place an antibiotic disc gently but firmly on the surface of the inoculated plates. The antibiotic discs used were: Amoxicillin/clauvulanic acid (AMC) 30 µg, Gentamicin (CN) 10 µg, Ciprofloxacin (CIP) 5 µg, Chloramphenicol (C) 30 µg, Cefotaxime (CTX) 5 µg, Ampicillin (AMP) 10 µg, Imipenem (IPM) 10 µg, Penicillin G (P) 10 µg, Ceftriaxone (CRO),

Levofloxacin (LBC), Cefuroxime (CXM), Azithromycin (AZN), Ofloxacin (OFX) (Oxoid). The plates were incubated at 37°C for 24 h. Zones of growth inhibition were measured and interpreted according to the standards of Clinical Laboratory Standards Institute (CLSI) 2021.

***In vitro* antimicrobial screening of plant extracts**

The antibacterial activity of the plant extracts was determined by agar-well diffusion method. A 0.5 McFarland standard equivalent suspension of each isolate was made in 0.85 % saline and 0.1 mL of resulting isolate suspension was used to inoculate the plates of Mueller Hinton Agar. Wells of equal distances were bored with the aid of a standard sterile 8mm cork borer and 100 µl of different concentrations of extracts and control were placed into the corresponding wells. Ciprofloxacin (5 µg) was used as the standard drug control. Diffusion of the extract was allowed by leaving the plates for about one hour at room temperature. The plates were then incubated at 37 °C for 24 h.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts using microbroth dilution method.

The MIC and MBC of the extracts and fractions were determined using microbroth dilution assay as described by Kuete *et al.,* 2008, with slight modification. The extracts and fractions were serially diluted in Tryptone Soy broth (TSB) to obtain concentrations ranging between 0.78125 - 100 mg/mL for the extracts and fractions. Each well contained 100 µl of each concentration, 90 µl of TSB and 10 µl of bacterial suspension adjusted to 0.5 McFarland standard. Wells containing 195 µl of TSB and 5µl of bacterial suspension served as growth control. Ciprofloxacin (10 µg/ml) served as the control drug. The plates were covered and incubated at 37℃ for 24 hours. After which about 50 µl of tetrazolium chloride salt (2 mg/ml) was added to each of the wells and re-incubated at 37℃ for 30 minutes. A purple coloration indicated the presence of viable bacteria. The lowest concentration that prevented this change was taken as the MIC. To determine the MBC, wells showing no visible growth were streaked aseptically on Mueller Hinton Agar plate using a sterile loop and incubated at 37℃ for 24 hours. The lowest concentration showing no bacterial growth was taken as the MBC.

Vacuum Layer Chromatography (VLC)

The methanol leaf extract was subjected to fractionation using VLC with solvents of varying polarities. Seven grams of *Ficus mucuso* was adsorbed with silica gel (60-200 mesh size) and 200 mL of different ratios of solvents ((hexane 100, hexane/ethyl acetate 80:20, 60:40, 40:60, 20:80; ethyl acetate 100, ethyl acetate/ methanol 80:20, 60:40, 40:60, 20:80 and methanol 100) was used for elution. The eluents were collected in clean tubes. Fractions were spotted on thin layer chromatographic (TLC) plates and developed with mobile phases of Dichloromethane/ethyl acetate ratio (4:1). The fractions were pooled together based on their TLC profiles. The pooled fractions were concentrated to dryness and weighed. The antimicrobial assay was carried out using agar-well diffusion technique as previously described for the crude extracts.

Combination testing using checkerboard assay

Checker board synergy testing was carried out as described by Cha *et al.,* 2009. Checkerboard synergy was performed by micro-dilution method in microtitre plates with Mueller Hinton broth. Fifty microliters each of the combinations of the most active fraction and antibiotics (ciprofloxacin and amoxicillin-clavulanate) were tested at different concentration (0.781 – 25 mg/mL for extracts and 0.125 – 10 µg/mL for ciprofloxacin and 0.938 – 30 µg/mL for amoxicillin-clavulanate). Each well contained unique combination of plant fraction/antibiotic concentration. Wells were inoculated with 5 µl of bacterial suspension adjusted to 0.5 McFarland standard. The microtitre plates were incubated at 37℃ for 24 hours. After 24 hours of incubation, 50 µl of tetrazolium dye (2 mg/ml) was added to each of the wells and re-incubated at 37 ℃ for 30 minutes. A pink coloration indicated the presence of viable bacteria. The MIC was defined as the lowest concentration of antimicrobial agents in combination at which visible growth was inhibited. The fractional inhibitory concentration (FIC) index was calculated using the formula shown below, which is an indication of the combination activity:

FIC index = (MIC of extract in combination / MIC of extract alone) + (MIC of control drug in combination / MIC of control drug alone).

The FICI values were interpreted as follows:

≤ 0.5= synergistic; > 0.5-1.0 = additive; >1.0-4.0 = indifferent (non-interactive); > 4.0 = antagonistic (Van Vuuren and Viljoen, 2011)

RESULT

Phytochemicals detected were saponins, alkaloids, tannins, coumarins, anthocyanins, terpenoids, phenols, anthraquinones, flavonoids, and steroids (Tables 1 and 2).

Table 1: Results of phytochemical analysis of *Ficus mucuso*

Parameters	Hexane	Ethylacetate	Methanol
Saponin	-	++	+
Alkaloid	-	+	+
Flavonoid	-	+	+
Tannin	-	+	+
Coumarin	-	+	+
Steroid	+	++	+
Terpenoid	+	+	+
Cardiac Glycosides	+	+	+
Quinones	+	+	+
Anthocyanin	+	-	-
Anthraquinone	+	+	+
Phenols	+	+	+

Key: Absence of metabolite, +: presence of metabolite, ++: high concentration of metabolite

Table 2: Results of quantitative phytochemical analysis of *Ficus mucuso*

Parameters	Ethylacetate fraction	Methanol fraction
Saponin (%w/v)	5.65 ± 0.21	2.68 ± 0.11
Alkaloids (%w/v)	14.81 ± 0.11	9.47 ± 0.19
mg/g Flavonoid (QE)	98.41 ± 0.13	66.05 ± 0.17
mg/g Phenol (GAE)	79.17 ± 0.23	37.14 ± 0.33
mg/g Tannin (GAE)	27.26 ± 0.18	30.76 ± 0.33

Key: GAE- Gallic acid equivalent, QE- Quercetin equivalent, SD- Standard deviation

Antibiogram results revealed that most of the test isolates employed in this study were multi-drug resistant, being resistant to more than two classes of antibiotics (Magiorakos *et al*., 2012) as shown in Tables 3.

The zone of growth inhibition results in Table 4 showed that the plant extracts and fractions had appreciable inhibitory activities against the antibiotic-resistant bacteria.

Table 3: Antibiogram profile of urinary tract clinical isolates and Type strains

Isolates Antibiotics (µg)	*P. a* 1	*P. a* 2	*P. a* 3	*P. a* ATCC 27853	*E.co* 1	*E.co* 2	*E.co* 3	*E.co* ATCC 25922	*K. p* 1	*K. p* 2	*K. p* 3	*K.p* ATCC 700603
Cefuroxime	R	R	R	R	R	R	R	R	R	R	R	R
Ceftriaxone	R	R	R	I	R	R	R	R	R	R	R	R
Gentamicin	S	I	S	S	S	S	S	S	R	R	R	S
Ciprofloxacin	S	S	S	S	I	S	S	S	S	S	R	S
Ofloxacin	S	S	S	S	S	S	S	S	S	S	S	S
Imipinem	S	R	R	R	R	R	R	R	R	R	R	R
Azithromycin	S	S	S	R	S	S	S	S	S	S	S	S
Levofloxacin	S	S	S	S	S	S	S	S	I	S	I	S
MDR status (CLSI)				MDR					MDR	MDR	MDR	

Key: **'R'** = Resistant, **'S'** = Susceptible, **'I'** = Intermediate, **'MDR'** = multi drug-resistant, **'CLSI'** = Clinical Laboratory Standards Institute, **'*E. co*'** = *Escherichia coli*, **'*P. a*'** = *Pseudomonas aeruginosa*, **'*K. p*'** = *Klebsiella pneumoniae*

Table 4: Antimicrobial screening of *Ficus mucuso* extract and fractions against clinical isolates

Isolates	Crude methanol extract				Aqueous fraction				methanol				Ethylacetate fraction				n-Hexane fraction				Cipro	10% DMSO
	Concentration of extract and fractions in mg/L																					
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	10µg	
	Zones of inhibition (in mm)																	
E. coli$_1$	14	12	10	-	12	10	-	-	14	12	10	-	-	-	-	-	12	-
E. coli$_2$	14	10	-	-	10	-	-	-	14	10	-	-	-	-	-	-	10	-
E. coli$_3$	16	14	10	-	10	-	-	-	16	14	12	10	-	-	-	-	12	-
E. coli$_{ATCC25922}$	18	16	14	10	10	-	-	-	20	16	12	10	-	-	-	-	16	-
K. p$_1$	18	16	14	-	-	-	-	-	22	12	10	-	10	-	-	-	20	-
K. p$_2$	12	10	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-
K. p$_3$	14	10	-	-	10	-	-	-	14	12	-	-	-	-	-	-	-	-
K. p$_{ATCC700603}$	14	-	-	-	10	-	-	-	12	10	-	-	-	-	-	-	12	-
P.a$_1$	12	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	14	-
P. a$_2$	14	10	-	-	-	-	-	-	14	12	10	-	-	-	-	-	-	-
P. a$_{ATCC\ 27853}$	14	12	10	-	10	-	-	-	10	-	-	-	-	-	-	-	14	-

Key: Cipro = Ciprofloxacin, DMSO = Dimethyl sulfoxide, ATCC = American Type Culture Collection, *E. coli* = *Escherichia coli*, *K. p = Klebsiella pneumoniae*,*P. a = Pseudomonas aeruginosa*, - = No Zone of growth inhibition

The DPPH scavenging of *Ficus mucuso* and ascorbic acid (reference standard) is shown in Table 5. The percentage of DPPH radical scavenged for the ethyl acetate fraction of *Ficus mucuso* was relatively fair, when compared to the reference standard used.

Table 5: DPPH Scavenging Activity of the Ethyl acetate extract of *Ficus mucuso* and Ascorbic acid

Concentration	% Inhibition	
(µg/mL)	*Ficus mucuso*	Ascorbic acid
400.00	62.50	96.40
200.00	47.39	79.95
100.00	40.00	55.30
50.00	35.07	36.12
25.00	32.18	24.42

The LC_{50} values of the crude methanol extract of *Ficus mucuso* evaluated by Brine Shrimp Cytotoxicity assay showed mild cytotoxicity, and this was done in comparison to the positive control (potassium dichromate) which was highly cytotoxic. This can be seen in Table 6 below.

Table 6: Brine Shrimp Cytotoxicity Assay of Crude Methanol extracts of *Ficus mucuso*

Plant extracts	Concentration (µg/mL)	Number of surviving nauplii (after 24 hours)			Total number of nauplii survivors	% Mortality	*LC* 50 (µg/mL)
		T_1	T_2	T_3			
Crude	1000	7	8	6	21	30.0	31.30
Methanol	100	8	7	7	22	26.7	
Ficus mucuso	10	8	10	10	28	6.7	
	1	10	10	9	29	3.3	
Potassium	1000	0	0	0	0	100	0.78
Dichromate	100	0	1	0	1	96.67	
	10	3	0	2	5	83.33	
	1	7	7	5	19	36.67	

The most active partitioned fractions (ethyl acetate) of the two plants were subjected to VLC, the fractions were developed on TLC plates, (solvent system-Dichloromethane: Ethyl acetate, 4:1). The fractions were pooled together based on their TLC profile. Table 7 shows the weight and the physical appearance of the pooled VLC fractions of ethyl acetate extract of *Ficus mucuso*

Table 7: Pooled fractions gotten from VLC of the ethyl acetate extract of *Ficus mucuso*.

Pooled fractions	Colour VLC Ethyl acetate fractions	Weight (g)
Fraction 1	Light yellow	0.182
Fraction 2	Light brown	0.234
Fraction 3	Light brown	0.335
Fraction 4	Brown	0.703
Fraction 5	Brown	4.430
Fraction 6	Dark brown	0.620
Fraction 7	Dark brown	0.715

The MIC and MBC ranged from 1.5625 to 12.5mg/mL and 3.125 to 25mg/mL respectively, as shown in Table 8 below.

Table 8: MIC and MBC of the most active fractions from VLC of ethyl acetate extracts of *Ficus mucuso*

Isolates	*Ficus* Fraction 1	*mucuso*	*Ficus* Fraction 2	*mucuso*	*Ficus* Fraction 6	*Mucuso*
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
E. coli$_3$	12.5	25	6.25	12.5	12.5	25
E. coli ATCC 25922	12.5	25	6.25	50	12.5	25
K. pneumoniae$_{1UTI}$	12.5	25	6.25	50	12.5	25
*K.pneumoniae*ATCC 700603	12.5	25	12.5	25	6.25	12.5
P. aeruginosa$_2$	25	25	6.25	12.5	12.5	25
P. aeruginosa ATCC 27853	12.5	25	6.25	12.5	1.5625	3.125

The effect of combining the most active VLC fractions with ciprofloxacin and amoxicillin-clavulanate is shown in Table 9. This was evaluated using the FIC index, which can be calculated as:

FIC index = (MIC of extract in combination / MIC of extract alone) + (MIC of control drug in combination / MIC of control drug alone). The FIC index values were interpreted as follows according to (van Vuuren and Viljoen, 2011):

$\leq$ 0.5= synergistic; > 0.5-1.0 = additive; >1.0-4.0 = indifferent (non-interactive); > 4.0 = antagonistic

Table 9: Combined effect of most active VLC fraction of *Ficus mucuso* and two Antibiotics

Isolates	Drug	MIC of extract (mg/mL)		FIC	MIC of Antibiotic (µg/mL)		FIC	FIC index	Outcome
		Combi	Alone		Combi	Alone			
P. aeruginosa ATCC 27853	Cipro	0.048	1.5625	0.03	10	2.5	4	4.03	Indifference
K. pneumoniae ATCC 700603	Cipro	0.3906	6.25	0.06	0.625	2.5	0.25	0.31	Synergistic
P. aeruginosa ATCC 27853	Amoxicillin-Clauvulanate	0.78125	1.5625	0.5	120	30	4	4.5	Antagonism
K. pneumoniae ATCC 700603	Amoxicillin-Clauvulanate	12.5	6.25	2.0	60	60	1	3.0	Indifference

Key: FIC = Fractional inhibitory concentration, ATCC = American Type Culture Collection, Cipro = Ciprofloxacin, , Combi = Combination,*E. coli = Escherichia coli, K. pneumoniae = Klebsiella pneumoniae, P.aeruginosa = Pseudomonas aeruginosa*

DISCUSSION

Antibiotics are very vital in combating bacterial infections, but their effectiveness is increasingly jeopardized by the emergence of strains of bacteria that are resistant to the existent antibiotics. This has resulted in many treatment failures (Xie *et al.,* 2015). Uropathogens have been major culprits, as antibiotic resistance of uropathogens has been rising (Ku *et al.*, 2024).

Medicinal plants have been widely used in the treatment of infections, including urinary tract infections, and some *Ficus* species (including *Ficus mucuso*) are purported to have activity against uropathogens.

It is hypothesized that phytochemicals in crude extracts can enhance or synergize the bacteriostatic or bactericidal actions of antibiotics, making their combination with antibiotics an alternative strategy to combat bacterial resistance. (Atta *et al.,* 2023)

In this study, we evaluated and confirmed the activity of *Ficus mucuso* against uropathogens and we reported synergism between ethyl acetate extract of *Ficus mucuso* and Ciprofloxacin against *Klebsiella* species.

The extraction method used has been shown to affect the classes of the bioactive constituents extracted. In research by (Cujic *et al.,* 2016), it was shown that cold maceration method results in high yields of phenolic compounds extracted. The process of extraction used was cold maceration, and further partitioning with solvents across varying polarity was done to ensure extraction of as many bioactive constituents of the plants as possible. (Iloki-Assanga*et al.,* 2015; Lezoul *et al.,* 2020). The extraction method employed also helped to ensure no loss of probable thermolabile bioactive constituents to heat. Also further successive partitioning of the extracts into solvents of varying polarities helped ensure extraction of non-polar, moderately polar and polar constituents in hexane, ethyl acetate and methanol respectively.

The phytochemical constituents present were similar with those from a study by Coker and Adeniyi-Aogo, (2021) on *Ficus mucuso* that showed the presence of saponins, alkaloids, flavonoids, steroids, terpenoids, tannins and anthraquinones, however, recorded the absence of cardiac glycosides.

The percentage inhibition of DPPH for the crude extract of *Ficus mucuso* was 62.5%. Findings by Oguntoye *et al.,* (2016) revealed that the ethyl acetate fraction of *Ficus mucuso* had a percentage inhibition of 48.7% at 400µg/mL, which was close to the percentage inhibition obtained in this study. The antioxidant qualities of these bioactive components in the plant have been linked to phenolic compounds that scavenge oxygen and react with catalytic metals and free radicals. (Olajuyigbe *et al.,* 2020).

The crude methanol extracts of *Ficus mucuso* was mildly cytotoxic, as it had an LC_{50} value of 31.30 µg/mL. LC_{50} values between >30 to < 100µg/ml depict mild cytotoxicity. According to Moshi *et al* (2010) mild toxicity implies a probability of no obvious danger of outright toxicity during acute exposure. So far, there have been no findings on the cytotoxicity of *Ficus mucuso*, however, a study carried out to evaluate the sub-chronic and chronic toxicity of a *Ficus* species, *Ficus thonningii* by Coker *et al.,* (2009) showed that it is safe when used in low doses.

When comparing the resistance profile of the clinical isolates employed in this study with a study done by Coker *et al.,* (2021), there were similarities. Their findings showed a 100% resistance of *Pseudomonas aeruginosa* isolates to third generation cephalosporins. The result of this research showed 100% resistance of

Pseudomonas aeruginosa to second generation cephalosporin (Cefuroxime), and a 75% resistance to third generation cephalosporin (Ceftriaxone). Additionally, they reported lesser resistance to ciprofloxacin and gentamicin, which was similar to the result observed in this research, with 25% resistance to gentamicin and 8.33% resistance to ciprofloxacin.
The resistant nature of these organisms to current antibiotics suggests an urgent need to explore alternative therapeutic agent in the treatment of infections caused by these microbes.

Crude extracts and methanol extract of *Ficus mucuso* had considerable activity, whereas the hexane extract was very poor. The diameter for zone of inhibition was similar to a study by Coker and Adeniyi-Aogo (2021), with hexane fraction of *Ficus mucuso* showing the least inhibition diameter. This could be due to the fact that the non-polar bioactive constituents did not diffuse adequately through the agar. A review by Ellon (2019), showed that non-polar substances will not diffuse as well as polar compounds because agar is an aqueous preparation. This explains the reason for the seeming inactivity of the hexane extract of both plants. The MIC however showed more considerable activity of the plant extract and fractions. This was most likely due to the method employed (microbroth dilution method).
The MIC of the crude extracts and partitioned fractions ranged from 12.5 to 50mg/mL, and the MBC values were between 12.5 and 100mg/mL. The ranges were similar to a study carried out by Coker and Adeniyi-Aogo (2021), where they reported MIC ranges 6.25 to 25mg/mL and MBC ranges between 50 and >100mg/mL. One of the VLC fractions tested showed an eight-fold reduction in the MIC and a four-fold reduction in the MBC. This is in line with a finding of Coker *et al.*, (2020), that the VLC fractions of the plant extract tested were more active than the crude extracts. This can be explained to be due to an increase in the concentration of the bioactive constituents of the plant extract when subjected to VLC.
The combination of the most active VLC ethyl acetate fraction of *Ficus mucuso* and ciprofloxacin against *K. pneumoniae* ATCC 700603 resulted in a fourfold reduction in the MIC of ciprofloxacin. The synergism observed, with an FIC index of 0.31 followed the same pattern of *Ziziphus mucronata* and ciprofloxacin combination in Olajuyigbe's study (Olajuyigbe *et al.*, 2020), which was also against a Type strain of *K. pneumoniae*. Overall, it can be deduced that when used in combination, the dose of the drug will be reduced, thereby minimizing the side effects of the drug. The study by Olajuyigbe *et al.*, (2020), on the interaction of methanol extracts of *Ziziphus mucronata* subsp. *mucronata* with first-line antibiotics, reported a 2.5% antagonism of the plant extracts with test drug.

CONCLUSION

Leaf extracts of *Ficus mucuso* have antimicrobial potentials which demonstrated enhanced synergistic activity against UTI organisms when combined with a standard chemotherapeutic, thus making the plant a potential source of novel antimicrobial.

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