

Phytochemical Screening and Antimicrobial Activity of the Plant of *Cissus Ibuensis* (Hook f.)

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Abstract

Background: *Cissus ibuensis* (hook f) used in folkloric treatment of human infections was used against five bacterial and two fungi isolates to test for the broad spectrum antimicrobial activities.

Methods: Methanol solvent was used to extract the bioactive components of the plant using Soxhlet extraction method. The extract was diluted at concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL and the diameter of zones of inhibitions was determine by well diffusion technique.

Results: The methanol extract of the plant at different concentration was active against *Staphylococcus aureus*, *Streptococcus feacacis*, and *Bacillus cereus* with zone of inhibition ranging from 27.00 mm \pm 0.32 to 11.00 mm \pm 0.10. The extract at different concentration also was active against *Candida albicans* with zone of inhibition ranging from 22.00 mm \pm 0.01 to 8.00 mm \pm 0.10. The antifungal analyses also showed activities with *Cissus ibuensis* showing fungal inhibition with zone from 22.00 mm \pm 0.01 to 8.00 mm \pm 0.10 against *Candida albicans*. Positive controls for bacteria and fungi using standard antibiotic and antifungal showed that most of the test organisms are resistance to antibiotic and antifungal. *S. aureus*, *S. facalis*, *B. cereus*, *E.coli* and *S. typhi* were sensitive to Sparfloxacin while *C. albicans* and *C. tropicalis* were sensitive to Fluconazole. Phytochemical screening revealed the presence of carbohydrates, tannins, saponnins, flavonioids, cardiac glycolysis, steroid and triterpenes.

Conclusion: The result of the study demonstrated that the extract of the *Cissus ibuensis* has significant antimicrobial properties and suggest that it may be useful in the treatment of microbial infections.

Keywords

Antimicrobial activity, Well diffusion, *Cissus ibuensis*, *Staphylococcus aureus*, Soxhlet apparatus

Introduction

The use of medicinal plants by human as therapeutic agents has been in practice for decades. Many people the world especially in developing countries depend on medicinal plants for the treatment or management of various ailments because of its effective curative properties [1-4]. Medicinal plants have played a vital role globally in the health care with about 80% of Africans depending on ethnomedicine in the treatment of ailments like malaria, HIV/AIDS, sickle cell anemia, diabetes and hypertension [5].

Phytochemistry is concerned with the chemical studies of plant secondary metabolites and required the assay of plant extracts using chemical means to determine the active ingredients of the plant. These active ingredients in plants have bactericidal or bacteriostatic effect on pathogenic microbes. The most important of these



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bioactive compounds of plants are alkaloids, cardiac glycosides, flavonoids, tannins, saponin, phenolic compounds, steroids and terpenes [6-9]. These compounds possess numerous pharmacological effects such as antibacterial, antifungal, anticarcinogenic and vasodilatory activities [10].

Cissus ibuensis Hook (F) a climber belongs to the *Vitaceae* family and is distributed in the tropical regions particularly in Nigeria, Niger, Togo, Benin and Ghana with medicinal properties of resveratrol. It is used in traditional medicine as an anti-diabetic, anti-cancer, gastrointestinal disturbance, rheumatism and arthritis and for cardiovascular disease [11-15]. The leaves of *C. ibuensis* contained Quercetin 3-O-rutinoside and flavanoids using ethanol extraction and butanol fractionation techniques [16]. The present study was aimed at evaluating the phytochemical and *in-vitro* antimicrobial activity of *Cissus ibuensis* plant extracts against clinical bacterial and fungi isolates obtained from human infections.

Materials and Methods

Collection and identification of samples

The plant *Cissus ibuensis* was collected from Makurdi, Benue State. They were authenticated by a taxonomist at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria - Nigeria. The test organisms were obtained from Medical Microbiology Department of Ahmadu Bello University Teaching Hospital Zaria, Nigeria. It was authenticated by subculturing, gram staining and biochemical analysis.

Preparation and extraction procedure of the plant of *Cissus ibuensis*

The plant materials were washed with distilled water and air-dried at room temperature for two weeks and then ground with wooden mortar and pestle to coarse powder. Air-dried and pulverized plant material (500 g) was weighed and put in a thimble, which was in turn loaded inside a Soxhlet extractor and then defatted with methanol. The supernatant were allowed to evaporate using a rotary evaporator at 40 °C. The extract obtained was transferred into a clean dry bottle, weighed and labelled. The extract was then reconstituted using dimethyl sulphoxide (DMSO). The extract was diluted serially to give stock percentage 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL and was used for agar well diffusion.

Phytochemical screening

The methanol extract and its fractions were subjected to standard phytochemical analysis for different constituents such as carbohydrates, tannins, saponins, flavonoids, cardiac glycolysis, steroid and triterpenes as described by Edeoga, et al. [7] and Daniels and Olu [17].

Test organisms

The microorganisms used in this study were clinical isolates obtained from Medical Microbiology Department of Ahmadu Bello University Teaching Hospital Zaria, Nigeria. The organisms were maintained on nutrient agar slant at 4 °C and subcultured on a fresh appropriate agar slant 24 hours prior to the antimicrobial test. *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans*, and *Candida tropicalis* were used for the bioassay.

Anti-Microbial Screening

Agar-well diffusion assay

The antimicrobial screening was carried out using the agar well diffusion method as described by Nostro, et al. [18]; Lino and Deogracious [19]; CLSI [20]. The bacterial cultures were inoculated in nutrient broth (Oxoid) and incubated for 24 hours at 37 °C while the fungal cultures were inoculated on Sabouraud dextrose agar (Oxoid) and incubated for 72 hours at 30 °C. Sparfloxacin 2 mg/mL and fluconazole 5 mg/mL



Table 1: Phytochemical screening of methanol extracts of plants.

Constituents	Methanol extract
Carbohydrate	+
Saponins	+
Reducing sugar	NT
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Resins	NT
Steroids/Triterpenes	+
Anthraquinone	-
Alkaloids	-

Key: + = Positive, - = Negative, NT = Not tested.

Table 2: Zone of Inhibition of the methanol extract against the test microorganisms.

Conc.	<i>S. aureus</i>	<i>S. facalis</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. tropicalis</i>
50 mg/mL	27.00 ± 0.32	25.00 ± 0.20	32.00 ± 0.10	22.00 ± 0.10	20.00 ± 0.20	22.00 ± 0.10	#
25 mg/mL	24.00 ± 0.20	23.00 ± 0.12	27.00 ± 0.22	20.00 ± 0.30	17.00 ± 0.30	18.00 ± 0.20	#
12.5 mg/mL	20.00 ± 0.10	19.00 ± 0.20	22.00 ± 0.10	15.00 ± 0.20	13.00 ± 0.20	14.00 ± 0.30	#
6.25 mg/mL	17.00 ± 0.30	15.00 ± 0.30	18.00 ± 0.30	#	#	8.00 ± 0.10	#
3.125 mg/mL	12.00 ± 0.20	11.00 ± 0.10	16.00 ± 0.10	#	#	#	#
Sparfloxacin 2 mg/mL	40.00 ± 0.30	29.00 ± 0.20	35.00 ± 0.20	30.00 ± 0.20	21.00 ± 0.10	#	#
Fluconazole mg/MI	#	#	#	#	#	26.00 ± 0.30	20.00 ± 0.20

Values represent diameter of zone of inhibition (mm); #: means no inhibition.

were used as positive controls for bacteria and fungi respectively. Mueller Hinton agar (Oxoid) was dispensed into sterile plates and allowed to solidify. Each of the test culture was then adjusted to 0.5 McFarland turbidity standard and 0.2 mL each inoculated onto Mueller Hinton agar plates. 5 mm diameter of sterile cork borer was used to make wells (4 mm depth) for different concentrations of the extract on each of the culture of different test organisms. After that, 0.2 mL of the appropriate extract concentration was placed in the well using sterile Pasteur pipettes and allowed to diffuse into the agar. All the tests were run in duplicates for quality results. The set up was incubated for 24 h at 37 °C for bacterial and at 30 °C for 72 hrs for fungi. The zones of inhibition were measured using a ruler and results reported in Millimeters (mm).

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the extract was determined using broth dilution method. Two-fold serial dilution of the extract in the Mueller Hinton broth was performed to obtain the concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. The initial concentration was obtained by dissolving 0.5 g of the extracts in the Mueller Hinton broth (10 mls). 0.1 mL of the standardized inoculum of the test organism was added into the different concentrations of the extracts in the Mueller Hinton broth in a test tube inoculated at 37 °C for 24 hrs for bacterial while 30 °C for 72 hrs for fungi and was observed for turbidity (growth). The lowest concentration of the extract which shows no turbidity was recorded as the minimum inhibitory concentrations.

Minimum bactericidal and fungicidal concentration

The Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) were determined by removing 1 mL of bacterial and fungal suspension from the MIC tubes that did not show any growth and subcultured onto Mueller Hinton agar plates and incubated at 37 for 24 hrs for bacterial cultures and 30 °C for 48 hrs for fungal cultures. After incubation, the concentration at which no visible growth was seen was recorded as the MBC or MFC.



Results and Discussion

Phytochemical screening of the methanolic extract of *Cissus ibuensis* (Table 1)

Table 3: Minimum Inhibition Concentration of the extract against the test microorganism.

Test organisms	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
<i>Staphylococcus aureus</i>	-	-	Ĩ	+	++
<i>Streptococcus faecalis</i>	-	-	Ĩ	+	++
<i>Bacillus cereus</i>	-	-	-	Ĩ	+
<i>Escherichia coli</i>	-	-	Ĩ	+	++
<i>Salmonella typhi</i>	-	-	Ĩ	+	++
<i>Candida albicans</i>	-	-	Ĩ	+	++
<i>Candida tropicalis</i>	-	-	Ĩ	+	++

Key: - = No turbidity (no growth); Ĩ = Minimum Inhibition Concentration; + = Turbid (Light growth); ++ = Moderate turbidity.

Table 4: Minimum Bactericidal/Fungicidal Concentration of the extract against the test microorganism.

Test organisms	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
<i>Staphylococcus aureus</i>	-	Ĩ	+	++	+++
<i>Streptococcus faecalis</i>	-	Ĩ	+	++	+++
<i>Bacillus cereus</i>	-	-	Ĩ	+	++
<i>Escherichia coli</i>	Ĩ	+	++	+++	++++
<i>Salmonella typhi</i>	Ĩ	+	++	+++	++++
<i>Candida albicans</i>	Ĩ	+	++	+++	++++
<i>Candida tropicalis</i>	Ĩ	+	++	+++	++++

Key: - = No Colony growth; Ĩ = MBC/MFC; + = Scanty colonies growth; ++ = Moderate colonies growth; +++ = Heavy colonies growth.

revealed the presence of Carbohydrate, Saponins, Flavonoids, Steroids/Triterpenes, Tannins and Cardiac glycosides. Alkaloids was not detected from this plant as it was reported in a study with *Ampelocissus* extracts which belong to the same family [21,22]. The antimicrobial activities of the extract of *Cissus ibuensis* examined in this study were assessed in terms of diameter of zones of inhibition (Table 2), MIC (Table 3), MBC and MFC (Table 4). The methanol extract had the yield of 7.7 g with the percentage of 1.54%.

The methanolic extract of *Cissus ibuensis* showed significant level of inhibition against some of the test organisms even at low concentrations. *S. aureus*, *S. faecalis* and *B. cereus* were the most susceptible bacterium to the extract with inhibition zone ranging from 11.00 mm ± 0.10 to 32.00 mm ± 0.10 while *C. albicans* was the most susceptible fungi with inhibition zone ranging from 8.00 mm ± 0.10 to 22.00 mm ± 0.10.

Sparfloxacin demonstrated greater activities against bacteria while Fluconazole has activities against fungi. Result of study showed that the methanol extract demonstrated a broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacterial and fungal strains tested.

Bacterial and fungal resistance to antimicrobials has become a growing concern globally [23]. Medicinal plants have been recognized as valuable resources of natural antimicrobial compounds [24]. The extract showed bactericidal and fungicidal activities on the bacterial and fungal isolates.

The broad spectrum antimicrobial activities of the plant extract may possibly be due to the identified phytochemicals in the plant such as carbohydrates, tannins, saponins, flavonoids, cardiac glycolysis, steroid and triterpenes.

Conclusion

The methanolic extract of *Cissus ibuensis* showed a broad-spectrum of activity against Gram-positive bacteria, Gram-negative bacteria and the fungi which are known to be associated with different types of infections. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections.

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Author Contributions

Q.O got the concept and design of the study. QO & BEI perform the study and data analysis. EPA & A.O.A participated in the interpretation of data; BEI and A.O critically revised the manuscript for important intellectual content; all the authors read and gave a final approval of the revised version of the manuscript.

Conflict-of-Interest Statement

The authors declare no conflict of interest related to this study.

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