In vitro antibacterial activities of crude and partially purified fractions of defatted ethanol extract of *Ficus capensis*

Dickson A. MUSA¹,², Sandra T. DORGU¹, Roger MARKWALDER², Alaba O. OSHE¹, Hussaini KANKI¹, Fred O.C. NWODO³ and Philipp KRASTEL²

¹Natural Products and Infectious Diseases Research Unit, Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria. ²Natural Products Unit, Centre for Proteomic Chemistry, Novartis Institute for BioMedical Research, Basel, Switzerland. ³Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.

Correspondence: Dickson Musa: dickson.musa@gmail.com; +2348030557007

**ABSTRACT:** *Ficus capensis* is used in Igala folk medicine for the treatment of several febrile ailments and infective disorders. This investigation is aimed at determining the antibacterial effect of crude and partially purified fractions of defatted ethanol extract of *F. capensis* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Acinectobacter baumanii* and *Burkolderia cepacia*. The defatted ethanol crude extract (FC_dEtOH) was obtained by successive cold maceration (cyclohexane to dichloromethane to ethanol). Six fractions (FC_dEtOH_FM1, FC_dEtOH_FM2, FC_dEtOH_FM3, FC_dEtOH_FM4, FC_dEtOH_FM5 and FC_dEtOH_FM6) were obtained from FC_dEtOH by flash chromatography. The antibacterial activity against was determined by spectrophotometric method. Chloramphenicol, Amoxicillin and Gentamicin were used as positive control. The crude extract and the fractions demonstrated a wide spectrum of activities against the test organisms. FC_dEtOH_FM1 (95.32% inhibition at 100 µg/ml) had the highest antibacterial activity against *E. coli*. Similarly, FC_dEtOH_FM4, FC_dEtOH_FM2, FC_dEtOH_FM2, FC_dEtOH_FM2, FC_dEtOH_FM3 and FC_dEtOH_FM3 produced the strongest antibacterial effect against *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *A. baumanii* and *B. cepacia* respectively. From the results of this investigation, *Ficus capensis* contain active principle(s) that may have broad-spectrum antibacterial activity. Fractionation of the crude extract led to increased potentiation. The antibacterial activities of the partially purified fractions of defatted ethanol extract of *F. capensis* compare favourably with standard antibiotics and provides pharmacological basis for the applications of *Ficus capensis* in folk medicine.

**KEYWORDS:** *Ficus capensis*, antibacterial, folk medicine, Igala, Flash chromatography
INTRODUCTION

Throughout the world, the number of deaths caused by pathogens and parasites is declining slowly, but there is nowhere in the world that infectious diseases have yet become a negligible cause of illness and death (Dye, 2014). Morbidity and mortality arising from infectious diseases caused by a number of pathogens such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumannii and Burkholderia cepacia continue to pose serious public health challenges to developing countries, impacting negatively on their already precarious economies. This is in spite of the vast array of antibiotics available in the market today. WHO reported in 2000 that approximately 12 million children mainly in developing countries die every year, 50% of which may be from infectious diseases (Rice et al., 2000). Diarrhoea alone accounts for 21% of all deaths of children aged under 5 years. (Kosek, et al., 2003).

E. coli can cause diarrhea in healthy adults which is often lethal to children especially in the developing world (Odonkor and Ampofo, 2013). P. aeruginosa is inherently resistant to many antimicrobial agents owing to impermeability, multidrug efflux and a chromosomal AmpC β-lactamase (Henwood et al., 2001). K. pneumoniae which usually causes hospital-acquired urinary tract infections, septicaemia and pneumonia, have been reported to cause rapidly fatal bacteremia cases (Decre et al., 2011). S. aureus is a major cause of bacteremia, and the associated morbidity and mortality is higher compared with bacteremia caused by other pathogens (Naber, 2009). Multidrug-resistant A. baumannii is recognized to be among the most difficult bacteria to control and treat (Maragakis and Perl, 2008; Cornejo-Juarez et al., 2015; Almasaudi, 2016). B. cepacia is an important emerging nosocomial pathogen that may result in substantial mortality, and this organism is difficult to treat because it is often resistant to multiple antibiotic classes (Wood et al., 2004; Torbeck et al., 2011).

Antibiotics forms the mainstay for the treatment of infectious diseases, however, development of resistance to the commonly prescribed antibiotics (El-Mahmood, 2009) has emerged and continues to be of clinical significance in developing countries (Musa et al., 2010). In developing countries, various factors such as wrong diagnosis, over prescription of antibiotics and failure of patients to complete antibiotic doses have contributed to the development of bacterial multi-drug resistance (Iscla et al., 2015). The drug resistant microorganisms are usually more pathogenic and with higher mortality rate (Kumar et al., 2010). There is therefore a need to develop new therapies for these diseases, especially from natural products.

Humans have frequently used plants to treat common infectious diseases (Dogruoz et al., 2008). Ficus capensis commonly known as fig tree (Oyeleke et al., 2008) is used in Igala folk medicine for the treatment of several febrile ailments, infectious diseases and for boosting the immune system (Daikwo et al., 2012). The aim of this present study was to determine the antibacterial activities of crude and partially purified fractions of defatted ethanol extract of F. capensis against six pathogenic bacteria.

MATERIALS AND METHODS

Collection of Samples

Fresh leaves of Ficus capensis were collected from around Anyigba, Kogi State, North-Central Nigeria. They were authenticated by a taxonomist at the herbarium section of the Department of Biological Sciences, Kogi State University Nigeria.

The microbial strains used in this study (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumannii and Burkholderia cepacia) were obtained from University of Lagos Teaching Hospital, Lagos.

Preparation of Samples

Fresh leaves of Ficus capensis were collected, washed and air-dried at room temperature and pulverized using high speed grinder (Creston).

To obtain defatted crude ethanol extract of the plant sample, 300 g were cold macerated in 3 L of Cyclohexane, allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The residue was cold macerated in 3 litres of dichloromethane, allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The residue obtained was similarly cold macerated in 3 litres of ethanol, allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The filtrate was concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-300). The yield of the crude extract (FC_dEtOH) was determined relative to the starting material.

Fractionation by Column Chromatography was carried out using flash chromatography (Interschim SPOT II Flash 430) with a flow rate of 200 ml/min, maximum pressure of 30bar and quaternary gradient system. Eighty fractions were obtained but based on the peaks on the chromatogram and thin layer chromatography using pre-coated silica gel TLC plates, the fractions were pooled together to form 6 different fractions.
The fractions are FC_dEtOH_FM1, FC_dEtOH_FM2, FC_dEtOH_FM3, FC_dEtOH_FM4, FC_dEtOH_FM5 and FC_dEtOH_FM6. The fractions were concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-300). The yield of the crude extract was determined relative to the starting material.

The inocula were standardised using the method of Rajarkaruna et al. (2002).

The antibiotics used in this study were Chloramphenicol (Kuka Consumer Healthcare, Nigeria), Gentamicin (Jinhing Pharmaceuticals, China) and Amoxicillin (Clarion Medicals, Nigeria), obtained from a reputable pharmacy store.

Each of the plant extracts was individually reconstituted by dissolving 1 mg of the extract in 2 ml of DMSO to obtain a stock solution of 0.5 mg/ml. Three test tubes were prepared for each of the extract and the standard antibiotics. A 1 ml sample of the stock solution was added to 4 ml nutrient broth in test tube A to give a concentration of 100 µg/ml. By double serial dilution with nutrient broths, concentrations of 50 µg/ml and 25 µg/ml were obtained in test tubes B and C respectively. Similar procedure was repeated for the standard antibiotics to obtain concentrations of 100 µg/ml, 50 µg/ml and 25 µg/ml. The negative control was obtained by adding 1 ml DMSO to 4ml nutrient broth.

**Antibacterial Studies**

The percentage inhibition of the bacteria by the extracts was determined by spectrophotometric method. The method of Banjara et al. (2012) was slightly modified as follows. Three test tubes were prepared for each of the extracts. The test tubes contained 4 ml samples of 100 µg/ml, 50 µg/ml and 25 µg/ml of the reconstituted extracts in nutrient broth. A drop (0.025 ml) of the standardised bacteria was then suspended in each of the test tubes. Similarly, three test tubes were prepared for each of the standard antibiotics, gentamicin, amoxicillin and chloramphenicol, used as positive control, and inoculated with the standardised bacteria. Three test tubes were also prepared containing 4ml of the negative control sample and one drop (0.025 ml) of the standardised bacteria. This procedure was repeated for all the six test bacteria. A test tube containing 4 ml nutrient broth agar inoculated with the bacteria was used as control. They were then incubated at 37 °C for about 18 h. The experiment was carried out in triplicates. The optical density was determined using a spectrophotometer (Biochrom UV 2800) at 540 nm, using nutrient broth as the blank. Prior to the incubation, the optical density of the test tubes were taken (T₀) and recorded after which, the cultures were incubated at 37 °C in the dark. The optical density was then taken at 3 h interval as T₃, T₆, T₉, T₁₂, T₁₅, T₁₈ and recorded. The cuvettes were disinfected with 50% nitric acid after taking each optical density reading.

![Figure 1: Growth rate of six bacteria using the measurement of their optical density at 540 nm](image)
The percentage growth inhibition for each extract at different concentrations was calculated using the formula below:

\[
\text{Percentage growth Inhibition} = \left( \frac{(A_{\text{control}} - A_{\text{control}0}) - (A_{\text{test}} - A_{\text{test}0})}{(A_{\text{control}} - A_{\text{control}0})} \right) \times 100
\]

Where \( A_{\text{control}} \) = absorbance of control culture at 12 h,
\( A_{\text{control}0} \) = initial absorbance control culture at start of the experiment,
\( A_{\text{test}} \) = absorbance test culture at 12 h,
and \( A_{\text{test}0} \) = initial absorbance of the culture at the start of the experiment.

**Statistical Analysis**

Three parallel measurements were taken in this study and result was recorded as mean ± standard deviation of the three measurements using Microsoft Excel 2010 Software. The results were further analysed using multivariate analysis of variance (MANOVA) performed with pair-wise comparison test at 5% significance level and verified using the Bonferroni multiple comparisons post-hoc test at same significant level. SPSS windows version 23 (2015) was employed for the analysis.

**RESULTS**

The kinetics of the microbial growth is as shown in Figure 1. The optical densities of the test tubes containing the six test bacteria, free of the plant extract and antibiotics, peaked at 12 hours then began to decline afterwards.

The negative control which contained DMSO without the plant extract or standard antibiotics produced no inhibition of any of the test organism at 12 hours post inoculation.

All the extracts and standard antibiotics inhibited the growth of the various test samples in a concentration dependent manner.

The plant extract and standard antibiotics inhibited the growth of *E. coli* as shown in Figure 2. FC_dEtOH_FM1 and FC_dEtOH_FM3 produced percentage inhibition of the test organism significantly (p<0.05) higher than the crude extract; however, the percentage inhibitions caused by other fractions were significantly (p<0.05) lower than that of the crude extract. The highest percentage inhibition of the fraction by FC_dEtOH_FM1 (95.32%) is favourably comparable (p>0.05) to the standard antibiotics, amoxicillin (94.94%) and gentamicin (93.08%).

![Figure 2: Percentage Inhibition of Growth of *E. coli* by Crude and Partially Purified Fractions of Defatted Ethanol Extracts of *F. capensis* and some Standard Antibiotics](image-url)
Figure 3: Percentage Inhibition of Growth of *P. aeruginosa* by Crude and Partially Purified Fractions of Defatted Ethanol Extracts of *F. capensis* and some Standard Antibiotics

Figure 4: Percentage Inhibition of Growth of *K. pneumoniae* by Crude and Partially Purified Fractions of Defatted Ethanol Extracts of *F. capensis* and some Standard Antibiotics
Figure 5: Percentage Inhibition of Growth of *S. aureus* by Crude and Partially Purified Fractions of Defatted Ethanol Extracts of *F. capensis* and some Standard Antibiotics

Figure 6: Percentage Inhibition of Growth of *A. baumannii* by Crude and Partially Purified Fractions of Defatted Ethanol Extracts of *F. capensis* and some Standard Antibiotics
All the fractions except FC_dEtOH_FM5 and FC_dEtOH_FM6 produced significantly (p<0.05) higher percentage of inhibitions of *P. aeruginosa* compared to FC_dEtOH as shown in Figure 3. FC_dEtOH_FM and FC_dEtOH_FM4 with a 100% inhibition at 100µg/ml were favourably comparable (p>0.05) to the standard antibiotic, gentamicin and chloramphenicol.

The percentage growth inhibition of *K. pneumoniae* by FC_dEtOH_FM5 was the only growth inhibition by a fraction that was not significantly (p<0.05) higher than that of the crude extract as shown in Figure 4. The standard antibiotic, gentamicin and FC_dEtOH_FM2 both completely inhibited (100%) the growth of *K. pneumoniae* at 100 µg/ml. The isolate of *S. aureus* tested was susceptible to the crude extract of *F. capensis* as well as its partially purified fractions. Figure 5 showed that FC_dEtOH_FM1 and FC_dEtOH_FM2 significantly (p<0.05) inhibited the growth of the test organism by the highest percentage, which was comparable (p>0.05) to that of gentamicin.

The percentage inhibition of *A. baumannii* by FC_dEtOH_FM3 was significantly (p<0.05) higher than that of all other extracts and the standard antibiotics as shown in Figure 6. FC_dEtOH_FM1 and FC_dEtOH_FM2 also significantly (p<0.05) inhibited the growth of the test organism compared to the standard antibiotics. When used at 100 µg/ml concentration, FC_dEtOH_FM3 and FC_dEtOH_FM4 produced 100% inhibition of *B. cepacia* as shown in Figure 7.

**DISCUSSION**

The growth rate of the six test organisms peaked at 12 hours on incubation, the percentage inhibition of the extracts; standard antibiotics and negative control were therefore calculated using the optical density values at 12 hours post incubation. This is to ensure that the results obtained are as a result of the activities of the extracts rather than due to natural decline in the bacterial growth rate.

The crude extract and the 6 partially purified fractions of the plant sample exhibited a wide spectrum of activities against the test organisms. The most active fraction against each of the bacteria had activity higher than that of the crude extract, which suggests that fractionation of the crude extract leads to increased antibacterial activity.

The increased activity of the partially purified fractions over the crude extract is not necessarily due to the fractionation process since loss of potentiation from fractionation of crude extract has been reported (Musa et al., 2014). In contrast, Banfi *et al.* (2014) have reported increased potentiation with purification process. The apparent increased potentiation of the partially purified fractions here could be explained by the fact that weight-for-weight, there is a higher concentration of the active principle responsible for the antibacterial activity in the active fraction than in the total crude extract.

![Figure 7: Percentage Inhibition of Growth of *B. cepacia* by Crude and Partially Purified Fractions of Defatted Ethanol Extracts of *F. capensis* and some Standard Antibiotics](image-url)
One fraction of the crude extract, FC_dEtOH_FM1, had above 95% inhibition against E. coli. Similarly, three, three, three, four and four fractions of the crude extract had above 95% inhibition against P. aeruginosa, K. pneumonia, S. aureus, A. baumanii and B. cepacia respectively. This suggests that the plant may contain more than one active principle responsible for the antibacterial activity against each organism or else it could be one active principle which sequesters into the various fractions in different concentrations. If it is one active principle, the crude extract would have contained a higher weight for weight concentration of the active principle than the fractions, and would have therefore demonstrated a stronger antibacterial activity, but that is clearly not so. A possible explanation to support a single active principle would be the presence of another substance whose presence in the mixture reduces the potency of the active principle (Musa et al., 2013). The other substance could have sequestered into fractions 5 and 6, but not into the first four fractions, which explain the increased antibacterial activities of the first four fractions compared to the crude extract, and the reduced antibacterial activities of fractions 5 and 6 compared to the crude extract.

As reported by Oyeleke et al. (2008) and François et al. (2010), the plant sample produced strong antibacterial activity against E.coli, which causes diarrhoea, with FC_dEtOH_FM1 having an inhibition of 95.32% which is higher than that of the most potent standard antibiotic used, Amoxicillin (94.94%). This suggests that the plant has potential for development as therapy against infective disorders caused by E. coli.

FC_dEtOH_FM2 had 100% inhibition of K. pneumonia and S. aureus which was higher than that of the standard antibiotics used. These organisms cause pneumonia (Podschun and Ullmann, 1998) and impetigo (Bowen et al., 2014) among many other diseases. Adebayo-Tayo and Odeniyi (2012) also reported that ethanol leaf extract of F. capensis exhibited strong antibacterial activities against K. pneumonia and S. aureus. This suggests that F. capensis bioactive substances that has potentials for development of therapy against diseases caused by K. pneumonia and S. aureus.

FC_dEtOH_FM3 and FC_dEtOH_FM4 had 100% inhibition of P. aeruginosa, same as the standard antibiotics. This agrees with the findings of Adebayo-Tayo and Odeniyi (2012). FC_dEtOH_FM3 also produced 100% inhibition of A. baumanii and B. cepacia. These three organisms are nosocomical pathogens (Woods et al., 2004; Stratueva and Yordanov, 2009; Almasaudi, 2016), these results therefore suggest that F. capensis contains active principle that may be useful in the management and therapy of nosocomical infections.

The findings of this investigation provide a basis for the development of the plant in Igala folk medicine for the treatment of a wide range of infective disorders.

**Conclusion**

From the results of this investigation, *Ficus capensis* contain active principle(s) that may have broad-spectrum antibacterial activity. Fractionation of the crude extract leads to increased potentiation against the test organisms used. The antibacterial activities of the partially purified fractions of defatted ethanol extract of *F. capensis* compares favourably with standard antibiotics.

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