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Antibacterial Investigation using Spectrophotometric Assay of the Polar Leaf Extracts of *Ficus capensis* (Moraceae)

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Abstract

This study was designed to explore new antibacterial agent(s) from the defatted methanol and water extracts of *Ficus capensis*. The extracts were obtained using various solvents, namely, cyclohexane, dichloromethane, methanol, and water by serial exhaustive extraction, after which both extracts were challenged with pure clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Burkolderia cepacia viz-a-viz* standard antibiotics of chloramphenicol, gentamicin, and amoxicillin at 25 µg/ml, 50 µg/ml, and 100 µg/ml concentrations using spectrophotometric method. Using the zone of inhibition as an inhibitory parameter, both extracts of *F. capensis* showed promising antimicrobial activity in a concentration-dependent manner comparable to the standard antibiotics for all tested microorganisms. This research holds promise for the exploration of various potentially active secondary metabolites which would help in developing pharmaceuticals, especially antibacterial drugs, for combating common microbial infections. This validates the use of the plant leaves in the treatment of broad-spectrum microbial infections in ethnomedicine.

Keywords: Antimicrobial, Defatted extracts, *Ficus capensis*, Spectrophotometric

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Introduction

Ficus capensis, a native of tropical Africa and the Cape Islands, belongs to the family Moraceae. The plant is a deciduous tree with spreading roots and branches and broad green leaves. It is known as "*uwaryara*" in Hausa, "*opoto*" in Yoruba, "*rima bichehi*" in Fulani, "*akokoro*" in Igbo, and "*obada*" in Edo.[1,2] Conventionally, preparations from the plant parts are used in the treatment of dysentery, edema, epilepsy, chest ailments, leprosy, tuberculosis, anemia, and rickets in infants among others.[2-5] Aside its tradomedicinal applications, *F. capensis* has been reported based on modern scientific investigations to possess anti-sickling,[6,7] antibacterial,[4] antiabortifacient,[8] immune-stimulatory,[9] antidiarrheal,[10] antioxidant,[11] pro-fertility in treating azoospermia,[12] as well as its leaf, stem, and roots recently established to manage and treat typhoid disease-causing *Salmonella* typhi organism.[13]

The world over relies one way or the other on medicinal plants as alternative sources of drugs for the treatment of several disease conditions[14] as a result of the fact that majority of the populations, especially in developing countries like Nigeria experience inadequate contacts with orthodox health-care facilities, the non-availability and unbearable side effects associated with synthetic drugs.[2] Phytochemicals are naturally occurring compounds in medicinal plants which offer defense mechanism and protection from various diseases.[15] They are active secondary metabolites playing an important role in the prevention of various diseases conditions.[14]

This study attempts to give scientific credence or otherwise to the claim that *F. capensis* leaf as used in Igala traditional medicine have positive effect for the treatment of febrile ailments and infective disorders, hence as part of a wider study, the broad-spectrum antibacterial activities of the polar extracts of the plant were investigated.

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Materials and Methods

Plant Collection and Authentication

Healthy and matured *F. capensis* leaves were collected from Anyigba town in North-Central Nigeria. They were identified by Prof. COC Agwu of the Biological Sciences Department, Kogi State University, Anyigba, Nigeria. They were collected in bags before them being washed with running tap water to remove earthy impurities. They were shade dried at room temperature for 2 weeks before being pulverized using high-speed Creston mechanical grinder. The pulverized samples were stored in airtight glass container until ready to use.

Collection of Microorganisms

Clinical microbial strains used in this study (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinectobacter baumanii*, and *Burkolderia cepacia*) from different specimens of patients referred to the Medical Microbiology Department, University of Lagos Teaching Hospital, Lagos State Nigeria. The bacteria were characterized to species level using different laboratory procedures, including gram's stain, cultural characterization, and biochemical tests, notably Indole, Methyl red, Vougues Proskeaur, Catalase, Citrate utilization, and coagulase tests.[16,17] The isolates were maintained on nutrient agar slants at 4°C until ready for use.

Standard Antibiotics Employed for the Study

Chloramphenicol (Kuka Consumer Healthcare, Nigeria), Gentamicin (Jinhing Pharmaceuticals, China), and Amoxicillin (Clarion Medicals, Nigeria) were the employed reference drugs employed for the study purchased from reputable drug outlets.

Extracts Preparation

The defatted methanol extract of *F. capensis* (FC_dMeOH) was obtained by cold macerating 300 g of the pulverized plant sample within 3 L of cyclohexane for 24 h with the resulting residue after filtration subjected to further cold maceration sequentially using 3 L of dichloromethane and methanol for 24 h each. The residue of the methanol extraction was cold macerated in distilled water, allowed to stand for 24 h and filtered with Whatmann No. 1 filter paper. The filtrate was concentrated to dryness using a freeze dryer. The yield of the crude extract (FC_dHOH) was determined relative to the starting material. The extracts were kept in an air-tight glass container and refrigerated at −4°C until use.

Preparation and Reconstitution of the Plant Extracts for Antibacterial Study

The inocula were reconstituted and standardized according to the procedure described by Rajarkaruna *et al*.[18] The FC_dMeOH and FC_dHOH were individually reconstituted by dissolving 1 mg of the extract in 2 ml of dimethyl sulfoxide (DMSO) to obtain a stock solution concentration of 0.5 mg/ml. One milliliter of the stock solution was added to 4 ml nutrient broth give a concentration of 100 µg/ml. By double serial dilution, the concentrations of 50 µg/ml and 25 µg/ml were obtained. Twelve test tubes were sorted into two groups (FC_dMeOH and FC_dHOH) with each group having six test tubes, three for the test concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml, respectively, while the other three test tubes were each for the standard antibiotics (positive control) prepared using the same concentration. The negative control was prepared by adding 1 ml DMSO to 4 ml nutrient broth.

Antibacterial Screening

The spectrophotometric method described by Banjara *et al*.[19], as modified by Musa *et al*.[20] was used to determine the antibacterial activity of the extracts.

Statistical Analysis

Data obtained from the study were subjected to a one-way analysis of variance, and variant means were separated *post hoc* using the least significant difference method. Statistical Package for the Social Sciences software, version 16.0, was used for the analysis. Significance was accepted at $P < 0.05$.

Results

The kinetics of the optical densities of the microbial growth of the six test bacteria is as shown in Figure 1.

The results of the percentage inhibitions of the FC_dMeOH and FC_dHOH in comparison to the standard antibiotics when challenged with the test organisms at the different investigated concentrations are represented in Figures 2a-f.

Discussion

Percentage Yield of the Extracts

12.30 g and 35.40 g of the methanol and aqueous extracts corresponding to a percentage yield of 4.1% and 11.8%, respectively, were obtained for the extraction of the pulverized leaves of *F. capensis*. These results are comparable to the findings of Dickson *et al*.[13] but higher than the 8.78% reported by Ayinde and Owolabi[2] and Ayinde *et al*.[10]

Antimicrobial Study

The microbial strains growth and inhibitions when monitored spectrophotometrically were seen to increase on an hourly basis before peaking after 12 h, the decline between 12 and 15 h before increasing during the 18 h monitoring period. The negative control which contained DMSO without the plant extract or standard antibiotics produced no inhibition of any of the test organisms at 12 h post-inoculation.

Both extracts exhibit an increase in the inhibition of all the microbial isolates in a dose-dependent manner. All the standard antibiotics exhibit a higher microbial inhibitions greater than both extracts when challenged with *E. coli*, *P. aeruginosa* and *K. pneumoniae* except for *E. coli* which exhibits a 100% inhibition at the highest concentration of 100 µg/mL. This, however differs, from that of *S. aureus*, *A. baumanii*, and *B. cepacia*, where the standard antibiotics exhibit a higher inhibition than both extracts at the highest investigated concentration. The disparity between the activities of the extracts and the standard antimicrobial drugs may be due to the mixtures of bioactive compounds present in the extracts compared to the pure compound contained in the standard antibiotics.[14] The antimicrobial activities of the extracts are in agreement with the report of Cooper *et al*.[21] who reported

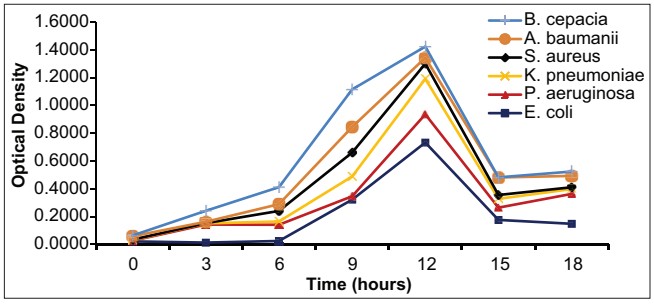

Figure 1: Growth rate of six bacteria using the measurement of their optical density at 540 nm

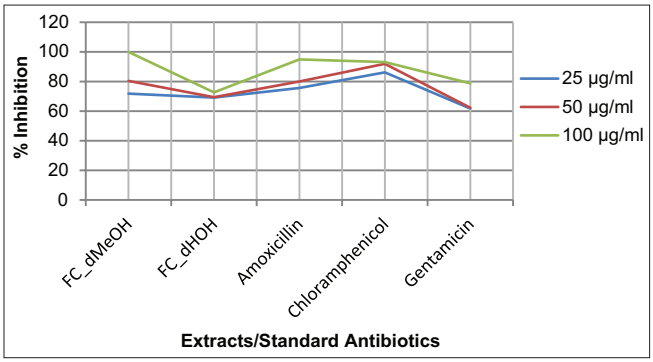

Figure 2a: Percentage inhibition of *Escherichia coli* when challenged with the plant extracts and the standard drugs at varying concentrations. FC_dMeOH: Defatted methanol extract of *Ficus capensis*, FC_dHOH: Defatted aqueous extract of *Ficus capensis*

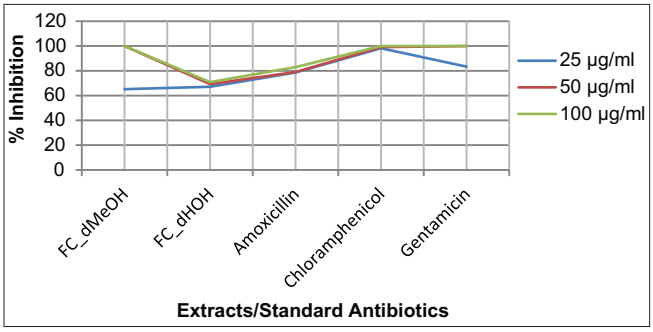

Figure 2b: Percentage inhibition of *Pseudomonas aeruginosa* when challenged with the plant extracts and the standard drugs at varying concentrations. FC_dMeOH: Defatted methanol extract of *Ficus capensis*, FC_dHOH: Defatted aqueous extract of *Ficus capensis*

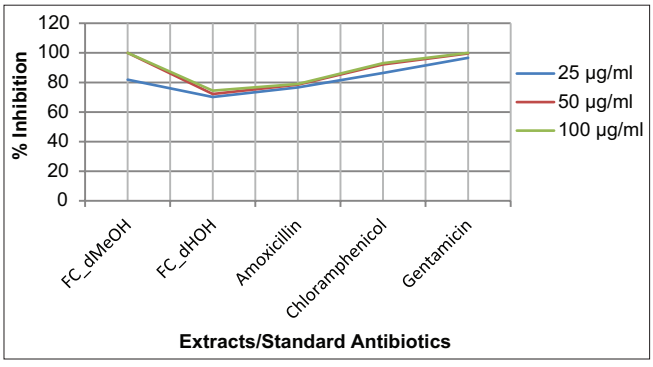

Figure 2c: Percentage inhibition of *Klebsiella pnuemoniae* when challenged with the plant extracts and the standard drugs at varying concentrations. FC_dMeOH: Defatted methanol extract of *Ficus capensis*, FC_dHOH: Defatted aqueous extract of *Ficus capensis*

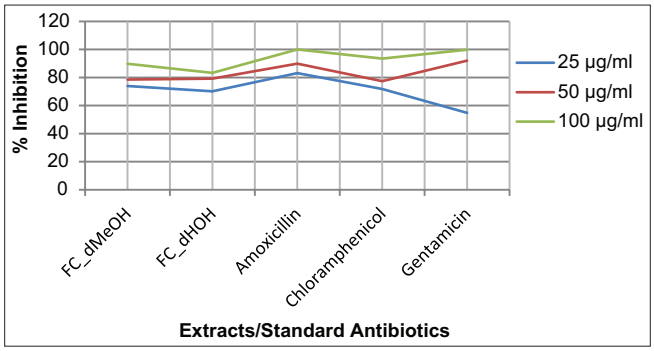

Figure 2d: Percentage inhibition of *Staphylococcus aureus* when challenged with the plant extracts and the standard drugs at varying concentrations. FC_dMeOH: Defatted methanol extract of *Ficus capensis*, FC_dHOH: Defatted aqueous extract of *Ficus capensis*

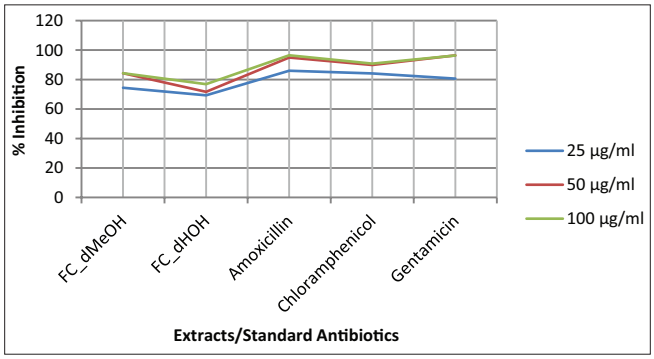

Figure 2e: Percentage inhibition of *Acinectobacter baumanii* when challenged with the plant extracts and the standard drugs at varying concentrations. FC_dMeOH: Defatted methanol extract of *Ficus capensis*, FC_dHOH: Defatted aqueous extract of *Ficus capensis*

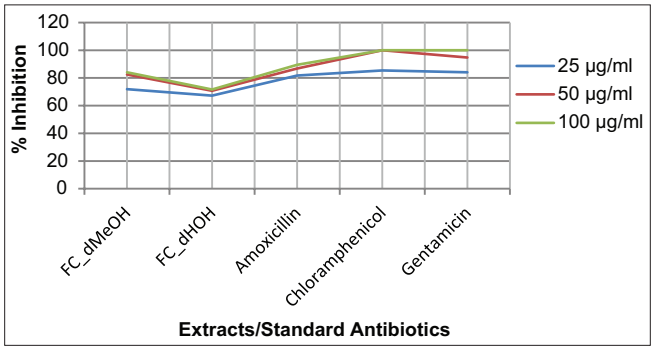

Figure 2f: Percentage inhibition of *Burkolderia cepacia* when challenged with the plant extracts and the standard drugs at varying concentrations. FC_dMeOH: Defatted methanol extract of *Ficus capensis*, FC_dHOH: Defatted aqueous extract of *Ficus capensis*

that the presence of more group of phytochemical diversity gives synergic effects in many biological applications.

The phytoconstituents present in the leaves of the plant, as reported by Dickson *et al*.[13,19] as well as Uzoekwe and Mohammed,[22] who confirms the presence of flavonoid, alkaloid, saponins, phenols, cardiac glycoside, terpenoid, steroids, and tannins. The presence of these phytochemicals in *F. capensis* leaf extracts has also been reported by many researchers[23-25] to confer the therapeutic potentials of all medicinal plants. Alkaloids, saponins, and tannins specifically have been reported to inhibit bacterial growth as well as offer protection to plants from microbial infections.[26] The leaves of the plant have been reported to exhibit remarkable inhibitory activities against the growth of enteric bacteria notably *E. coli, B. subtilis*, *S. aureus*, and *Shigella* species as reported by Oyeleke *et al*.,[4] François *et al*.,[27] and Solomon-Wisdom *et al*.[28]

Conclusion

The standard antibiotics showed a better antibacterial activity than both extracts thou not statistically significant. All tested concentrations of FC_dMeOH have a better antibacterial agent than FC_dHOH except when challenged with *P. aeruginosa* and *S. aureus* at 25 µg/mL and 25 µg/mL, respectively. These reports in combination with the results of the present work may justify the use of the *F. capensis* leaves in the treatment of broad-spectrum microorganisms in ethnomedicine as well as encourages researchers to do further *in vitro* and *in vivo* research that will explore the role of the bioactive constituents responsible for these activities as well as carry out studies at molecular level.

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