



Comparison of Diffusion and Spectrophotometric Methods for *in Vitro* Antibacterial Evaluation of Extract of *Ficus capensis* on Six Bacteria

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ABSTRACT

As part of an ongoing study to provide pharmacological basis for the application of *Ficus capensis* in Igala traditional medicine for the treatment of several febrile illnesses and infective disorders, the antibacterial activity of the plant extract was investigated at low concentrations using diffusion and spectrophotometric methods. This was aimed at determining the sensitivity of the methods at low concentrations. The millimetre zone of inhibition was determined by agar well diffusion method and the percentage growth inhibition was determined by spectrophotometric assay. The minimum inhibitory concentration (MIC) was determined by broth diffusion method. At 25 µg/ml the percentage growth inhibition of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Burkholderia cepacia*, obtained by spectrophotometric method were 69.16%, 67.1%, 70.18%, 70.14%, 69.34% and 67.24% respectively. At the same concentration the diffusion methods indicated that the extract had no antibacterial activity on the test organisms. As the concentration of the extract increased, the diffusion method showed concentration dependent activity on the test organisms. The MIC of the extract for each of the test organisms; *E. coli* (100 µg/ml), *P. aeruginosa* (100 µg/ml), *K. pneumonia* (50 µg/ml), *S. aureus* (50 µg/ml), *A. baumannii* (100 µg/ml) and *B. cepacia* (100 µg/ml) indicated that the extracts inhibited the growth of some organisms at concentrations where zone of inhibition was zero. This suggests that spectrophotometric determination of antibacterial studies is more sensitive than dilution methods, and the broth dilution method is more sensitive than the solid agar well diffusion method.

Keywords: *Ficus capensis*, Igala, spectrophotometric assay, agar well diffusion, MIC.

INTRODUCTION

Natural products from plants have served as the source of inspiration for most of the current pharmacopeia. There is therefore a growing interest in discovering and developing new antibacterials from medicinal plants. This has led to paying great attention to the methods of evaluating the antibacterial potentials of these natural products. There are several methods available to detect the antibacterial activity of plant extracts and since they are not all based on the same principles, the results obtained may be influenced by the selected method, as well as by the microorganisms used, and by the extraction method or the degree of solubility of each test compound (Klančnik *et al.*, 2010).

Well known disc diffusion, agar well diffusion and broth agar diffusion have been commonly used (Balouiri *et al.*, 2015). Spectrophotometric assays are also increasingly being employed in the determination of antibacterial activity (Arthington-Skaggs *et al.*, 2002; Hern *et al.*, 2009; Akujobi and Njoku, 2010; Fagbohun *et*

al., 2015). Other methods such as flow cytofluorometric and bioluminescent methods are not widely used because they require specified equipment (Balouiri *et al.*, 2015).

Ficus capensis commonly known as fig tree or cape fig belongs to the moraceae family. In Igala traditional medicine, it is used for the treatment of several febrile ailments, infectious diseases and for boosting the immune system (Daikwo *et al.*, 2012). It is also used as therapy for a wide range of infective disorders in order parts of Nigeria (Igoli *et al.*, 2005; Ojokuku *et al.*, 2010). There is a study ongoing to determine the pharmacological basis or otherwise for the traditional application of *F. capensis* for the treatment of infective disorders. This current study is aimed at determining the sensitivity of

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two methods of evaluating antibacterial studies at low extract concentrations.

METHODOLOGY

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, 100 g were cold macerated in 1 L of Cyclohexane, allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The residue was cold macerated in 1 L of dichloromethane, allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The residue obtained was similarly cold macerated in 1 litres of methanol, allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The residue obtained was then cold macerated in 1 L of distilled water and allowed to stand for 24 h and filtered with Whatmann No 1 filter paper. The filtrate was concentrated to dryness using a water bath. The yield of the crude extract was determined relative to the starting material.

Antibacterial Studies

The zone of inhibition of the extract was determined by the agar well diffusion technique described by Odama *et al.* (1986) as modified by Musa *et al.* (2011). A suspension of the standardized inoculum (0.2 ml) was seeded into 600 ml of molten Mueller-Hinton agar at a temperature of 40°C. The seeded agar was poured aseptically into sterile Petri dishes allowed to set at room temperature. The solidified agar was bored with a sterile 8 mm cork borer to create 5 wells on the agar plate about 10mm deep. The wells were filled with 0.1 ml of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml of the reconstituted crude extract and the fifth well was filled with distilled water to serve

as solvent control. A plate was similarly prepared for Gentamicin to serve as the positive control. The plates were incubated for 24 hours at 37°C. The resulting zones of inhibitions in mm were then measured using a transparent ruler. The experiment was carried out in triplicates. The results are presented as the mean values.

The percentage inhibition of the bacteria by the extracts was determined by spectrophotometric method of Banjara *et al.* (2012) as modified by Musa *et al.* (2017). s follows. Four test tubes were prepared for each for the extract and the standard antibiotic, gentamicin. The test tubes contained 4 ml samples of 200 µg/ml, 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. Three test tubes were also prepared containing 4ml of the negative control sample and one drop (0.025 ml) of the standardized 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 was 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.

The MIC was determined using the serial broth dilution method. Four test tubes each were prepared for the extracts and the standard antibiotic. The test tubes contained 3ml of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml of reconstituted crude extract in nutrient broth agar. A drop (0.025ml) of the standardized bacteria was then suspended in each of the test tubes. They were then incubated at 37°C for about 15 to 24 hours. The experiment was carried out in triplicates and results recorded as mean values. The MIC was determined as the lowest concentration of crude extracts that inhibited visible growth (turbidity) of the test organisms after 18 hours.

RESULTS

Water which served as the negative control for the measurement of diameter zone of inhibition did not inhibit the growth of the microorganism. The kinetics of the microbial growth is as shown in Fig. 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 nutrient broth without the plant extract or standard antibiotics produced no inhibition of any of the test organism at 12 hours post inoculation.

At 25 µg/ml and 50 µg/ml concentrations, defatted aqueous extract of *F. capensis* had zero millimeter zone of inhibition of the six bacteria isolates as presented in Tables 1 and 2, whereas Figures 1 and 2 showed that the extract inhibited the growth of the test organisms ranging from 67.24% to 79.2% at the same concentration.

Tables 3 and Figures 3 and 4 showed a concentration dependent increase in antibacterial activity of the extract against the test organisms. The MIC of the extract against the test organisms are as presented in Table 5.

Table 1: Diameter Zone of Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 25 µg/ml concentration

| Test Organism | <i>F. capensis</i> | Gentamicin |
|----------------------|--------------------|------------|
| <i>E. coli</i> | 0 | 0 |
| <i>P. aeruginosa</i> | 0 | 1 |
| <i>K. pneumoniae</i> | 0 | 2 |
| <i>S. aureus</i> | 0 | 0 |
| <i>A. baumannii</i> | 0 | 1 |
| <i>B. cepacia</i> | 0 | 1 |

Table 2: Diameter Zone of Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 50 µg/ml concentration

| Test Organism | <i>F. capensis</i> | Gentamicin |
|----------------------|--------------------|------------|
| <i>E. coli</i> | 0 | 0 |
| <i>P. aeruginosa</i> | 0 | 2 |
| <i>K. pneumoniae</i> | 0 | 3 |
| <i>S. aureus</i> | 0 | 0 |
| <i>A. baumannii</i> | 0 | 1 |
| <i>B. cepacia</i> | 0 | 1 |

Table 3: Diameter Zone of Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 100 µg/ml concentration

| Test Organism | <i>F. capensis</i> | Gentamicin |
|----------------------|--------------------|------------|
| <i>E. coli</i> | 0 | 1 |
| <i>P. aeruginosa</i> | 1 | 4 |
| <i>K. pneumoniae</i> | 2 | 4 |
| <i>S. aureus</i> | 1 | 5 |
| <i>A. baumannii</i> | 2 | 3 |
| <i>B. cepacia</i> | 1 | 3 |

Table 4: Diameter Zone of Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 200 µg/ml concentration

| Test Organism | <i>F. capensis</i> | Gentamicin |
|----------------------|--------------------|------------|
| <i>E. coli</i> | 2 | 2 |
| <i>P. aeruginosa</i> | 1 | 6 |
| <i>K. pneumoniae</i> | 2 | 5 |
| <i>S. aureus</i> | 3 | 5 |
| <i>A. baumannii</i> | 2 | 3 |
| <i>B. cepacia</i> | 1 | 4 |

Table 5: Minimum Inhibitory Concentrations of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms

| Test Organism | <i>F. capensis</i> | Gentamicin |
|----------------------|--------------------|------------|
| <i>E. coli</i> | 100 µg/ml | 100 µg/ml |
| <i>P. aeruginosa</i> | 100 µg/ml | ≤25 µg/ml |
| <i>K. pneumoniae</i> | 50 µg/ml | ≤25 µg/ml |
| <i>S. aureus</i> | 50 µg/ml | 50 µg/ml |
| <i>A. baumannii</i> | 100 µg/ml | ≤25 µg/ml |
| <i>B. cepacia</i> | 100 µg/ml | ≤25 µg/ml |

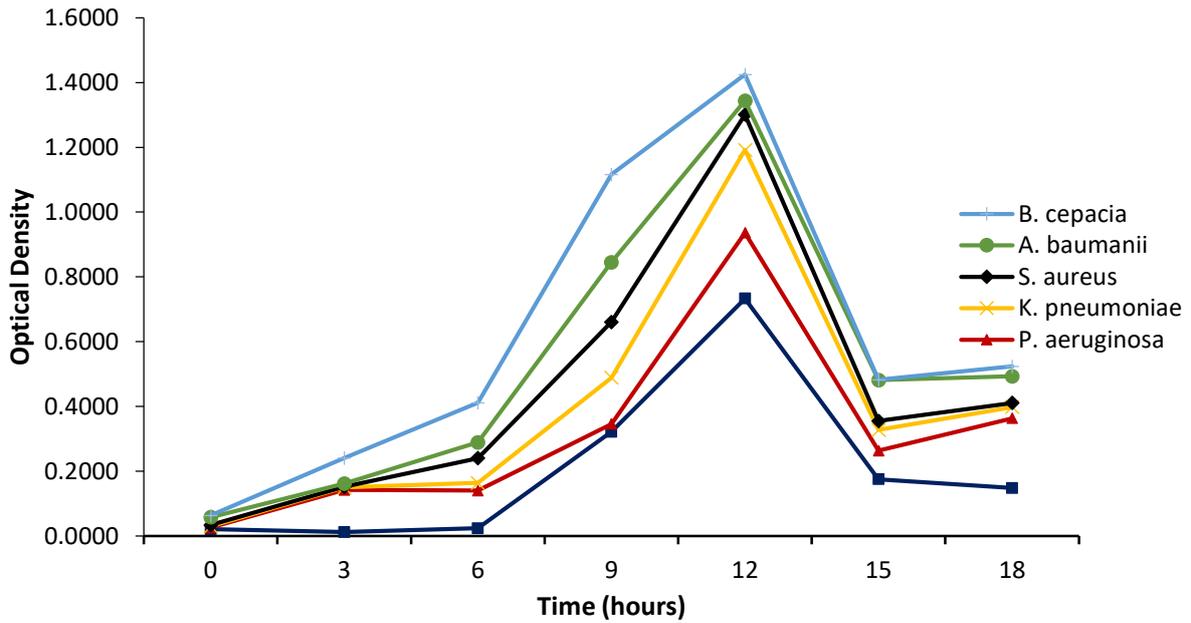


Fig. 1: Growth rate of six bacteria using the measurement of their optical density at 540nm

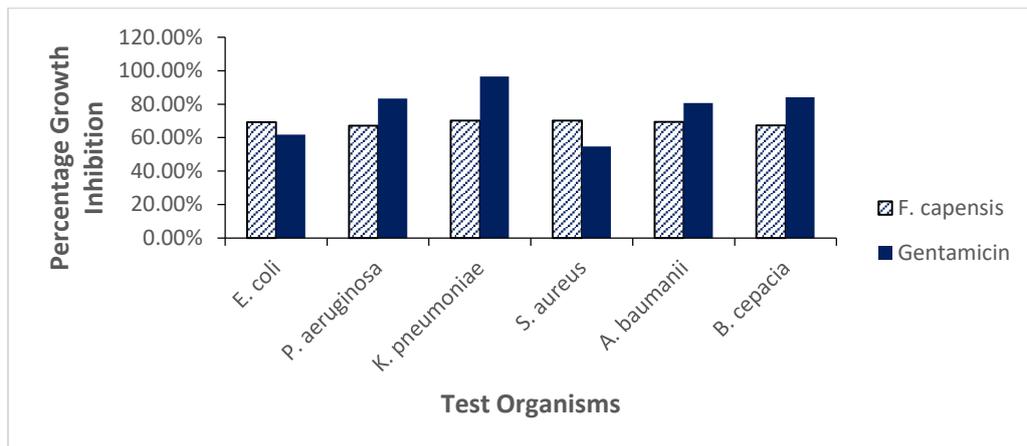


Fig. 2: Percentage Growth Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 25 µg/ml concentration

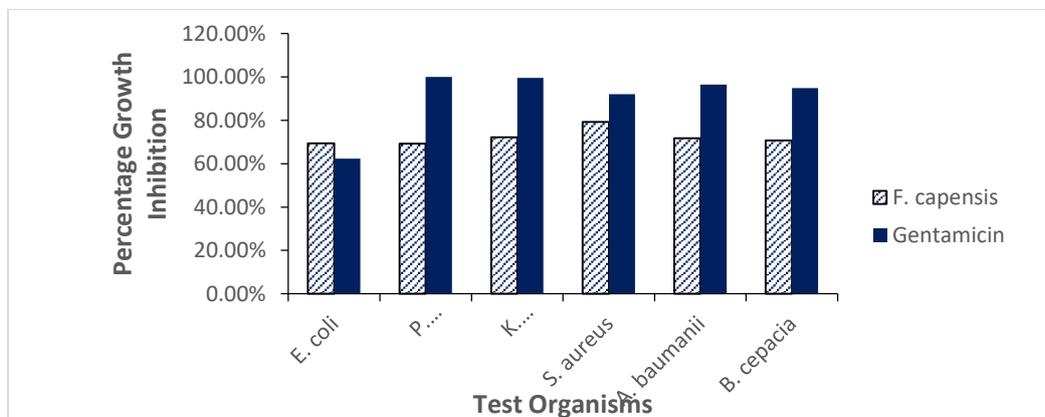


Fig. 3: Percentage Growth Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 50 µg/ml concentration

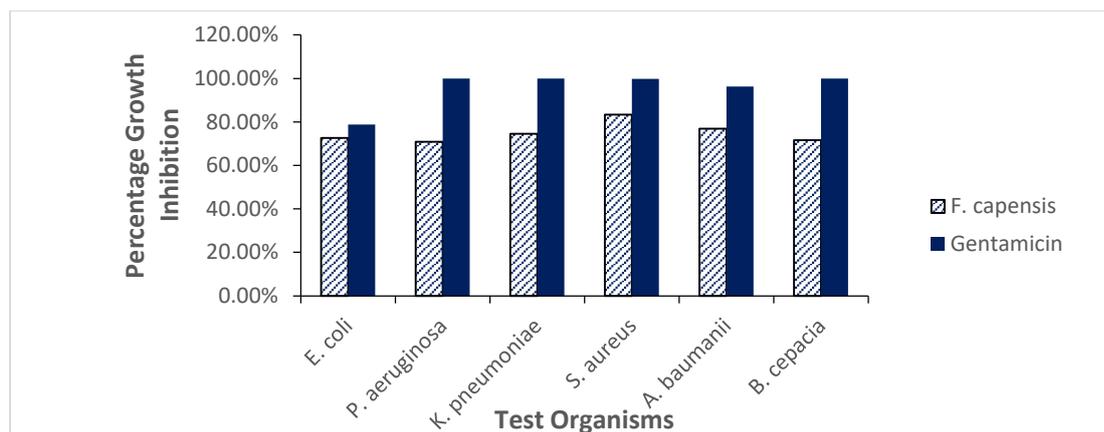


Fig. 4: Percentage Growth Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 100 µg/ml concentration

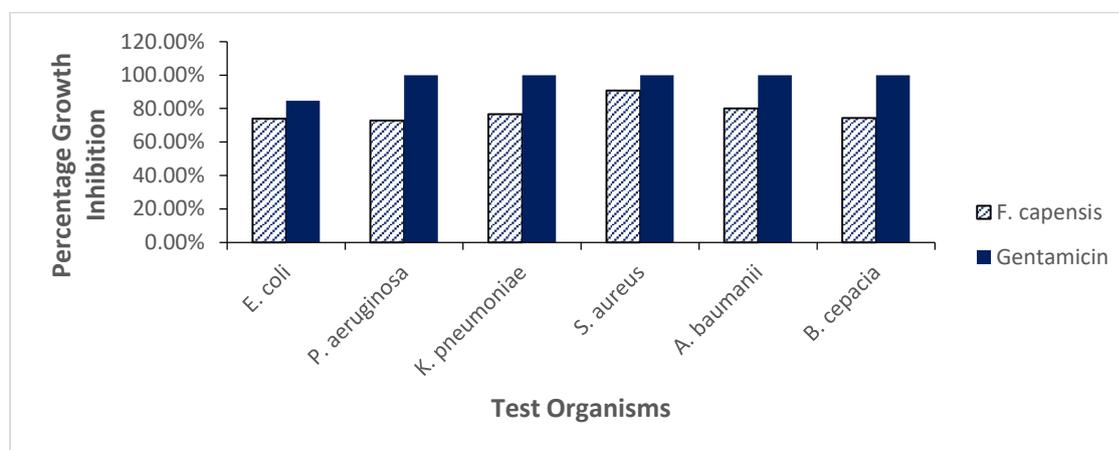


Fig. 5: Percentage Growth Inhibition of Deffated Aqueous Extract of *F. capensis* and standard antibiotic on Six Test Organisms at 200 µg/ml concentration

DISCUSSION

Water and nutrient broth which served as negative controls for the two methods did not inhibit the growth of any of the test organisms. This implies that all bacterial growth inhibition demonstrated by the extract and standard antibiotic was not as a result of the solvent in which they were reconstituted.

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 extract demonstrated a concentration dependent antibacterial activity against all the test organisms, this suggests that the extract might have potential to be investigated further for the development of a broad spectrum antibiotic. Although the activities of the extract on the test organisms were all lower than that of gentamicin, a standard antibiotic, it should be noted that the extract is in a crude form, and purification of the active principle may lead to potentiation (Nwodo *et al.*, 2010; Tamokou *et al.*, 2011).

The diffusion methods employed in this studies were agar well diffusion method and serial broth dillution method. The well diffusion method is based upon a solid cuture medium and measured the diameter zone of inhibition, whereas the

serial dilution method is based on a liquid broth medium and measured the MIC. MIC results showed that at 50 µg/ml the extract inhibited growth of *K. pneumonia* and *S. aureus*, meanwhile the extract had 0 mm zone of inhibition on the same bacteria at that concentration. Similarly, MIC results showed that the extract inhibited the growth of *E. coli* at 100 µg/ml, but the agar well diffusion method indicated no inhibition of the bacteria at the same concentration. This suggests that serial broth dilution method is more sensitive than well diffusion method in determining the antibacterial activity of plant extracts at low concentrations. This can be explained by the fact that it is easier for the active compound to traverse a liquid medium and produce its effect than through a solid medium.

The percentage inhibition of growth of test organisms obtained by spectrophotometric means showed that the extract inhibited *E. coli* (69.16%), *P. aeruginosa* (67.1%), *K. pneumonia* (70.18%), *S. aureus* (70.14%), *A. baumannii* (69.34%) and *B. cepacia* (67.24%) at 25 µg/ml, whereas, the diffusion methods indicated that the extract had no activity on the test organisms at this concentration. This suggests that spectrophotometric determination of antibacterial studies is more sensitive than dilution methods.

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