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Antibacterial and Phytochemical Potentials of *Ficus capensis* Leaf Extracts Against Some Pathogenic Bacteria

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ABSTRACT

Natural products represent an alternative source of potent antimicrobial to combat the increasing antimicrobial resistance (AMR) to synthetic drugs. Plants in particular contain metabolites which have been widely employed in traditional settings to treat ailment. However, there is a need for scientific knowledge on their bioactivity. This study is aimed at elucidating the phytochemicals, and antibacterial activity of *Fiscus capensis*. The leaves of *F. capensis* were pulverized and extracted successively using n-hexane, acetone, methanol and distilled water. The plant extracts were evaluated for their antibacterial activity against selected Gram-positive and Gram-negative bacteria (*Salmonella* Typhi, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella* sp.) using the agar-well diffusion technique. The minimum inhibitory concentrations (MIC) of the plant extracts were established using the microbroth dilution technique. The phytochemical analysis showed the presence of alkaloids in all extracts. Saponins, phenols, terpenoids and tannins were present in all extracts except n-hexane extract. The antimicrobial studies showed varying levels of activity, with acetone extract having the highest activity against *S. aureus* and *S*. Typhi with MIC of 6.25 mg/mL. Methanol had an MIC activity of 12.5 mg/mL against *E.coli*. While some prior studies reported no activity of methanol extract against *S*. Typhi, our finding showed that methanol extract of *F. capensis* exhibited antimicrobial activity against *S.* Typhi due to the extraction process. Our study concludes that the overall antimicrobial activity of the crude extract of *F. capensis* leaf provided evidence that future antimicrobial agents could be isolated from this plant leaf.

Keywords: Antibacterial, Minimum inhibitory concentration, Drug resistance, Microbroth dilution, p-iodonitrotetrazolium chloride.

Introduction

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi, and parasites evolve and lose their ability to respond to antibiotics, making infections more difficult to cure and raising the risk of disease transmission, severe illness, and death.[1] AMR has become one of the twenty-first century's most serious public health issues, posing a threat to the efficient prevention and treatment of a growing number of infections caused by bacteria, parasites, viruses, and fungi that are no longer susceptible to commonly used antibiotics.[2]New resistance mechanisms are evolving and spreading worldwide, posing a danger to our ability to treat infectious diseases. In addition, AMR substantially impacts a country's economy and healthcare system, limiting patient and handler productivity due to prolonged hospitalization, which may necessitate a more expensive and complicated treatment method.[3]

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Some groups of pathogenic microbes have reportedly exhibit AMR and multidrug resistance; the emergence of "superbugs" such as *Staphylococcus aureus*, *Enterobacter* spp, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* has raised much concern due to the decline in the efficiency of antimicrobials to combat such pathogenic microbes. The growth of AMR in today's human society will lead to greater usage of older, less effective infection-control measures on an individual basis.[4] For example, Methicillin-resistant *Staphylococcus aureus* (MRSA) is impervious to methicillin and other antibiotics such as chloramphenicol and aminoglycosides. This is enhanced by the reduced discovery of new effective antimicrobials.

This made man look for novel antimicrobial agents that will have great potency against pathogens. In recent times, there has been an increase in the use of natural products for the development of therapeutics, which has reduced the side effects characterized by the reliance on synthetic drugs.[5]For centuries, Africans have used medicinal herbs to heal illnesses. According to the World Health Organization (WHO) research, approximately 80 % of developing nations use herbal medicine in primary healthcare.[6]

Ficus capensis Thunb (Moraceae) is also called "Ficus sur Forssk". It is a medicinal plant commonly found in the tropics and subtropics.[7] The extract of its leaves can be used to improve sterility in men[8] and treat diseases such as epilepsy, gonorrhoea and respiratory disorders.[9] It has also been reported that leaves contain pharmacological characteristics like anti-inflammatory, antioxidant and antimicrobial effects. It has phytochemicals such as terpenoids, tannins, alkaloids, cardiac glycosides and flavonoids.[10] This current study

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explored continuous successive extraction for stratification of extract based on polarity to determine the antimicrobial properties of the different phytochemical components of *F. capensis*.

Materials and Methods

Sample collection

The leaves of *F. capensis* were collected between March to June 2020 from Omu-Aran forest in Kwara State, Nigeria. Identification of the plants was done at the Department of Botany, University of Ilorin and a voucher specimen was archived with the reference number UILH/002/1417/2021 (*Ficus capensis*).

Extraction

F. capensis leaves weighing 600 g were washed and dried at 16 to 18°C. The leaves were ground into a fine powder and kept in an airtight container. The modified Rubab *et al.* (2021) approach was used to remove the crushed material. The dried pulverized plant leaves were successively extracted with n-hexane, acetone, methanol, and water as solvents, in the ratio 1:10 i.e. 600 g of leaves in 6 L of solvent.[11] The maceration extraction was done for 72 hours for each solvent at ambient temperature (27 ± 2.5°C) on an Orbital Shaker at 100 rpm. The solution was filtered through a Whatman 1 filter paper and the filtrate was concentrated using a rotary evaporator under reduced pressure. The final concentrated leave extracts were weighed and kept in a glass container.

Phytochemical screening

Qualitative phytochemical screening

Phytochemicals like alkaloids, saponins, cardiac glycoside, sterols, phenols, terpenoids, quinones, flavonoids, and tannins were evaluated in crude solvent-free extracts of *F. capensis* leaves using the established method described by Ali *et al.* (2018).[12]

Quantitative phytochemicals screening

Determination of total alkaloid

The gravimetric technique described by Onochie *et al.*[13] was used. In 50 mL of 10% ethanolic acetic acid solution, 5 g of each extract was dissolved. Before filtering, the solution was agitated for roughly 10 minutes and left to stand for 4 hours. The alkaloid present in the solution was precipitated with concentrated NH_4OH. Afterwards, the filtrate from the solution was allowed to evaporate to 25% of its original volume (drop-wise addition). Next, the precipitate was filtered out of the solution and washed with 1% NH_4OH solution. The resultant precipitate was dried at 60°C for 30 minutes in a hot air oven to evaporate all liquid and then reweighed to determine the alkaloid concentration of each extract.

Determination of total terpenoid content

A certain quantity of the leaf extract (2 g) was weighed into a beaker containing 50 mL of 95% ethanol for 24 h. The leaf extract was filtered, and the recovered filtrate was re-extracted with petroleum ether (60-80°C) and dried. Total terpenoids were calculated from the dried mass in mg/g.[14]

Determination of total phenolic content

The FolinCiocalteu technique reported by Singleton *et al.*, was used to quantify the total phenolic content of the leaf extracts.[15] Gallic acid was used as the standard and varying concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL were used for its calibration curve. Then 50 µL of crude extract was measured into 250 µLFolin-Ciocalteu reagent, mixed with 3 mL of double distilled water, and allowed to stand for 5 minutes on the bench. Next, about 750 µL of 20% Na_2CO_3 was added to the standing mixture and mixed for 2 – 4 mins, after which it was incubated at room temperature for 30 mins. Finally, the absorbance was measured in triplicates using a UV-visible spectrophotometer at 760 nm.

Determination of total saponin content

The leaf extract (5 g) was dissolved in 100 mL 20% acetic acid (in ethanol) and placed in a water bath at 50°C for 24 hours. The resultant solution was filtered and concentrated to a quarter of its original volume in a water bath. Then, drop by drop, concentrated NH_4OH was added to the solution until it entirely precipitated. Before the precipitate was filtered and weighed, the solution containing the precipitate was allowed to stand for the precipitation to settle.[11]

Determination of total tannin content

A slightly modified Folin-Ciocalteu assay technique was used to determine the total tannin content of the leaf extracts. First, a Gallic acid calibration curve was made using varying concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 $mg \cdot mL^{-1}$. Then 5 ml of already prepared leaf extract was added to 5ml of distilled water, Na_2CO_3 (35%) and Folin-Ciocalteu reagent (500 µL) was mixed for 15 min and allowed to stand for 30 min at ambient temperature. The absorbance of standard solutions was measured against a blank at 725 nm with a UV-visible spectrophotometer, and the total tannin content was extrapolated from the calibration curve and represented as mg/g.[12]

Organisms used in this study

The bacteria used in this experiment were strains sourced from the Microbiology laboratory, Landmark University, Omu-Aran, Kwara State, namely *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella* Typhi (ATCC 20971), *Escherichia coli* (ATCC 25922) and *Klebsiella* spp.

Antimicrobial screening

Antimicrobial activity of extracts

The antimicrobial potential of all crude extracts (200 mg/mL) was tested using the agar well diffusion technique. The agar well diffusion technique was used to examine the antibacterial properties of all crude extracts (200 mg/mL). For each organism in replicates, a 24 hour old culture on nutrient broth was utilised as inoculum, and the closed mesh was streaked on solidified Mueller-Hinton agar plates. In the Mueller-Hinton agar, four wells were drilled with a 9mm cork borer. 100 microliters of each leaf extract were poured into each well. The positive control was ciprofloxacin, and the negative control was DMSO. Before incubating at 37 °C for 18 - 24 hours, the prepared plates were allowed to stand for the extract to diffuse into the medium. Zones of inhibition were observed on the plates, and antimicrobial activity was calculated as the average of the diameters of the zones of inhibition.[14]

INT colorimetric assay for MIC determinations

The MIC of *F.capensis* leaf extracts on *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *P. aeruginosa* (ATCC 9027), *S.* Typhi (ATCC 20971), *E. coli* (ATCC 25922), and *Klebsiella sp*. was determined using the INT colourimetricmicrobroth dilution method published by Eloff 1998,[16] with modification. The leaf extracts were dissolved in DMSO at a concentration of 200 mg/mL. All 96 wells of the microtiter plates were filled with 100 µL of Mueller Hinton Broth. 100 µL of the extract solution was poured into the first well of the column and stirred several times before 100 µL was removed and injected into the corresponding well to achieve a two-fold dilution; this was repeated until the tenth well, at which point 100 µL was discarded. Thus, each well received 20 µL of broth containing the test organism.
For 16-18 hours, the plates were covered and incubated at 37°C. The experiment was carried out in multiples. As a negative control, DMSO was used. After 18 hours, 10 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) was added to all wells and incubated for another 30 minutes. Wells, where the yellow INT solution could not be reduced to pink, was taken as the MIC.

Statistical analysis

Data obtained were analyzed using a one-way analysis of variance (ANOVA) with the aid of IBM-SPSS Statistics version 22 (IBM Corp., USA) software and Microsoft Excel 2016. Results are expressed as mean ± standard deviation ($p > 0.05$).

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Results and Discussion

Phytochemical screening

Dried leaves of *F. capensis* were successively extracted using four (4) solvents of varying polarity from non-polar to polar. The extraction yield varied based on polarity, with acetone extract having the highest yield (111 g) of all solvents used while the lowest was n-hexane extract (11 g) as shown in Figure 1. Quantitative analysis of the phytochemicals in the leaf of *F. capensis* extract showed the presence of alkaloids concentration (0.05 ± 0.004 mg/g) highest and as the only phytochemical of interest tested in the n-hexane extract. The acetone, methanol and water extracts have all five secondary metabolites tested for in the plant extracts. Acetone extract was highest in Saponin (0.13±0.1 mg/g), Methanol contained more Phenols and Tannins at 0.04±0.05 mg/g and 0.04±0.04 mg/g respectively, while the Water extract was highest in Terpenoids (0.04±0.01 mg/g).

Selected phytochemicals based on their antimicrobial properties were quantified in the plant extracts. The varying concentration of the five secondary metabolites (alkaloids, saponins, phenols, terpenoids, and tannins) quantified in the extracts of *F. capensis*, leaves could be responsible for the variation in the degree of antimicrobial potential of the extracts against the selected microbial isolates.[24]

Phytochemicals derived from plants have been the subject of interest for researchers due to their potential in helping to combat the emergence of antimicrobial-resistant organisms.[17] The antibacterial, antifungal, antiviral and antioxidant activities of secondary plant metabolites have been reported and can restore the potency of older antibiotics while also limiting the possibility of the development of resistance.[18] As most traditional treatments rely on water extraction and a mid-polar solvent such as ethanol, the yield of the active phytochemicals needed to be compared for availability. From the observation recorded in Table 1, it is evident that phytochemicals extracted from plant leaves varied with the polarity of the solvent used. The result for *F. capensis* showed a higher yield of acetone extract followed by methanol extract, and this could indicate that more bioactive constituents reside in the mid polar region of the leaf matrix. This is likewise supportive of the result from the quantitative phytochemical analysis that showed the presence of more phytochemicals in the mid polar region, with non-polar n-hexane having only alkaloids of the phytochemicals tested. Considering the extraction technique used (in this case successive extraction) n-hexanes being the first solvent for extraction significantly impacted the yield, and could also mean the leaf contains less fat constituent and non-polar metabolite. *F. capensis*, leaf extract, was tested for the presence of secondary plant metabolites such as alkaloids, saponins, phenols, terpenoids, and tannins. According to Snehlata *et al.* (2018), the presence of these phytochemicals is an indication that the extracts of the plant leaves possess antimicrobial properties.[19]

Antimicrobial screening

The antimicrobial potentials of the extracts of *F. capensis* leaf were examined using agar well diffusion techniques against six selected bacterial isolates; *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *P. aeruginosa* (ATCC 9027), *S.*Typhi (ATCC 20971), *E. coli* (ATCC 25922), and *Klebsiella sp*. DMSO was used to dissolve the extract and also used as the negative control. The loading vector did not show any detectable inhibitory effect against any of the bacteria isolates. The positive control used was ciprofloxacin (CPR) which showed a good inhibitory effect on all test isolates (Table 2). All extracts except n-hexane extract showed a similar zone of inhibition of 11.33 mm against *P. aeruginosa*. Water extract of *F. capensis* had the highest diameter against *S.* Typhi (22.33 ± 3.18 mm) which is significantly higher than that of n-hexane extract (13.33 ± 0.33 mm). Against *S. aureus*, methanol extract of the plant had the highest activity of all extract with 19.67±0.8 mm zone of inhibition, which was also not significantly different from the 26.33 ± 0.67 mm diameter exhibited by the control drug (CPR) despite methanol extract being lower. Activities of all extracts against *E. coli* and *B. subtilis* were not significantly different except for water extract that did not show any visible zone of inhibition against *E. coli*. Acetone extract had the highest zone of inhibition 18.67 ± 0.33 mm against *Klebsiella sp.* and was not significantly different from the activity observed for ciprofloxacin.

Contrary to Oyeleke *et al.* and Obonga *et al.* reports on the antimicrobial activities of *F. capensis* leaf methanol and water extract against *S.* Typhi,[20,21]we observed that methanol and water extract showed antimicrobial properties against *S.*Typhi with a zone of inhibition of 20.67 ± 2.40 mm and 22.33 ± 3.18 mm, respectively and MIC of 12.5 mg/ml for the two extracts. This new observation could be as a result of the extraction technique. Musa *et al.* (2019) used a similar technique and also reported the antimicrobial activity of extracts of *F. capensis* against *S.* Typhi. Oyeleke *et al.* and Obonga *et al.* extracted directly using methanol and water as solvent. This work reported zones of inhibition by water extract of *F. capensis* leaf against *S. aureus*, *B.subtilis* and *P. aeruginosa* at a 200 mg/mL concentration. This finding is in tandem with the reports of Umeokoli *et al.*.[22] The extracts were combined to see if metabolites of varying polarity may have synergistic, additive, or antagonistic effects against the activities of the test microorganisms.

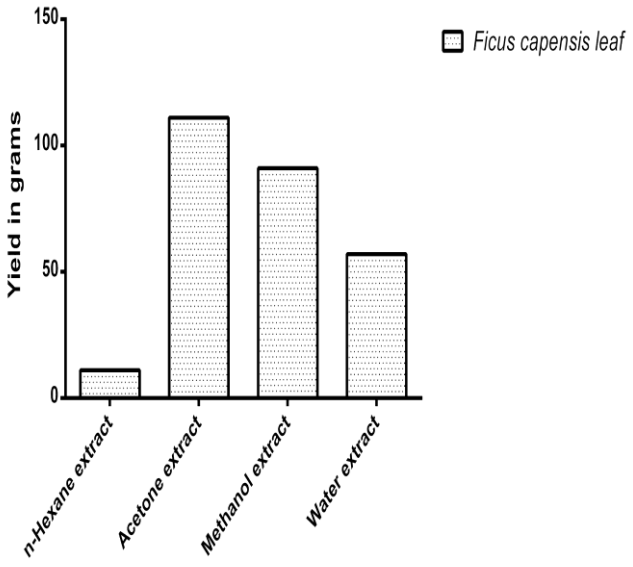

Figure 1: Extraction yield from successive extraction procedure using solvent of varying polarity.

Table 1: Quantitative estimates of five selected phytochemicals in the leaf of *F. capensis* extracted with solvents of different polarities

Ficuscapensis	Alkaloids (mg/g)	Saponins (mg/g)	Phenols (mg/g)	Terpenoids (mg/g)	Tannins (mg/g)
n-Hexane extract	0.05 ± 0.004	-	-	-	-
Acetone extract	0.04 ± 0.005	0.13 ± 0.10	0.03 ± 0.04	0.03 ± 0.01	0.02 ± 0.03
Methanol extract	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.05	0.01 ± 0.01	0.04 ± 0.04
Water extract	0.02 ± 0.005	0.07 ± 0.05	0.01 ± 0.03	0.04 ± 0.01	0.01 ± 0.03

Note: The mean ± standard deviation (triplicate) of the estimated quantity of each phytochemical in *F. capensis*was reported in the table above, - = the phytochemical is absent.

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In the case of *P. aeruginosa*, all combined extracts have synergistic effects except for the combination of "acetone and methanolic" extract that displayed an antagonistic effect. The combination of the extracts showed an antagonistic effect when tested against *S.* Typhi, except for "n-hexane and acetone" extract combination presented a synergistic effect. *S. aureus* was more susceptible to the combination of the extracts as most of the combinations had synergistic effects except "acetone and methanol" extract and "methanol and water" extract combinations. They both had antagonistic interactions. The combined extracts showed antagonistic, synergistic and additive effects against *E. coli*. "n-hexane and acetone" extract, "acetone and methanol" extract combinations displayed antagonistic effects when tested against *E. coli*. While "n-hexane and methanol" extract, "n-hexane and water" extract and "methanol and water" extract combinations showed an additive effect. Only the "acetone and water" extract combination had a synergistic effect. All combinations of the extracts expressed antagonistic effects when tested against *B. subtilis* and *Klebsiella sp.* except for "n-hexane and methanol" extract, which showed a synergistic effect when tested against *B. subtilis*.

The antagonistic effect observed when methanol and water extracts of *F. capensis* leaf were combined with other extracts of *F. capensis* leaf could be responsible for the lack of activity against *S.* Typhi previously reported.[20,21] This further gives credence to the advantages of successive extraction techniques. Obonga *et al.* also reported no antimicrobial activity by methanol extract of *F. capensis* leaf against *P. aeruginosa, S. aureus, K. pneumonia, E. coli* which is contrary to the observations in this work. In *F. capensis*, the combination of two medium polarity extracts (acetone with methanol) produced inhibition of antibacterial effect (Table 3). Relatively lower zones of inhibition (relative to the referent drug CPR) are achieved by combining either polar with medium polar extracts or non-polar with medium non-polar extracts.

This trend further buttresses the efficiency of the successive extraction technique. It implies that the full potential of the active compound in a plant extract cannot be determined until it is isolated and tested as a pure compound. Previous works indicate that certain factors can contribute to the activity of a plant extract in agar well diffusion, according to Eloff *et al.*[28] They include the polarity of the extract, ability to diffuse within the media (for example, if the media is water-based), the thickness of the media and others. Minimum Inhibitory Concentration assay was performed using the microbroth dilution techniques to evaluate the lowest effective concentration of each *F. capensis* leaf extract and presented in Table 4. Acetone extract of *F. capensis* had the lowest MIC against *P. aeruginosa, S. aureus and S.*Typhi (12.5 mg/mL, 6.25 mg/mL and 6.25 mg/mL, respectively). The acetone extract also shared the lowest MIC with other extracts against *B. subtilis* and *Klebsiella sp*. However, methanol extract of the leaf of *F. capensis* had the lowest MIC against E. coli (12.5 mg/mL). The acetone extract of *F. capensis* had the lowest MIC of the extracts across the selected isolates. The acetone extracts had a MIC of 6.25 mg/mL against *S.*Typhi and *S. aureus*. This report is similar to the observation of Famuyide *et al.*, who found that acetone was the best extraction solvent for plant antimicrobial metabolites.[23] The result of Antimicrobial Susceptibility testing of *F. capensis*, plant leaf extracts reported in Tables 2 through 4 presented activities against both gram-positive and gram-negative bacteria. This could imply that the antimicrobial activities of the extracts were not limited by the structural differences in the bacterial cell walls, as earlier reported by Gonelimali *et al.*[25] The susceptibility of the test organisms to these leaf extracts may also suggest that the microbes have not developed resistance to the bioactive phytochemicals in the plant leaf. Furthermore, the pathogens may not have developed the usual efflux pumps (EPs) by which pathogens excrete detected antibiotics out of their cytosol.

Table 2: Diameters of the zone of inhibition by *F. capensis* leaf extracts made in different solvents

		Diameter (in mm) of inhibition zone:				
Test Isolates	**CPR**	**N-hexane**	**Acetone**	**Methanol**	**Water**	**DMSO**
Pseudomonas aeruginosa	30.17 ± 0.44^{c}	0 ± 0.00^{a}	11.33 ± 0.33^{b}	11.33 ± 0.17^{b}	11.33 ± 0.17^{b}	0 ± 0.00^{a}
Salmonella Typhi	43 ± 0.00^{e}	13.33 ± 0.33^{b}	15.83 ± 1.59^{bc}	20.67 ± 2.40^{cd}	22.33 ± 3.18^{d}	0 ± 0.00^{a}
Staphylococcus aureus	26.33 ± 0.67^{c}	13 ± 1.00^{b}	15.33 ± 7.67^{b}	19.67 ± 0.88^{bc}	14.6 ± 0.40^{b}	0 ± 0.00^{a}
E. coli	36.33 ± 2.4^{c}	14.17 ± 0.44^{b}	13.5 ± 0.50^{b}	13.83 ± 0.17^{b}	0 ± 0.00^{a}	0 ± 0.00^{a}
Bacillus subtilis	33.33 ± 1.2^{c}	14.5 ± 0.76^{b}	14.67 ± 0.93^{b}	16.5 ± 0.50^{b}	13.67 ± 1.33^{b}	0 ± 0.00^{a}
Klebsiella sp.	25.33 ± 5.42^{c}	17 ± 0.00^{b}	18.67 ± 0.33^{bc}	17.33 ± 0.33^{b}	13.67 ± 0.60^{b}	0 ± 0.00^{a}

The mean ± SEM (triplicate) was reported, CPR = ciprofloxacin; well size = 9.0 mm; diameters were measured in mm; DMSO was the loading solvent and concentration of extracts used was 200 mg/mL. The alphabets show significant differences.

Table 3: Diameters of inhibition (mm) resulting from combining *F. capenesis* leaf extracts of different polarities

Test Isolates	n-hexane and acetone extracts	n-hexane and methanol extracts	n-hexane and water extracts	Acetone and methanol extracts	Acetone and water extracts	methanol and water extracts	CPR	DMSO
Pseudomonas aeruginosa	14.00 ± 0.58^{b}	13.33 ± 0.44^{b}	16.33 ± 0.17^{b}	5.00 ± 5.00^{a}	16.00 ± 0.58^{b}	14.50 ± 0.5^{b}	30.17 ± 0.44^{c}	0.00 ± 0.00^{a}
Salmonella Typhi	16.83 ± 0.17^{d}	14.33 ± 0.33^{bc}	14.50 ± 0.5^{bc}	15.33 ± 0.44^{c}	15.67 ± 0.60^{cd}	13.67 ± 0.88^{b}	43.00 ± 0.00^{e}	0.00 ± 0.00^{a}
Staphylococcus aureus	19.67 ± 0.33^{c}	17.00 ± 0.76^{c}	18.83 ± 0.93^{c}	0.00 ± 0.00^{a}	19.67 ± 0.88^{c}	10.33 ± 5.17^{b}	26.33 ± 0.67^{d}	0.00 ± 0.00^{a}
E. coli	10.00 ± 5.03^{b}	13.00 ± 0.58^{b}	14.17 ± 0.83^{b}	0.00 ± 0.00^{a}	15.00 ± 0.76^{b}	13.67 ± 0.33^{b}	36.33 ± 2.4^{c}	0.00 ± 0.00^{a}
Bacillus subtilis	12.50 ± 1.04^{bc}	14.33 ± 0.6^{c}	7.33 ± 3.67^{b}	8.00 ± 4.00^{bc}	12.17 ± 0.73^{bc}	13.67 ± 0.33^{bc}	33.33 ± 1.20^{d}	0.00 ± 0.00^{a}
Klebsiella sp.	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.67 ± 3.67^{a}	3.67 ± 3.67^{a}	25.33 ± 5.42^{b}	0.00 ± 0.00^{a}

CPR = ciprofloxacin, well size = 9.0 mm, dimeters were measured in mm, DMSO was the loading solvent. The concentration of extracts used was 200mg/mL and combinations were 1:1.

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Table 4: Minimum inhibition concentration (MIC) of *F. capensis* leaf extracts against selected isolates

	Pseudomonas aeruginosa	***Staphylococcus aureus***	***Salmonella* Typhi**
n-Hexane extract (mg/mL)	25	25	12.5
Acetone extract (mg/mL)	12.5	6.25	6.25
Methanol extract (mg/mL)	25	50	12.5
Water extract (mg/mL)	25	12.5	12.5
	Bacillus subtilis	***Escherichia coli***	***Klebsiellasp***
n-Hexane extract (mg/mL)	25	25	25
Acetone extract (mg/mL)	25	25	25
Methanol extract (mg/mL)	25	12.5	25
Water extract (mg/mL)	25	25	25

All assays were done in replicates (n = 2).

The overexpression of efflux pumps has been reported to be a major precursor to the development of antibiotic resistance in bacteria.[24] Despite the distinction in the mechanisms of action of plant metabolites, the cytoplasmic membrane appears to be the most natural site of action. Mostly, they cause lysis of the cell, resulting in loss of cellular contents and eventually death of the pathogenic organism.[26] In some cases, antimicrobial agents also disrupt the protein synthesis process and genetic mechanisms, thereby resulting in the abnormal transcription and incoherent function of the pathogen's cellular system. The activities observed in the plant extract could also result from the antimicrobial potentials of the phytochemicals present.[27] Phenolic compounds (Flavonoids, polyphenols, and tannins) have been reported to exhibit two lines of action which could be on the cell wall and membrane or the cytosol resulting in coagulation of cellular content.[24] The antimicrobial effect of saponins is a function of the hydrophilic core, made up of one or more sugar moiety and the lipophilic (aglycon) part of the phytochemical structure.[24] The combination of the extracts did not show a better zone of inhibition than observed for individual extracts. Thus, the combinations of the extracts are not additive or synergistic. However, the combinations of polar with non-polar leaf extracts of the three test plants produce inhibitory effects lesser than individual extracts; that is, antagonistic effects when compared to individual extract (Table 3).

Conclusion

In conclusion, antimicrobial activities reported against the selected bacterial isolate may explain the plant's medicinal use by locals to treat diverse ailments. Despite the antimicrobial activity of the plant against both Grams positive and negative bacteria reported in this work, it is expedient to establish that crude extract needs to be further analysed to establish the bioactive components. To achieve the goal of discovering new and potent antimicrobials, further research into the activity of the bioactive compounds of *F. capensis in-vivo*, toxicity and clinical studies are still required.

Conflicts of Interest

The authors have no competing interests.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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