



## INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY

Evaluation of Antioxidant Potency, Antimicrobial Activity and Phytochemical Screening of *Fiscus capensis* Leaves (Cape Fig)Adaji M. U., \*Dauda J. A., Ameh E. M., Ocheme G. W. and Aaron P. E.  
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Received on: August, 2020

Revised and Accepted on: September, 2020

Published on: October, 2020

## ABSTRACT

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into Africa as medicinal plants constituents are effective source of natural products. Phytochemical screening, antioxidant and antimicrobial analyses were carried out on the leaves extract of *Fiscus capensis*. It was extracted selectively using water, methanol and ethanol by maceration. The phytochemical screening of the extracts revealed the presence of flavonoids, saponins, total phenol and tannins with cardiac glycoside and alkaloid in trace amount. The results of the quantitative analysis revealed that the extracts (aqueous, ethanol, methanol) contains in mg/g; tannins (4.23, 12.84, 23.89), total phenol (144.95, 461.28, 662.30), saponins (4.44, 70.44, 115.04), flavonoids (13.22, 20.94, 84.78) and alkaloids (1.70, 1.82, 2.12) respectively. Antimicrobial analysis was conducted on the ethanol extract and it revealed inhibitory activity against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Pseudomonas aeruginosa* at 250 mg/mL concentration with no inhibition against *Escherichia coli*. The zone of inhibition of growth produced by the ethanol extract on the selected microorganism at 50, 100, 150, 200 and 250 mg/mL ranged from 1 – 13 mm. *In vitro* antioxidants analysis using DPPH at 25, 50, 100, 200, 400, 800, 1600 and 3200 mg/mL revealed methanol extract (IC<sub>50</sub> of 90.52 mg/mL). Results obtained from this research have provided preliminary evidence for the use of *Fiscus capensis* leaves in traditional medicine for treatment of diseases associated to free radicals and microbial infections.

**Keywords:** Phytochemicals, *Fiscus capensis*, Antimicrobial, Antioxidants and IC<sub>50</sub>

## 1.0 INTRODUCTION

The use of plants in Nigeria traditional practice as either extracts or infusion is a widespread practice in the treatment of common infections such as malaria, epilepsy, infantile convulsion, diarrhea, dysentery, flatulence, tonsillitis, bacterial and fungal infections and worm infections (Nweze *et al.*, 2004). The medicinal use of these plants range from the administration of the plant roots, stem, stem-bark, leaves and seed as extracts in forms of infusion or decoction (Ogbulie *et al.*, 2004). Nigeria diverse floras produce wide spectrum of medicinal plants and *Ficus capensis* happens to be one of them. *Ficus capensis* is a fast-growing, deciduous or evergreen tree. It usually grows from 5-12 metres in height. It can be found in tropical forests and grassy woodlands, and occurs in higher densities within well-watered, temperate upland habitats (Palgrave, 1984). But it is unfortunate that only a small portion of these plants had been thoroughly investigated for their medicinal values. In Nigeria and other parts of

the world, the practice of herbal medicine in modernized form is now gaining momentum with various health official and other persons coming to realize the potency and efficacy of some of the indigenous plant. Plants possess chemical substances that have effects on the animal system, but some have therapeutic properties, which can be and have been utilized in the treatment of human disease (Sofowora, 1976). Herbal medicines also provide new antimicrobial agents, which are effective against microorganisms that have developed resistance to some common and less expensive antimicrobial agents. As a result of these reasons, herbal remedies in several parts of the world are being incorporated into orthodox medicinal practices (Olukemi and Kandakai-olukemi, 2004). To achieve this, science and technology is employed in active research to investigate the potency and activities of these plants. On this note, the aim of this research is to evaluate the phytochemicals, antioxidants and antimicrobial potency of *Fiscus capensis* leaves (Cape fig).

## 2.0 MATERIALS AND METHODS

### 2.1 Sample Collection and Treatment

The fresh leaves of *Ficus capensis* (also called Ogbaikolo by the Igalas ) was collected from the school farm located at the Faculty of Agriculture, Kogi State University, Anyigba. The collected plant was identified as *Ficus capensis* (*cape fig*). Prior to analyses, the sample was identified by Mr Momoh T. B. at the Department of Animal and Environmental Biology, Kogi State University, Anyigba, Nigeria. The sample was air dried and pulverized, it was then stored in a polythene bag to prevent moisture.

### 2.2 Preparation and extraction of the plant

The powdered plant (50 g) was extracted selectively with 200 mL each of water, ethanol and methanol for 48 hours using maceration method. The samples were then filtered with the aid of vacuum pump. The filtrate was poured into a beaker and placed on a steam bath to evaporate the solvents. The percentage yield of each extract was calculated.

### 2.3 Qualitative Phytochemical Screening of the Plant Extracts

The aqueous, methanolic and ethanolic extract of *Ficus capensis* leaves were screened for the presence of phytochemicals using standard procedures to identify the constituents.

#### 2.3.1 Test for Tannins

The 5 drops of 0.1% ferric chloride was added to 2 mL of the plant extracts, and a brownish green or blue-black coloration indicates a positive result (Sofowara, 1993).

#### 2.3.2 Test for Saponins

The 2 mL of the plant extracts was diluted with 2 mL distilled water. It was then agitated in a test tube for 5 min. About 0.1 cm layer of foam indicates a positive result (Mbatchou and Kosoono, 2012).

#### 2.3.3 Test for Flavonoids

The 2 mL of 10% sodium hydroxide was added to 2 mL of the plant extracts in a test tube. An intense yellow color was formed which turned colorless upon addition of 2 mL of dilute hydrochloric acid indicating a positive result (Mbatchou and Kosoono, 2012).

#### 2.3.4 Test for Alkaloids

To 2 mL of the plant extracts, 2 mL of 10% hydrochloric acid was added. To the acidic medium, 1 mL of dragendroff's reagent was added. An orange precipitate indicates a positive result (Mbatchou and Kosoono, 2012).

#### 2.3.5 Test for Glycosides

The 2 mL of acetic acid was added to 2 mL of the plant extracts. The mixture was cooled in cold water bath. The 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Color development from blue to bluish green indicates the presence of glycosides (Sofowara, 1993).

#### 2.3.6 Test for Phenols

The 2 mL of the plant extracts was mixed with 2 mL of 1% ferric chloride. The formation of deep blue or blue-black coloration is an indication of a positive result (Harborne, 1998).

## 2.4 Quantitative Phytochemical Screening of the Plant Extracts

### 2.4.1 Determination of Total Alkaloid Content

The total alkaloid content was measured using Harborne, (1993) and Obadoni and Ochuko (2001). The 5 g each of the extracts was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. It was filtered and the extracts were concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

$$\text{Alkaloid (mg/g)} = \frac{W_2 - W_1}{\text{weight of sample}} \times 100$$

### 2.4.2 Determination of Total Flavonoid Content

The total flavonoid content was determined using the method of Harborne (1993). The 2 g each of the extract was mixed with 25 mL of 80% aqueous methanol. The solution was filtered individually through the whatman filter paper. The filtrates were transferred to a crucible and evaporated into dryness over a water bath and weighed.

$$\text{Flavonoid (mg/g)} = \frac{\text{weight of residue}}{\text{weight of sample}}$$

### 2.4.3 Determination of Total Saponin Content

This was measured using the method of Obadoni and Ochuko (2001). 5 g each of the extract was introduced into a conical flask and 25 mL of 20% aqueous ethanol was added. The sample was heated over a water bath for 1 hour with continuous stirring at about 55 °C. The concentrate was transferred into a 250 mL separatory funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. Then 15 mL of n-butanol was added followed by 2.5 mL of 5% aqueous sodium chloride. The remaining solution was heated over a water bath. After evaporation, the extracts were dried in the oven to a constant weight.

$$\text{Saponin (mg/g)} = \frac{\text{weight of residue}}{\text{weight of sample}}$$

### 2.4.4 Determination of Total Tannin Content

The tannin content of the sample was determined using the Folin-Ciocalteu phenol reagent. Accurately weighed 0.1 mL of the extracts was added individually with 7.5 mL methanol. Then 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% sodium carbonate solution were also added. The mixtures were diluted to 10 mL with distilled water. The mixtures were well shaken, kept at room temperature for 30 min and the absorbance was measured at 725 nm. Blank was prepared with distilled water. A set of standard solutions of tannic acid was read against a blank. Total tannin content was determined as mg of tannic acid equivalent per gram of the sample using the equation obtained from a standard tannic acid calibration curve

$$y = 0.021x + 0.343.$$

### 2.4.5 Determination of Total Phenolic Content

The total phenolic content of *Ficus capensis* leaf extracts were determined by Folin-Ciocalteu spectrophotometric method (McDonald *et al.*, 2001). Then 0.1 mL of Folin-Ciocalteu reagent was added to 2 mL of the extracts. The mixtures were allowed to stand for 15 min. Then, 5 mL of saturated sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added. The mixture was allowed to stand for 30 min at room temperature and the total phenolic content was determined spectrophotometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of mg of gallic acid equivalent per gram of the sample using the linear regression equation

obtained from the standard gallic acid calibration curve

$$y = 0.006x + 0.038.$$

### 2.5 In vitro Antioxidant Analysis

The free radical scavenging activities of the methanolic, ethanolic and aqueous extract of *Ficus capensis* were tested against 1,1-diphenyl-2-picrylhydrazyl (DPPH), using the method described by Adesegun *et al.* (2008). Various concentrations (25, 50, 100, 200, 400, 800, 1600, 3200 mg/mL) of the extract were prepared in methanol. The mixture was shaken vigorously and allowed to stand in the dark for 10 minutes at room temperature. The absorbance was read at 517 nm for the extracts and the blank (without extract). The percentage free radical scavenging ability was measured using the equation below:

$$\%I = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100$$

### 2.6 Antimicrobial Screening of Ethanolic Extract of *Ficus capensis*

#### 2.6.1 Collection of Test Organisms

The test organisms were collected from the microbiology laboratory of Kogi State University, Anyigba, Nigeria. The organisms include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*.

#### 2.6.2 Evaluation of Antimicrobial Activity

The antimicrobial activity of the ethanolic extract of *Ficus capensis* was determined using the agar well diffusion method as described by Irobi *et al.* (1994). The nutrient agar was prepared using standard method and sterilized at 120 °C. The Agar plates and a sterile cork borer of 6 mm in diameter were used to create wells on the inoculated plate. Each of the wells was filled with 0.1 mL of the extract concentrations of 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL and 250 mg/mL of ethanol extract. Distilled water was used to fill one of the wells to serve as control. These were allowed to diffuse into the agar at room temperature for one hour and then incubated at 37°C for 24 hours. The zones of inhibition were measured to the nearest millimeter using a metric rule. The readings were taken in triplicate and the mean values recorded.

### 2.6.3 Determination of Minimum Inhibitory Concentration

The estimation of the minimum inhibitory concentration was carried out using the method described by Akinpelu and Kolawole (2004). 0.5 mL of varying concentrations of the extract in mg/ml were dispensed into each test tube containing nutrient broth, inoculated with each test organism. A tube containing nutrient broth with test organism but no extract served as control. The minimum inhibitory concentration was taken after 24 hours of incubation at 37°C.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Results

The results of the qualitative and quantitative phytochemical screening of the extracts are presented in Tables 1 and 2. The in vitro antioxidant analysis results of the extracts are presented in Table 3 and Figure 1. The results of the inhibitory concentration (IC<sub>50</sub>) are presented in Table 4. The results of antimicrobial tests are presented in Tables 5, 6, 7 and Figure 2.

**Table 1: Phytochemical Screening of *Ficus capensis* leaves**

Phytochemicals	Methanolic extract	Ethanolic extract	Aqueous extract
Tannins	++	++	+
Total phenol	+++	+++	+++
Saponins	+++	+++	+
Flavonoid	++	++	++
Cardiac glycoside	+	+	+
Alkaloid	+	+	+

Keys: + = Trace, ++ = Abundant, +++ = Very abundant

**Table 2: Concentration of some phytochemicals in *Ficus capensis* leaves**

Phytochemicals	Concentration (mg/g)		
	Aqueous Extract	Ethanol Extract	Methanol Extract
Tannins	4.23	12.84	23.89
Total Phenol	144.95	461.28	662.30
Saponins	4.44	70.44	115.04
Flavonoids	13.22	20.94	84.78
Alkaloids	1.70	1.82	2.12

**Table 3: Percentage free radical scavenging activity of *Ficus capensis***

Concentration (mg/mL)	% Inhibition of Methanolic extract	% Inhibition of Ethanolic extract	% Inhibition of Aqueous extract	% Inhibition of Standard
25	34.11	21.66	4.69	85.55
50	35.64	22.68	5.41	90.28
100	38.69	24.72	6.84	91.09
200	43.27	27.77	8.98	95.85
400	49.38	31.84	11.84	96.96
800	57.02	36.92	15.42	98.48
1600	66.19	43.03	19.51	98.99
3200	75.35	50.15	24.50	99.49

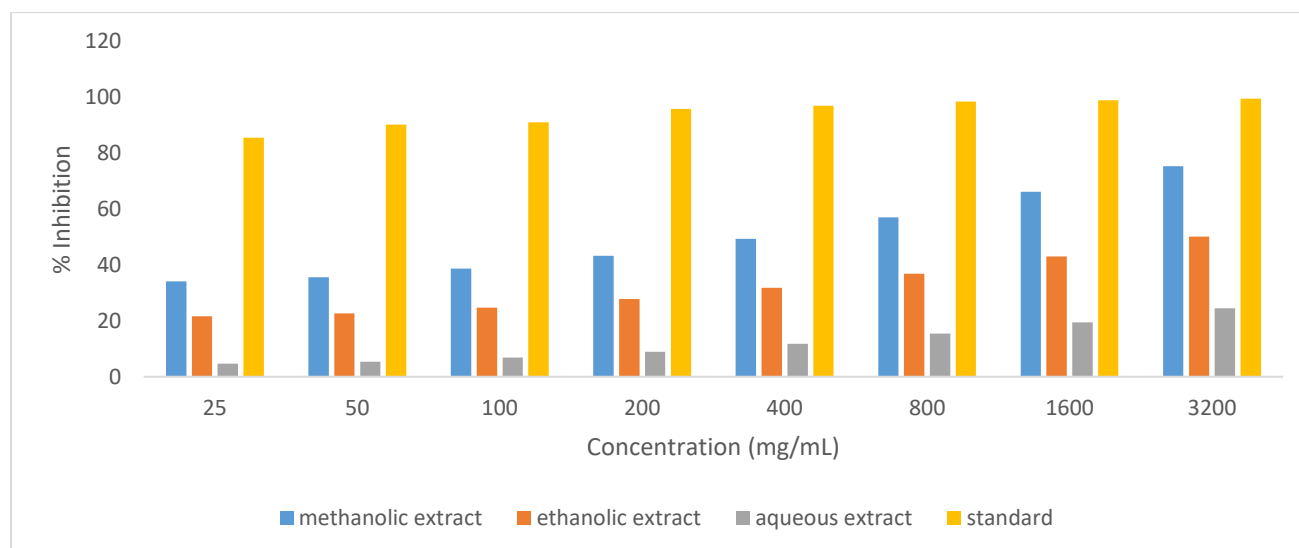


Figure 1: Free radical scavenging activity of *Ficus capensis* leaves

Table 4: IC<sub>50</sub> value of extracts

Extracts	IC <sub>50</sub> (mg/mL)
Aqueous	1487.79
Ethanol	119.52
Methanol	90.52
Standard (vitamin C)	9.557 x10 <sup>-11</sup>

Table 5: Antimicrobial Activity of Ethanolic extract of *Ficus capensis*

Concentration (mg/mL)	Zone of inhibition (mm)				
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
50	6	1	2	2	3
100	8	1	4	5	5
150	10	2	6	6	6
200	12	2	6	7	8
250	13	4	8	9	12

Table 6: Minimum inhibitory concentration of Ethanolic Extract of *Ficus capensis*

Test Organism	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	250 mg/mL
<i>S. aureus</i>	G	G	NG	NG	NG
<i>Escherichia coli</i>	G	G	G	G	G
<i>Salmonella typhi</i>	G	G	G	G	NG
<i>P. aeruginosa</i>	G	G	G	G	NG
<i>Bacillus subtilis</i>	G	G	G	NG	NG

Key: G means growth; NG means no growth

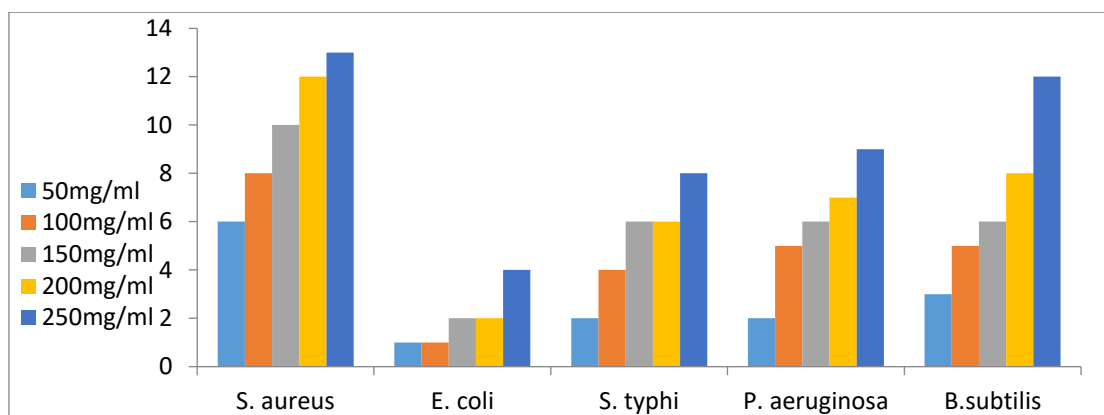


Figure 2: Zone of inhibition of the ethanol extract on the test organisms

### 3.2 Discussion

The qualitative phytochemical screening of the extracts revealed the presence of tannins alkaloids, flavonoids, saponins, total phenol and cardiac glycosides (Table 1). The extracts indicate that alkaloids and cardiac glycosides are present in small concentration. From the results of quantitative phytochemical screening the methanol extract possess high concentration of the secondary metabolites (Table 2).

From the results of the quantitative phytochemical screening, the total phenolic content of the extracts (aqueous, ethanol, methanol) in mg/g is (144.95, 461.28, and 662.30) respectively. The concentration of flavonoids is (13.22, 20.94, 84.78) respectively. The saponin content of the extracts (aqueous, ethanol, methanol) is (4.44, 70.44, 115.04) in mg/g respectively and the tannin content in mg/g is (4.23, 12.84, 23.89) respectively. The presence of phenols and flavonoids indicates that the extracts will be good for the management of cardiovascular diseases and oxidative stress because flavonoids and phenols are biological antioxidants. The presence of flavonoids in *Ficus capensis* leaves accounts for its antioxidant property as reported by Ramesh et al. (2005). Flavonoids also provide protection against these diseases by contributing to the total antioxidant defense system of the human body. The presence of saponins in the extracts accounts for its use in maintaining high density lipoprotein cholesterol (HDL-C) levels and lower low density lipoprotein cholesterol (LDL-C) levels as reported by World Health Organization (WHO) in 1992. Saponins aid in reducing cholesterol levels by forming complexes with cholesterol and bile acids (Shereen, 2011) which prevent them from being absorbed through the small

intestine hence, lowers the cholesterol level in the blood and liver. The presence of tannin in extracts accounts for its antimicrobial properties as reported by Shittu et al. (2007). Tannin has antibacterial, antiviral, and astringent properties (Akiyama *et al.*, 2001; Liu *et al.*, 2004).

The concentration of the phytochemicals in the extracts is in the order methanol extract > ethanol extract > aqueous extract. This difference in concentration may be due to the degree of solubility of phytoconstituents in different types of solvents, depending on their solubility (Halilu *et al.*, 2008).

The *in vitro* antioxidant ability of *Ficus capensis* leaf extracts (Table 3 and Figure 1), showed that, at all concentrations (25, 50, 100, 200, 400, 800, 1600, 3200 mg/mL), the methanol extract shows the highest free radical scavenging ability, followed by the ethanol extract and the aqueous extract showing the least activity. The IC<sub>50</sub> value revealed that the methanol extract is more potent than the other extracts. The IC<sub>50</sub> value of the methanol extract is 90.52 mg/mL (Table 4). The lower the IC<sub>50</sub> the more potent is the extract.

The ethanolic extract of *Ficus capensis* leaves revealed inhibitory activity against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis* and *Pseudomonas aeruginosa* with no inhibition against *Escherichia coli* (Tables 5, 6, 7 and Figure 2). *Staphylococcus aureus* was the most sensitive and *Escherichia coli* showing some resistance. The zone of inhibition of growth produced by the ethanolic extract on the pathogens at 50, 100, 150, 200 and 250 mg/mL ranged between 1mm to 13mm (Table 5). The inhibitory activity was potent at high concentration (250 mg/mL) against selected

pathogens except for *E. coli*. This implies that the inhibitory capacity of the ethanolic extract of *Ficus capensis* leaves is concentration dependent, because the inhibitory potency increases with increasing concentration. The antimicrobial activity demonstrated by the extracts may be due to the presence of the phytoconstituents present in the extracts.

#### 4.0 CONCLUSION

*Ficus capensis* leaves have been known to possess medicinal properties. However this study has revealed that the leaves of *Ficus capensis* have certain active agents that show inhibitory potency towards certain microorganisms. The inhibition of the ethanolic extract of *Ficus capensis* against the bacteria isolates of *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis* indicates its efficacy in the treatment of ailments caused by these organisms. *Ficus capensis* leaves could also serve as effective free radical inhibitor, which makes it a good candidate for pharmaceutical plant-based products.

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