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Screening of Phytochemicals and Biological Potential of Aqueous, Methanol and Hexane Extracts of *Cylicodiscus gabunensis* Stem Bark

E. L. Ayuk[1,*], A. N. Njokunwogbu[1], S. U. Ilo[1], G. A. Engwa[1], U. C. And T. O. Oni[2]

[1]Department of Chemical Science, Faculty of Natural and Applied Sciences Godfrey Okoye University, Enugu, Nigeria
[2]School of General studies, Delta State Polytechnic, Ogwashi – Uku, Nigeria

Abstract This paper covers the preliminary investigation of different phytochemicals and the anti-microbial analysis of *Cylicodisusgabunesis* stem bark extracts as well as the thin layer chromatography (TLC) analysis of the methanol and hexane extracts respectively. The phytochemical screening of the aqueous, methanol and hexane extracts of the plant above revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, and steroids at varying amount depending on the intensity of the colors observed. Anthraquinones were absent inaqueous extract while alkaloids, glycosides, phlobatannins and reducing sugars were absent in hexane extract. However, all the above mentioned chemical compounds were present in methanol extract. The presence of these compounds suggests that this plant has medicinal value. The antimicrobial analysis was carried out using the three extracts above on two test organisms, *Salmonella, typhi* and *Staphylococcus aureus* using streptomycine as a reference standard drug. The results showed appreciable inhibition of these organisms compared to the reference. The (TLC) analysis indicated the presence of other complex compounds whose identities need to be established by spectroscopic and elemental analysis.

Keywords *Cylicodiscusgabunansis*, Phytochemical, Antimicrobial, Salmonella typhi, Staphylococcus aureus

1. Introduction

Cylicodiscusgabunensis is a large tree of about 60m high and 11m in girth with widely spread, fairly open crown. This tree is a genus of only one species. It has different names in Nigerian local languages. The Boki people of Cross River State calls it "Kendum" Efik (Anyan), Igbo (Uzi) Yoruba (Olosan), Edo (Okan), Etsako (Koain) and Ishan (Ekan) [1].

This species of a tree is found in the high rainforest zone of West Africa and extends from Ivory Coast to Gabon [2]. It is well known for its medicinal values and is widely used in Boki (Cross River state of Nigeria) for the treatment general fevers, infections, stomach problems, diabetes, stroke etc. Apart from its medicinal value, it is also use for timber and fuel (fire wood).

There is very little report on the phytochemical and antimicrobial activity of the above plant. So far the only work on phytochemical screening and antimicrobial analysis of *C.gabunensis* was reported by Laure and co-workers (2006) using ethyl acetate stem bark extract [3]. Casadeval and co-workers reported only on the triterpenesaponnins from *C. gabunensis* [4]. In this work we decided to move a step further by using three different solvents (water, methanol andn-hexane) for extraction, phytochemical evaluation and antimicrobial analysis of the stem bark extracts of the above plant.

* Corresponding author:
eugeneayuk@yahoo.com (E. L. Ayuk)
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2. Experimental

Materials and Methods

The plant was obtained from the thick rainforest of Bodam village in Boki local government area of Cross River State, Nigeria on the 2nd of May 2013. The roots and leaves of the plant were observed by staff of the Biological science Department (Botany unit), Benue State University, Makurdi and was declared to be *Cylicodiscusgabunensis*.

The stem bark of the plant was removed and air dried in the chemistry laboratory on the table for two weeks after which the dried material was crushed into powder using a clean mortar and a pestle and then sieved. The powder was stored in an air-tight polyethene bag and kept in a cupboard for subsequent use.

Aqueous Extraction

The powdered material (20.0g) was heated to boil with distilled water (400ml) for 30 minutes to ensure complete extraction. The chaff was removed and the extract was

cooled, filtered into a conical flask, concentrated and made to dry in water bath at about 80°C, sealed with cellophane paper and stored in the freezer ready for phytochemical test and antimicrobial analysis. This extract is also referred to cold or aqueous extract.

Extraction of Plant Material with Methanol and Hexane

The extraction of methanol and hexane extracts was done with the use of a soxhlet apparatus using methanol and n-hexane respectively. The plant material (50.0g) was used for each of the solvents. It was exhaustively extracted with methanol and hexane (300ml) respectively for seven hours. The solvents were distilled and the concentrates (extracts) were transferred into pre-weighed beakers and then made to dry in a water bath at 80°C. These were preserved in a refrigerator for phytochemical and antimicrobial analysis as well.

Phytochemical Screening

The three extracts were screened qualitatively for the presence of alkaloids, anthraquinones, flavonoids, glycosides, phlobatannins, saponins, steroids and the reducing sugars; these were identified using characteristic color changes using standard procedures previously described by Harbon, 1973 [5], Odebiyi and Sofowara, 1978 [6]. Each test was qualitatively expressed as negative (−) or positive (+); the intensity of the characteristic color was expressed as (++) or (+++).

Preparation of Media for Antimicrobial Analysis

- **Nutrient Agar:** - Powdered Agar (28.0g) was weighed and poured into a conical flask and deionized water (25ml) was added and was allowed to soak for 10mins then swirled and mixed. This was sterilized by autoclaving for 15mins at 121℃. It was cooled to 47℃, mixed and the plates were prepared by adding the molten agar (20ml) into the sterilized petri dishes. This medium was used for antimicrobial activity test [7].
- **Deoxychlolate Agar:** - Powdered agar (45.5g) was weighed and dispersed in deionized water (250ml) contained in a conical flask and heated with frequent swirling. This was cooled to 47°C and poured into plate, no autoclaving was done. This media was used to isolate salmonella typi [7].
- **Blood Agar:** - Nutrient agar (100ml) and Agar base (100ml) were melted in a steamer and were allowed to stand in bottles placed on a bench for 15minutes. They were then placed in a water bath at 50°C for 10minutes and a layer of the base was poured into sterilized Petri dishes and allowed to set. Defibrinated blood (25ml) was added to the nutrient agar (100ml) at 56°C. This was mixed well and poured onto the agar base immediately. The medium was about 4mm deep. The blood agar level was 2mm deep so that haemolysis can easily be seen. This medium was used to isolate staphylococcus aureus [8].

Innoculation of Organisms

A sterile loop (sterilized by Bunsen flame) was used to smear a loopful of specimen (stool) on the deoxycholate agar medium. This was streaked and kept in the oven at 37℃ for 24hours.

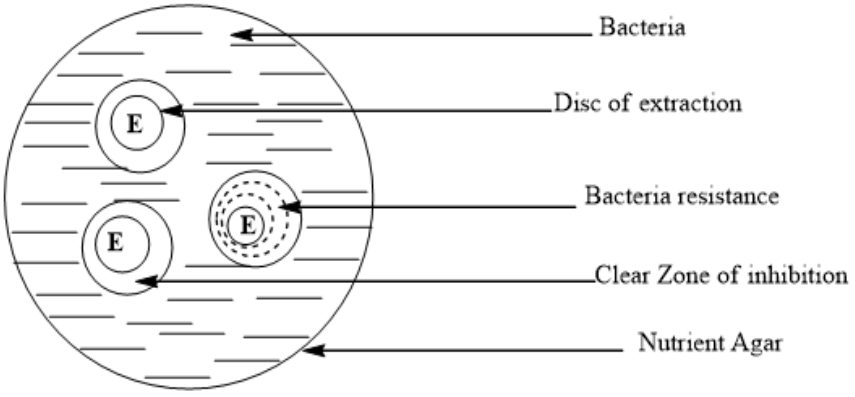
A loop was flamed again to sterilized and used to smear another loopful of the second specimen (semen) prepared as the medium containing blood agar medium. This was streaked and kept in the oven at 37℃ for 24 hours as well.

All the materials used (Petri dishes, conical flask, sample bottles etc.) were sterilized at 165℃ for 30 minutes while inoculating loops and distilled water were sterilized using autoclave at 121℃ for 15 minutes. [8]

Preparations of Samples for Anti-Microbial Test

For the anti-microbial analysis, three samples were used. These are methanol and hexane crude extracts respectively dissolved in (chloroform) (5ml) and the aqueous extract.

These extracts were prepared into three different concentrations of 50mg/ml, 5.0mg/ml and 0.5mg/ml respectively to test for the sensitivity of the study organisms (*Salmonella typhus and Staphylococcus aureus*) [9].



Optimum zone of inhibition 8mm-15mm
Minimum zone of inhibition 5mm-8mm
No inhibition 2mm-5mm

Figure 1. Antimicrobial sensitivity pattern of extracts zone of inhibition

Anti-Microbial Activity Test

The media surfaces (which were kept in the refrigerator) were dried to obtain desecrate colonies of the organisms. The drying of the plates was performed by placing the flat surface of the lid onto an incubator at 37℃ and angling the media – containing dishes (media downward) on the edge of the lid. After drying the nutrient agar plates, the organisms isolated (*Salmonella typhiand Staphylococcus aureus*) to be tested were seeded on the plates. Then the blotting paper disc impregnated with different concentrations of 50mg/ml, 5.0mg/ml and 0.5mg/ml of aqueous were placed on the preseeded plates. The diagram below illustrates the anti-microbial sensitivity pattern as observed with the extracts [9].

Thin Layer Chromatography of Methanol and Hexane Extracts of *Cylicosdiscus Gabunensis*

Chromatography refers to processes that are based on differences in rates at which individual components of a mixture migrate through a stationary medium under the influence of a moving phase. These methods enable a chemist to separate, isolate and identify components that might otherwise be difficult to be resolved [10].

The reason for embarking on the TLC analysis of the above is to ascertain the presence of other complex components that may be present in this medicinal plant but could not be identified through phytochemical analysis.

The crude dried methanol extract was dissolved in chloroform (5ml). A solvent system constituting of chloroform and methanol (4:1) was used for the methanol extract TLC. The crude dried hexane extract was also dissolved in chloroform (5ml). A mixture of benzene and ethylacetate (4:1) was used as the solvent system for the hexane extract TLC analysis. Acetone was first of all used to rinse the capillary tubes before the spotting was done to eliminate any impurity that may be present.

Two 250ml beakers were improvised as the chromatographic tanks which also served as developing chambers for the methanol and hexane extract respectively. The spotted plates were placed in the developing chambers with the spotted ends downwards. The plates were carefully positioned to ensure that the spotted parts did not touch the solvent combinations and the chambers were then covered. The solvents were allowed to move up the chromatoplates by capillary action. As the solvent ascended the chromatoplates, the samples components separated out. When the solvents have ascended to 1cm marks at the upper edges of the plates, the process was stopped and the chromaloplates were removed and air-dried on a bench. The air-dried plates were placed in an iodine tanks so that the spots can be seen more clearly. Iodine is a common no selective visualization reagent which reacts which organic compounds to (pi) complexes which are either yellow or brownish in colour [10].

The iodine stained plates were then removed after few minutes and the spots were seen more clearly, for the methanol extract TLC analysis, four spots were revealed, while three spots were obtained for the hexane extract TLC analysis. The retention factors were measured using a meter and calculated by using the formula.

$$R_f = \frac{\text{Distance moved by spot } (\text{cm})}{\text{Distance traveled by solvent front } (\text{cm})}$$

3. Results and Discussion

The qualitative chemical test of *Cylicosdiscusgabunensis* stem bark aqueous extract was carried out, it was observed that the methanol extract showed the presence of alkaloids, anthraquinones, flavonoids, glycosides, phlobatannins, tannins, saponins, steroids and reducing sugars. In aqueous extract, only anthraquinones were absent while n-hexane extract, alkaloids, glycosides, phlobatannins and reducing sugars were absent The result is given in the table (1) below.

Table 1. Phytochemical screening of extracts of *Cylicodicusgabunensis* stem bark

Class of compound indicated	Aqueous Extract	Methanol Extract	Hexane Extract
Alkaloids	+ +	+ + +	-
Anthraquinones	-	+ + +	+
Flavonoids	+	+ + +	+
Glycosides	++	+ + +	-
Phlobatannins	+	+ + +	_
Reducing sugars	+	+++	-
Saponins	+ + +	++	+
Steroids	+	+	++
Tannins	+	+++	++

(-) Absence
(+) Presence of compound
+ + + > + + >+: Measure of intensity of the characteristic color

The presence of these compounds justifies the use of this plant as a medicinal plant [11].

The three extracts of *Cylicodiscusgabunensis* were screened for their antimicrobial activity against *Salmonella typhi and Staphylococcus aureurs,* using Streptomycin as a standard drug [12] and the result isshown in table (2) below. The antimicrobial activity is given in terms of activity index thus:

$$\text{Activity index} = \frac{\text{Inhibition diameter of test sample}}{\text{Inhibition diameter of the standard}}$$

The methanol extract showed the highest sensitivity in both organisms, which suggests that most of the antibiotics of the plant were extracted by methanol, followed by the aqueous extract while the hexane extract showed the least activity against the organisms.

Table 2. Antimicrobial activity of *C. gabunensis* stem bark extracts against *Salmonella typhi and Staphylococcus aureus*

Antibiotic	Concentration	*S. typhi*	*S. aureus*
Aqueous extract	50.0mg/ml	0.98	0.95
"	5.0mg/ml	0.51	0.49
"	0.5mg/ml	0.09	0.087
Methanol extract	50.0mg/ml	1.05	1.00
"	5.0mg/ml	0.62	0.56
"	0.5mg/ml	0.15	0.11
Hexane extract	50.0mg/ml	0.74	0.70
"	5.0mg/ml	0.23	0.20
"	0.5mg/ml	0.03	0.01

Table 3. Result of chromatogram development for methanol extracts (chloroform and methanol (4:1)

Number of spots	Distance moved By spot (cm)	Distance moved By solvent (cm)	Rf value	Color of spots
A	4.2	5.8	0.72	yellow
B	5.1	5.8	0.88	brown
C	5.6	5.8	0.97	light green
D	5.7	5.8	0.98	reddish brown

Table 4. Result of chromatogram development for hexane extracts (Benzene and Ethylacetate (4:1)

Number of spots	Distance moved By spot (cm)	Distance moved By solvent (cm)	Rf value	Color of spots
A	2.8	5.8	0.07	brown
B	4.5	5.8	0.78	Green
C	5.0	5.8	0.86	yellow

From the above observation, methanol is the best solvent to be used for the extraction of antibiotics from plant compared to hexane and water.

The percentage yields obtained from the extraction of the powdered plant material support the above claim. For the 50.0g of the powdered plant material extracted with methanol, 11.30g of the crude extract was obtained, while that of hexane was only 5.0g. Which represent 23% and10% yield respectively.

For the TLC analysis, the methanol extract revealed four spots while the hexane extract showed only three spot. Their results are shown in table (3) and (4) above.

The above chromatographic results suggest that this plant contains other complex components which needed to be isolated, characterized and studied using spectroscopy as well as elemental analytical methods.

4. Conclusions

Cylicodiscusgabunensis has been discovered to possess promising medicinal potentials. From the antimicrobial analysis result above, it is observed that the methanol, aqueous and hexane extracts of the above plant showed significant activity against *S. typhi* and *S. aureus* respectively. TLC analysis indicated the presence of other complex compounds; characterization studies will be done in our subsequent research to establish the actual identities of these compounds.

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