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# Phytochemical, Antimicrobial and Free Radical Scavenging Activity of *Ficus capensis* Thunb Leaves

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To evaluate the phytochemical constituents of the ethanol leaf extract of *Ficus capensis* using GC-MS, its antimicrobial and in vitro antioxidant activities.
**Study Design:** The study was designed to identify the phytochemicals present in *Ficus capensis*, to test the inhibitory ability of the plant extract on human pathogens and to ascertain its antioxidant activities.
**Place and Duration of Study:** Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri. Imo state and Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia. Abia state, between June to November, 2013.
**Methodology:** The phytochemicals from the stem of *Ficus capensis*Thunb were extracted with ethanol and subjected to GC/MS analysis and the identification of compounds was done by comparing spectrum of the unknown component with the spectrum of the known components stored in the NIST library. The antibacterial activity was performed by filter paper disc diffusion technique. The antioxidant activity of the extract was tested using DPPH and FRAP assays.

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**Results:** The results of the analysis revealed that ethanol extract of *Ficus capensis* contains twelve compounds with n- Hexadecanoic acid forming the bulk of the oil (40%). Other compounds identified include: glycerin, 4-(2, 6, 6-trimethylcyclohexa-1, 3-dienyl) but-3-en-2-one, 3-Acetoxy-4-cyano-2, 5-dimethylpyridine, tetradecanoic acid etc. The ethanol extract inhibited all the tested organisms *S. aureus, P. mirabilis, K. pneumoniae*, *Salmonella typhi* and *E. coli* though not to a high extent. The antioxidant activity of the extract increased with increasing concentration of the extract. The optimum activity was observed at 200 µg/ml.
**Conclusion:** The constituents of this plant suggest its varied medicinal uses in ethno-medicine. Further research is needed to determine the possible mechanism of action of these phytoconstituents.

*Keywords: Human pathogens; antioxidant; ethno-medicine; pytoconstituents; antimicrobial; Ficus.*

## 1. INTRODUCTION

The use of plants as source of medicine is as old as man's existence. There has been a continuous search for new phytochemicals and bioactive principles of medicinal plants that could be used for both pharmacological and nutritional purposes [1]. Extracts from plants have been used as alternative remedies for many infectious diseases [2]. Nigeria is blessed with many of such plants which include *Ficus capensis* Thunb.

*Ficus capensis* commonly known as fig tree is a medicinal plant found in terrestrial zones mostly along rivers. It belongs to the family of *Moraceae*; it produces fruits throughout the year with broad and green leaves [3]. It is a spreading deciduous or evergreen tree with a hick bole and spreading roots. The plant has been used extensively in traditional medicine for the management and treatment of leprosy, epilepsy, rickets, infertility, gonorrhoea, oedema, circumcision, respiratory disorders and as emollient [4]. In Nigeria, *F. capensis* has been used for the treatment of dysentery and for wound dressing [5]. It is used as a vegetable in foods with a reported blood boosting effect and an anti-sickling effect of red blood cells [6]. It is also used in herbal medicines to treat threatened abortion [7]. Oyeleke et al. [3] reported the inhibitory effect of the leaves and stem bark of *F. capensis* against *Esherichia coli* and *Shigella* species. Adebayo and Odeniyi [8] reported that the bark extracts had the highest inhibitions on *P. aeruginosa, C. albicans* and *S. aureus.* A study carried out by Njoku-oji et al. [9] showed that the aqueous leaf extract of *F. capensis* decreased body weight, testicular weight and serum testosterone levels yet increased sperm counts in normal adult male wistar rats. There is little or no documented literature on the bioactive components and free radical scavenging effect of the leaves of *F. capensis* and this has necessitated this research work.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Identification

Fresh matured leaves of *F. capensis* were collected from Uzuakoli in Bende LGA of Abia state. The leaves were authenticated by Mr. Ibe K. Ndukwe, a forester in the Herbarium Unit of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria. The voucher specimen number is FHI 25135.

### 2.2 Sample Preparation

The leaves of *F. capensis* were cut into pieces and dried in an oven (SD 93114624, Gallenkamp, United Kingdom) at 40°C. It was ground to powder with the aid of a hammer mill. It was extracted in ethanol for 48 hours, filtered and evaporated to dryness in an oven. The sample used for the free radical scavenging activity was extracted in methanol in a similar manner. The extracts were stored in a refrigerator until it was ready for use.

### 2.3 Gas Chromatography- Mass Spectrum Analysis (GC-MS)

Gas chromatography analysis was performed using GC-MS SHIMADZU QP 2010, JAPAN gas chromatography 5890-11 with a fused GC column ( OV- 101) coated with polymethyl silicon (0.25 nm x 50 m) and the conditions were as follows: Temperature programming from 80-200°C held at 80°C for 1 minute, rate 5°C / min and at 200°C for 20 mins. FID temperature 300°C, injection temperature 250°C, carrier gas

nitrogen at a flow of 1 ml /min, split ratio 1:75. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 kV and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, and hexane were all analytical grades and procured from Merck, Germany.

The interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Subsequently, the details about their molecular formula, molecular weight, structure were also obtained [10].

## 2.4 Antibacterial Evaluation

A concentration of 100 mg/ml of the extract was prepared by dissolving 0.1g of extract in 1.0 ml of dimethylsulphoxide (DMSO).

### 2.4.1 Microorrganisms

Five bacteria organisms used for this study were obtained from the stock culture of the Federal Medical Center, Umuahia. The organisms used include: *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Proteus mirabilis* and *Escherichia coli.* They were brought to laboratory conditions by resuscitating them in peptone water. Thereafter, they were subcultured into nutrient agar medium and incubated at 37°C for 24 hours.

### 2.4.2 Antibacterial activity

Paper disc diffusion method was employed for antimicrobial susceptibility test against the bacteria isolates. Filter paper disc (whatman No1, 6 mm diameter) were placed in glass petri dishes and sterilized in hot air oven [11]. The media (10 g nutrient agar in 200 ml distilled water, auto-claved at 115°C for 30 minutes) was cooled to 50°C. The sterile nutrient agar media were poured into the sterile petri dishes and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2 ml of plant extracts. Discs with DMSO (100 mg/ml) served as a control. The discs were used after drying them in an incubator at 40°C to remove any trace of solvent [12]. Discs were introduced onto the surface of the medium. The plates were incubated at 37°C for 24 hours to obtain zones of inhibition. Ciprofloxacin was used as the standard. The experiments were repeated two times for each extract and the average of these values were Tabulated in Table 2.

## 2.5 Free Radical Scavenging Effect of *F. capensis* Using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assay

This was analysed by the method described by [13]. To 2 ml of varying concentrations (50, 100, 200 and 400 µg/ml) of extract in a test tube were added 1ml of 0.5 mM DPPH (in 1 ml of methanol) each. All the concentrations were prepared in triplicates. They were shaken and kept in the dark for 30 minutes. The blank constituted a mixture of 1ml of methanol and 2ml of the extract while ascorbic acid served as the control. Percentage antioxidant activity was calculated as follows:

$$\% \text{ antioxidant activity} = 100 - [\{(\text{absorbance of sample} - \text{absorbance of blank}) \times 100\} \times (\text{absorbance of control})^{-1}]$$

## 2.6 Ferric Reducing Antioxidant Power (FRAP) Assay

The method described by [14] was employed. From a quantity of freshly prepared FRAP reagent, 3ml was collected and mixed with 100 µl sample solutions in methanol at concentrations of 25, 50, 100, 200 and 400µg/ml. The reaction was monitored for 4 min at 593 nm using spectrophotometer (Spectrum labs, USA) at 37°C. The assay was done in triplicates. Ascorbic acid was used as standard. Calculations were made by using a standard curve.

FRAP value of sample (µMol) = (Changes in absorbance of sample from 4 mins- 0 mins / Changes in absorbance of standard from 4 min- 0 min) × FRAP value of standard (1000 µm)

## 2.7 Statistical Analysis

All values were expressed as mean ± Standard Error of Mean. Statistical analysis was performed using one way analysis of variance (ANOVA).

## 3. RESULTS AND DISCUSSION

### 3.1 GC-MS Analysis

Fig. 1 represents the chromatogram obtained from the GC-MS analysis of the ethanol extract of *F. capensis* Thunb. Table 1 depicts the compounds identified by GC-MS in ethanol extract of *F. capensis.* Twelve compounds were identified with n-Hexadecanoic acid being the most abundant (40%). Other compounds identified include: glycerine; 4-(2,6,6-trimethylcyclohexa-1,3-dienyl)but-3-en-2-one; 3-Acetoxy-4-cyano-2,5-dimethylpyridine; tetradecanoic acid; phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy, pentadecanoic acid, 14-methyl-, methyl ester; 11-Octadecanoic acid, methyl ester; nanodecanoic acid; Z-11-Hexadecanoic acid, Octadecanoic acid; and 13-Docosenamide.

GC-MS analysis is a valuable technique for the identification of the bioactive ingredients in plants which serve as raw materials for pharmaceutical and cosmetic industries. Glycerin, the first compound identified is a very important raw material for cosmetic industry. It has ability to hydrate dry skin and aids desquamation in humans. It also has the ability to alleviate skin irritation caused by surfactants [15]. 4-(2,6,6-trimethylcyclohexa-1,3-dienyl)but-3-en-2-one is an oxygenated monoterpene. Terpenes are very important pharmacological agent with antifungal, antibacterial, antioxidant, anticancer, anti-spasmodic, hypotensive, and vasorelaxant properties [16]. Monoterpenes are useful in cosmetic industry because of their characteristic fragrance. Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy has antioxidant properties. Two ester compounds, Pentadecanoic acid, 14-methyl-, methyl ester and 11-Octadecanoic acid, methyl ester were also identified. Esters are well known for their antioxidant properties and aroma. Three fatty acid compounds tetradecanoic acid, nanodeconoic acid, Z-11-hexadecanoic acid and octadecanoic acid were identified. They have good antimicrobial, antioxidant, hypercholesterolemic, anticancer and hepatoprotective properties [17,18,19]. Oils rich in n-hexadecanoic acid are used to treat rheumatism and inflammation in India [20]. 13-Docosenamide has antimicrobial properties.

### 3.2 Antimicrobial Study

The result of the antimicrobial activity of ethanol extract of *F. capensis* is shown in Table 2. The zones of inhibition of all the microbes tested were much lesser than the control, Ciprofloxacin. Ethanol extracts of the leaves of *F. capensis* exhibited antibacterial activities on the pathogens tested as shown in Table 2. The extracts inhibited all the tested organisms *S. aureus, P. mirabilis, K. pneumoniae*, *Salmonella typhi,* and *E. coli*. This is in line with the reports of previous researchers that *F. capensis* showed significant antibacterial properties [3,21].

### 3.3 DPPH Assay

The result of the free radical scavenging activity of *F. capensis* using DPPH is as depicted on Table 3. The result showed increasing antioxidant activity with increasing concentration of extract. The antioxidant activity of the extract compared favourably with the control.

### 3.4 FRAP Assay

Table 4 presents the result of the free radical scavenging activity of *F. capensis* using FRAP. There was an increase in the antioxidant activity as the concentration of the extract increased. Antioxidants are scavengers of free radicals. They can be synthesized endogenously or exogenously from diet. Most plants are known to possess antioxidative properties [22]. The free radical scavenging activity of ethanol extract of *F. capensis* using DPPH showed that the extract had high antioxidant activity which is comparable to vitamin C, the reference antioxidant. The optimum activity was observed at concentration of 200 µg/ml. At 400 µg/ml of the extract, there was a drop in the antioxidant scavenging activity of the extract. In FRAP antioxidant assay, increase in absorbance reading indicates increase in antioxidant activity. The FRAP antioxidant activity in this study gave a similar trend with that of DPPH. The antioxidant compounds identified in the GC-MS analysis of *F. capensis* gives credence to the observed antioxidant activity of the plant extract. Antioxidants are any species that mop up free radicals. Free radicals have been implicated in a number of disease conditions such as Parkinson and Alzheimer diseases [23]. They cause

premature ageing of skin. However consumption of the leaves of *F. capensis* may play a role in preventing human diseases in which free radicals are involved such as cancer, Parkinson disease, cardiovascular disease and ageing. A combination of the phytoconstituents of this plant may be responsible for the traditional uses of this plant as medicine.

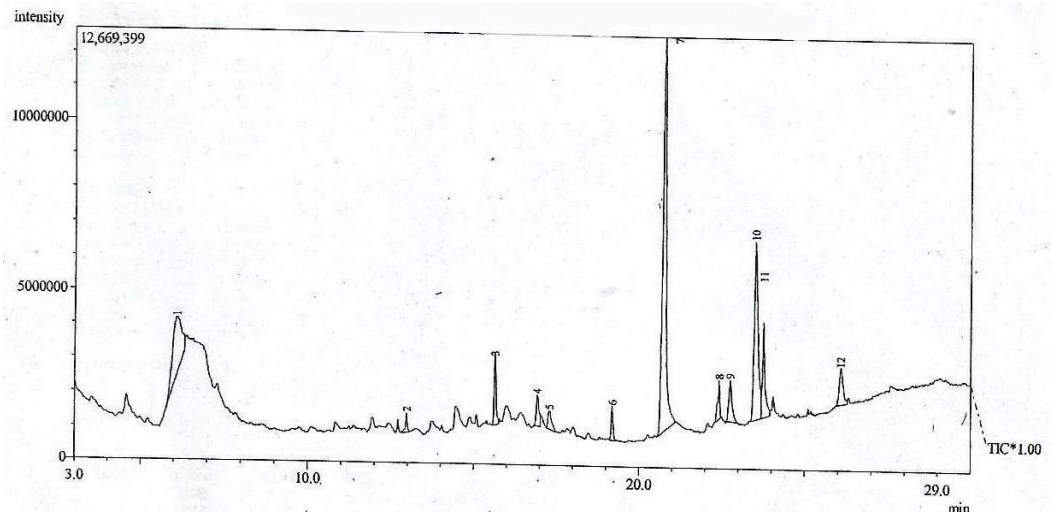


**Fig. 1. Chromatogram of *F. capensis***

**Table 1. Phyto-constituents identified in the ethanol leaf extract of *F. capensis***

| No | RT (min) | Name of compound | Molecular formula | Molecular weight | Peak area % |
|---|---|---|---|---|---|
| 1 | 6.086 | glycerine | C3H8O3 | 92 | 14.04 |
| 2 | 12.970 | 4-(2,6,6-trimethylcyclohexa-1,3-dienyl)but-3-en-2-one | C13H18O | 190 | 1.03 |
| 3 | 15.641 | 3-Acetoxy-4-cyano-2,5-dimethylpyridine | C10H13N2O2 | 190 | 3.70 |
| 4 | 16.920 | Tetradecanoic acid | C14H28O2 | 228 | 2.48 |
| 5 | 17.296 | Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy | $C_{10}H_{12}O_3$ | 180 | 1.73 |
| 6 | 19.187 | Pentadecanoic acid, 14-methyl-, methyl ester | $C_{17}H_{34}O_2$ | 270 | 1.80 |
| 7 | 20.766 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 40.33 |
| 8 | 22.416 | 11-Octadecanoic acid, methyl ester | $C_{19}H_{36}O_2$ | 296 | 2.13 |
| 9 | 22.755 | Nanodecanoic acid | $C_{19}H_{38}O_2$ | 298 | 3.61 |
| 10 | 23.502 | Z-11-Hexadecanoic acid | $C_{16}H_{30}O_2$ | 254 | 19.70 |
| 11 | 23.749 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 6.72 |
| 12 | 26.088 | 13-Docosenamide | $C_{22}H_{43}NO$ | 337 | 3.74 |

**Table 2. Antimicrobial activity of ethanol extract of *F. capensis* leaves**

| Microbes tested | Zone of inhibition (mm) | Zone of inhibition for ciprofloxacin (Control) |
|---|---|---|
| *Staphylococcus aureus* | 8.00 | 15.3 |
| *Klebsiella pneumoniae* | 5.00 | 11.3 |
| *Escherichia coli* | 5.00 | 12.5 |
| *Proteus mirabilis* | 10.0 | 14.0 |
| *Salmonella typhi* | 7.50 | 15.3 |

**Table 3. Free radical scavenging activity of *F. capensis* using DPPH**

| Concentration (µg/ml) | *F. capensis* (%) | Ascorbic acid (%) |
|---|---|---|
| 25 | 89.45±1.31 | 95.50±0.19 |
| 50 | 92.92±0.25 | 95.67±0.03 |
| 100 | 93.38±0.43 | 95.75±0.18 |
| 200 | 94.20±0.29 | 95.17±0.16 |
| 400 | 86.06±5.54 | 94.97±0.14 |

*Values are mean ± SEM of triplicate determinations*

**Table 4. Free radical scavenging activity of *F. capensis* using FRAP**

| Concentration (µg/ml) | *F. capensis* (µmol) |
|---|---|
| 25 | 0.17±0.09 |
| 50 | 0.37±0.22 |
| 100 | 0.84±0.05 |
| 200 | 1.69±0.92 |
| 400 | 0.67±0.20 |
| Ascorbic acid (125 mg/ml) | 2µM |

*Values are mean ± SEM of triplicate determinations*

## 4. CONCLUSION

This study has shown that the plant *F. capensis* is a rich source of pharmacological important compounds which could be extracted for further studies. The plant can be seen as a good antioxidant agent. Though 12 different compounds have been identified, it may have many more unidentified compounds in the same extract which can be isolated by adopting different extraction procedures. The antimicrobial activity of the extract should be studied using higher concentrations of the extract. Possible mechanism of action of the observed antioxidant compounds should be considered for future research.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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