

Full Length Research Paper

Antibacterial activity of *Ficus capensis*

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Accepted 13 March, 2008

The leaves and stem bark of *Ficus capensis* were investigated for antibacterial activity against some selected organisms at a concentration of 2000 µg/ml using agar diffusion method. The crude leaf extract inhibited the growth of *Escherichia coli* and *Shigella* sp. but no activity against *Salmonella typhi*. The stem bark extracts also had activity against *E. coli* and *Shigella* sp. but no activity against *S. typhi*. The phytochemical screening of the extracts reveals the presence of alkaloids, balsams, tannins, carbohydrates, resins, flavonoids, Sterols and terpenes. Glycosides were absent in the leaf extract while the stem bark extract had glycosides. Both extracts did not possess free arthaquinones and saponins. The Minimum inhibitory concentration (MIC) of the extracts range from 500-2000 µg/ml. The fractions obtained from the thin layer chromatography had no activity on the test organisms. The results from the activity of the crude extracts suggest that *F. capensis* could be used in treatment of diseases caused by these bacteria except *S. typhi*.

Key words: *Ficus capensis*, antibacterial, phytochemical.

INTRODUCTION

Plants parts, which have one or more of its organs containing substances that can be used for therapeutic purpose, are called medicinal plants (Sofowora, 1982). Plants have been used as medical agents from the earliest days of man's existence (Hansch, 1990; Shellard, 1987) and has made it necessary to study them in details in order to discriminate the kinds employed for different purposes (Ghani, 1986). The seeds of *Opium poppy* (*Papaver somniferum*) and castor oil seeds (*Ricinus communis*) were excavated from some ancient Egyptian tombs which indicated their use as far back as 1500 BC (Sofowora, 1986). Oboh and Abula (1997) reported the antimicrobial activity of the extracts of *Citrus aurantifolia* leaves. The fruit juice has also been shown to inactivate *Escherichia coli*. Garlic (*Allium sativum*) is used to reduce cholesterol levels and to boost immune system; it lowers high blood pressure and its oil has been seen to have antibacterial properties (Juurlink, 2001). Milk thistle *Silybum marianum* has also been used to protect the liver by treating a wide range of liver conditions; it can be used for increasing breast milk production (Juurlink, 2001).

Ficus capensis commonly known as fig tree is a medicinal plant found in terrestrial zones mostly along rivers (www.foa.org, 2003). *F. capensis* is a spreading deciduous or evergreen tree with a thick bole and spreading roots. It produces fruits throughout the year and the leaves are broad and green (Thomas 1988). In Nigeria, *F. capensis* has been used by the Igbo people as treatment for dysentery and in wound dressing (Igoli et al., 2005). It is also used in circumcision, leprosy and epilepsy (Joshua, 2006).

The objectives of this study are to identify the phytochemical components of *F. capensis*, determine the antibacterial activity of the extracts of the leaves and stem bark on selected bacterial species, and to determine the activity of the purified fractions of extract on thin layer chromatography.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves and stem bark of *F. capensis* were collected from New Nyanya area of Nasarwa state. The plant materials were identified by Professor Z. I. E. Ezenwa of the School of Agriculture and

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Processing of plant material

The identified leaves and stem bark of *F. capensis* were washed and air-dried over the period of two weeks. The dried samples were milled into fine powder by pounding manually with a clean and sterile mortar. The powder were each collected into sterile cellophane bags and labeled to prevent mix up. The samples were kept in a cool dry place till further use.

Extraction of plant material

Shoxlet extraction method was employed. 40 g of sample was weighed. 400 ml of methanol and water were used as extractant (methanol because it is a polar solvent and distilled water was used because it is a universal solvent). The process was run for 6 h after which the sample was evaporated to dryness using steam evaporator. The dried extracts were weighed and kept in a well labelled sterile specimen bottles.

Phytochemical screening

Several tests were carried out to detect the phytochemical components present in the extracts using standard procedures of Evans (1989) and Sofowora (1982).

Screening of test organisms

The microorganisms used (*Escherichia coli*, *Shigella* sp. and *Salmonella typhi*) were collected from National Institute for Pharmaceutical Research Development (NIPRD) Idu Abuja and General Hospital Minna. The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24 h. The microorganisms were repeatedly subcultured in order to obtain pure isolates. Morphological and biochemical reactions were carried to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4°C.

A loop full of test organism was inoculated on nutrient broth and incubated for 24 h. 0.2 ml from the 24 h culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3-5 h to standardize the culture to 10⁶ cfu/ml.

Screening of extracts for antimicrobial activity

The agar diffusion method was used. Sterile nutrient agar plates were prepared. 1 ml of test organism was added to 19 ml of the nutrient agar each plate was properly labeled. A sterile cork borer (4 mm) was used to make wells in each plate for the extracts. The base of each ditch was filled with sterile molten nutrient agar to seal the bottom and left for some time to allow it to gel. 0.2 ml of the extract was dispensed into each well. The plates were left to allow diffusion of the extracts before being placed in the incubator at 37°C for 24 h. The zones of inhibition produced after incubation was measured and recorded.

Determination of minimum inhibitory concentration (MIC)

The agar dilution method was used. 0.2, 0.1, 0.05 and 0.025 g of the extracts were dissolved in 5 ml of sterile distilled water. 1 ml each of the extracts was added to 19 ml of sterile molten nutrient

agar, mixed thoroughly and poured into pre-labeled sterile Petri dishes to make a final concentration of 2000, 1000, 500 and 250 ug/ml respectively a loop full of the standardized bacteria culture was used to inoculate the plates which were incubated at 37°C for 24 h. Growth of organisms on each concentration was checked to determine the minimum concentration that inhibits growth of test organism.

Determination of minimal bactericidal concentration (MBC)

Samples were taken from the nutrient agar plates that showed no visible growth after 24 h incubation and sub cultured into freshly prepared sterile nutrient agar. The least concentration that did not produce growth after 24 h was regarded as the MBC.

Thin layer chromatography (TLC)

Mini thin layer chromatography plates were prepared using microscopic slides. The slide were cleaned with acetone, and washed with hot water to remove all stains, dirt and oil marks. 2 g of silica gel (Merek AR60) was mixed with 4 ml of distilled water and ground in a mortar until it began to thicken. The slurry was then poured on the slide and spread evenly with a glass spreader. It was allowed to set for 5 min and then dried at 110°C in the oven for at least 15 min to activate the plates and allowed to cool by exposing the plates to the atmosphere for 30 min excessive silica gel was carefully removed from the slides using a razor blade. Preparation of the macro plates involved the same procedure; using 20 x 10 cm glass plates. 25 g of silica gel was mixed with sterile distilled water.

Chromatographic separation of the methanol extracts of *F. capensis* leaves and stem bark on the preparative macro plates involved the use of a solution of dried extract which was made by dissolving 1 g of extract in 3 ml of the solvent. A capillary tube was used to make a concentrated band of the solution on the TLC plate about 2 cm from the base of the plate the mobile phase used was a mixture of ethylacetate, methanol and triethanolamine in a ratio 16:2:2 this was put in a glass tank. The tank was closed and allowed to stand for about 10 min so that the tank becomes saturated with solvent. The plate was inserted in to the tank with the origin spot towards the bottom of the tank. The spot was higher than the level of the solvent. The glass tank was covered tightly and the solvent allowed to ascend until it gets close to the top. The plates are removed and dried in the oven. They are placed into an iodine tank which makes the spot visible. Distance moved by the solvent and that moved by the extract were measured.

Determination of antimicrobial activity of fractions separated from the preparative macro TLC plates

The activity of reconstituted extract from the resultant bands was determined against the test organisms using standard culture. 0.01 g of each extract was weighed and dissolved in 5 ml of water to give a concentration of 100 µg/ml. Sterile nutrient agar was prepared and the standard culture inoculated wells were made and filled with the extracts the plate were incubated for 24 h at 37°C after which zones of inhibition were observed.

RESULT

Results of the thin layer chromatography on leaf and stem bark of *F. capensis*

In the thin layer chromatography using ethylacetate,

Table 1. Phytochemical screening of *Ficus capensis*.

Test carried out	Leaf extract	Stem bark extract
Alkaloids	+	+
Balsams	+	+
Carbohydrates	+	+
Flavonoids	+	+
Free Anthraquinones	-	-
Glycosides	-	+
Tanins	+	+
Terpenes	+	+
Resins	+	+
Sterols	+	+
Saponins	-	-

+ = Present; - = Absent.

Table 2. Antibacterial activity of leaf and stem bark extracts of *Ficus capensis* (zones of inhibition in mm).

Organism	Leaf extract		Stem bark extract		Ampiclox (control, mm)
	Methanol extract (mm)	Aqueous extract (mm)	Methanol extract (mm)	Aqueous extract (mm)	
<i>Escherichia coli</i>	16	21	16	16	19
<i>Shigella sp</i>	15	16	14	18	21
<i>Salmonella typhi</i>	-	-	-	-	-

- = No inhibition.

triehanolamine and methanol (16:2:2), three spots were obtained from the methanol extract of the stem bark while for the methanol extract of the leaf, four spots were obtained. The fractions obtained from the leaves are Rf of 0.24, 0.4, 0.35 and 0.5. Those of the stem bark are Rf 0.4, 0.3 and 0.6. All did not exhibit any antimicrobial activity against the tested organisms

Photochemical screening

The result of the photochemical screening reveals the presence of a number of components. Result is shown in Table 1.

Antimicrobial activity of the crude extracts of *F. capensis*

The result of the antimicrobial activity of the crude extract of leaf and stem bark *F. capensis* on test organisms are shown in Table 2.

Minimum inhibitory concentration of the crude extracts

The results of the MIC screening of crude aqueous and methanolic extracts of the stem bark of *F. capensis* is

shown on Table 2. The results reveal that the minimum inhibitory concentration was between 1000 to 2000 µg/ml. The results of the screening for minimum inhibitory concentration of methanolic extracts of the leaf of *F. capensis* is shown on Table 3. The results reveal that the minimum inhibitory concentration was between 500 to 2000 µg/ml.

DISCUSSION

The preliminary photochemical screening of leaves and stem bark extracts of *F. capensis* revealed the presence of alkaloids, balsams, carbohydrates, flavonoids, free anthraquinones, tanins, glycosides, terpenes, resins, sterols and saponins. Glycosides were not present in the leaf but present in the stem bark. The presence of alkaloids reveals its activity against pathogenic bacteria. According to Ebana et al. (1991), alkaloids inhibit pathogenic bacteria. Tannins are important in herbal medicine in treating wounds and to arrest bleedings (Nguyi, 1988). This also confirms the use of *F. capensis* for wounds in circumcision (Joshua, 2006).

The extracts had varying degrees of antimicrobial activity against the test organisms. The leaf aqueous extract had the highest activity with a zone of inhibition between 16 – 21 mm as compared to that of the stem bark (16 – 18 mm). The methanol extracts of the leaf had

Table 3. Minimum inhibitory concentration of the extracts of *Ficus capensis*.

Organism	Leaf extract		Stem bark extract	
	Methanol extract (µg/ml)	Aqueous extract (µg/ml)	Methanol extract (µg/ml)	Aqueous extract (µg/ml)
<i>Escherichia coli</i>	500	500	1000	1000
<i>Shigella</i> sp	1000	1000	2000	2000
<i>Salmonella typhi</i>	-	-	-	-

- = No inhibition.

zone of inhibition of 15 – 16 mm and stem bark in 14 – 16 mm. The strong activity of the leaf suggests that it may be used for treatment of infections caused by these organisms except *S. typhi*. The minimum inhibitory concentration (MIC) obtained reveals the effectiveness of the plant extract as chemotherapeutic agents. The organisms were inhibited at concentrations ranging from 500 – 2000 µg/ml. *S. typhi* was resistant to the concentrations ranging from 250 – 2000 µg/ml. The most susceptible organism to the antimicrobial activity of *F. capensis* is *E. coli*. The fractions obtained from thin layer chromatography were found not to have any antimicrobial activity against the test organisms and the separated fractions indicated that the plant *F. capensis* possesses more than one active component. This agrees with Harbourne (1984) who reported that the activity of plant extracts can sometimes change after fractionation and a pure component eventually obtained may lack the activity of the original extract.

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