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# Phytochemical Screening, Antioxidant and Antibacterial Activities of the Root Extract of *Cyphostemma adenocaule* (Steud. ex A. Rich.) Wild & R.B.Drumm

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**Abstract**

Plant secondary metabolites have provided important bioactive principles for developing new lead compounds. Within their confinement, they exhibit unique chemical diversity, which influences their diverse biological properties. The *Vitaceae* family is known for its potent antioxidant and antibacterial phytoconstituents, among other biological properties. *Cyphostemma adenocaule* is one of the family members explored for its ethnomedicinal properties. This study undertook the evaluation of the phytochemical, antioxidant, and antibacterial properties of the root extract of *Cyphostemma adenocaule*. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, carbohydrates & glycoside, saponins, and tannins. The methanol root extract had the highest activity in the DPPH assay, providing $IC_{50}$ (50% inhibition) of 10.87µg/ml, followed by n-Hexane ($IC_{50}$ 74.10µg/ml) and chloroform ($IC_{50}$ 74.31µg/ml) extract. In the antibacterial assay, the chloroform extract was active against *E. coli* (24.00±0.15) and had moderate activity against *Staph. aureus* (12.5±0.18). The n-Hexane extract was completely inactive against the test organisms while the methanol extract showed poor activity against the test organisms. The present study adds to the existing literature on *Cyphostemma adenocaule* with scientific evidence into its biological properties.

**Keywords:** *Cyphostemma adenocaule*; Phytochemical screening; antioxidant and antibacterial activity.

**Abbreviations**: CA1 – Cyphostemma adenocaule 1; CHCl3 – Chloroform; COSY – Correlation Spectroscopy; DEPT – Distortions Enhancement by Polarization Transfer; EtA – Ethyl acetate; HMBC – Heteronuclear Multiple Bond Correlation; HSQC – Heteronuclear Single Quantum Correlation; KHSO4 – Potassium bisulphide; MeOH – Methanol; MOA – Mechanism of Action; nHex – Hexane; NMR – Nuclear Magnetic Resonance; TLC – Thin Layer Chromatography; WHO – World Health Organization.

## INTRODUCTION

Natural products have been in existence for ages and evolved with unique chemical diversity, which results in their diverse biological activities and drug-like properties. These compounds present as important resources for developing new lead compounds and scaffolds (Galm & Shen, 2007).

Morphine from the opium poppy plant is considered the first pharmacologically active compound isolated by Friedrich Sertürner (Hamilton & Baskett, 2000; Joo, 2014).

Natural products are important for the development of new drugs, and these products have been in constant use. Drugs used as anticancer, antihypertensive, and antimigraine medication, have benefited greatly from natural products (Joo, 2014; Newman et al., 2003)

Plants have been part of traditional medicine systems, which have been used for thousands of years (Iwu, 2014). These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization (WHO) that approximately 80 % of the world's inhabitants rely mainly on traditional medicines for their primary health care (WHO, 2017)

*Cyphostemma adenocaule* (Steud. ex A. Rich.) Wild & R.B.Drumm is a climbing, scrambling, or trailing herb that belongs to the *Vitaceae* family (Bello et al., 2019; Wickens & Burkill, 1986) and is locally known as yáákùwár fátàákéé (Hausa, Nigeria) (Wickens & Burkill, 1986). The plant is a popular, non-cultivated vegetable eaten in many parts of Africa i.e., Nigeria, Ghana, Congo, Uganda, Ethiopia, and Eritrea (Bello et al., 2019). The plant had been documented for its

ethnomedicinal value, with a comprehensive review given by Bello and colleagues (2019).

The effectivity of Plant bioactive compounds against oxidative stress-related diseases and as an anti-infective had been well explored. This study entails investigating the phytochemical, antioxidant, and antibacterial properties of the root extracts of *C. adenocaule* by employing standard protocol. The results from the study will justify the ethnomedicinal uses of the plant and underscore its potentials as a source of antioxidants and antimicrobial agents.

## MATERIALS AND METHOD

### Plant Collection and Identification

Fresh root parts of *C. adenocaule* were collected aseptically in July 2019 from Shuwarin town, Dutse LGA, Jigawa State, Nigeria, and identified at the Medicinal Botany section, of the Department of Biology, Ahmadu Bello University, Zaria, Nigeria.

### Preparation of Plant Extract and Its Fractions

The preparation of plant material and fractions employed in our previous work on *C. adenacaule* was adopted with modifications (Yakubu et al., 2020). In this study, one and a half kilograms (1.5kg) of the pulverized sample material was extracted with Hexane, Chloroform, and Methanol.

### Preliminary Phytochemical Screening

Phytochemical screening was carried out on the crude extracts to detect the presence of plant secondary metabolites; alkaloids, anthraquinones, flavonoids, glycosides, steroids, tannins, terpenoids, and carbohydrates using standard procedures as described in the literature (Brain, KR and Turner, 1975; Evans, 2009; Markham, 1982; Sofowora, 1996; Vishnoi, 2009).

### Biological Activity

### Test for Antioxidant Activity: DPPH Assay

The radical scavenging potential was done using DPPH assay (Brand-Williams et al., 1995). 3 mL of 0.004% DPPH working solution (prepared using DPPH stock solution and methanol in correct proportions to give 0.899 abs) was added per every 100 µL of different concentrations of the extract and incubated at 37 ℃ for 30 minutes in dark. Then absorbance was taken at 517 nm wavelength in a UV spectrophotometer. The negative control contained 100 µL of methanol in place of the sample solution.

The percentage antioxidant inhibition (AI) was obtained by the equation:

$$\% \text{ AI} = \text{Control (Abs)} - \text{Sample (Abs)} \div \text{Control (Abs)} \times 100$$

Ascorbic acid (AA) was used as the positive control. Inhibition curves were made and $IC_{50}$ value per sample was calculated.

### Antimicrobial Assay

### Test Organisms

The organisms employed in this study are; *Escherichia coli, Pseudomonas aeruginosa, staphylococcus aureus,* and *streptococcus epidermis*, clinical isolates got from the Microbiology department, University of Maiduguri, and were stored at 2-8 ℃ until required.

### Preparation of Extract solutions for Pathogenic Assay

A stock solution of the extracts was prepared by dissolving 10 g of extract in 10 mL of distilled water, and a 1000 mg/mL solution was obtained. A two-fold serial dilution was carried out to obtain working solutions of varying concentrations.

### Preparation of Test Organisms

Test organisms cultured for 24 hours were suspended in a sterile bottle containing pure broth. Normal saline was added gradually to it and the turbidity was observed and compared to that of 0.5 Mcfarland standard which corresponds to approximately $10^8$ cells/mL. This was then diluted to produce $10^6$ cells/mL and used in the experiments. The dilution ratio was 1:1000 and 1:1500 for Gram-positive and Gram-negative organisms respectively (Usman et al., 2009).

### Preparation of Agar Plates

Nutrient agar was prepared accurately to the manufacturer's specification (i.e. by dissolving 18.5 g powder in 500 mL of distilled water) and sterilized at 121 ℃ for 15 min. The sterilized agar was allowed to cool to 50 ℃ in a water bath. The test organism (1 mL) ($10^6$ cells/mL) was inoculated into pre-labeled Petri plates (90 mm diameter), then 19 mL of the molten agar was added to each Petri plate, shacked, and allowed to sit at room temperature on a flat surface.

### Antimicrobial Susceptibility Assay (agar well diffusion method)

The antibacterial activity of the crude extracts was determined by following the agar-well diffusion method described by Igbinosa and colleagues (Igbinosa et al., 2009) with modification. The bacterial isolates were grown for 18 h in a nutrient broth and standardized to 0.5 McFarland standards (10 6 $cfuml^{-1}$). Two hundred microliter of the standardized cell suspensions were spread on Mueller-Hinton agar (Oxoid) and wells were bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 100, 75, 50, and 25 $mgml^{-1}$ were introduced into the wells, allowed to stand at room temperature for about 2 hr, and then incubated at 37 ℃. Controls were set up in parallel

using the solvents that were used to reconstitute the extract. After 24 hr, the plates were observed for the zones of inhibition. The effects were compared with those of Ciprofloxacin at a concentration of 5 mg/ml. Antibacterial activity was evaluated by measuring the diameters of zones of growth inhibition in triplicates and results were presented as Mean±SEM.

### Statistical Analysis

The obtained antioxidant and antibacterial results were expressed in mean ± standard error with observation recorded in triplicates. Analysis of variance for individual parameters was performed based on mean values to determine the significance at $p < 0.05$ using SPPS v20. Regression analysis was deployed to calculate and obtain the $IC_{50}$ from the regression equation using Excel 2016.

### Percentage Yield

In this experiment, 1.5kg of the resultant size reduced root powder of *C. adenocaule* was used. The MeOH extract showed the highest yield of 44.6g. The percentage yield is given in the Table1

**Table 1**. Percentage yield of *C. adenocaule* root extracts.

| Extract | Weight of extract (g) | Percentage (%) yield |
|---|---|---|
| nHex | 5.4 | 0.36 |
| $CHCL_3$ | 12.5 | 0.83 |
| MeOH | 44.6 | 2.97 |

### Phytochemical Screening of Root Extract of *C. adenocaule.*

The result for the preliminary Phytochemical screening of root Extract of *C. adenocaule* is shown in Table 2.

**Table 2.** Preliminary Phytochemical screening of Methanolic extract of *C. adenocaule*.

| Phytoconstituent | Test | Result | | |
|---|---|---|---|---|
| | | nHexane | Chloroform | Methanol |
| Alkaloids | Dragendorff's | + | + | + |
| | Mayer's | + | + | + |
| Anthraquinones | | | | |
| Free- anthraquinones | Borntrager's | - | + | - |
| Combine anthraquinones | Borntrager's | - | - | - |
| Carbohydrates | | | | |
| General test | Molisch's | + | + | + |
| Monosaccharide | Barfoed's | + | + | + |
| Free reducing sugar | Fehling's | - | + | + |
| Combine reducing sugar | Fehling's | - | - | + |
| Cardiac glycosides | | | | |
| Steroidal nucleus | Salkowsi's | - | + | + |
| Steroidal nucleus | Liebermann-Buchard's | - | + | + |
| Terpenoids | | - | - | + |
| Flavonoids | Lead acetate | - | + | |
| | Ferric Chloride | - | + | + |
| | Shinoda's | - | + | + |
| | Sodium Hydroxide | - | + | + |
| Saponins glycosides | Frothing | - | - | + |
| Tannins | Ferric Chloride | + | - | + |
| | Lead acetate | + | - | + |

key: + = present: - = absent

### Biological Activity

### DPPH assay: *In-vitro* Antioxidant Activity

**Table 3**. *In vitro* antioxidant activity (DPPH assay) of *C. adenocaule* root extract fractions.

| Concentration (µg/ml) | % of DPPH scavenging activity | | | |
|---|---|---|---|---|
| | nHex | $CHCl_3$ | MeOH | AA |
| 6.25 | 22.8 | 33.5 | 32.8 | 46.5 |
| 12.5 | 29.9 | 35.9 | 55.2 | 53.7 |
| 25 | 36.8 | 39.4 | 67.2 | 64.1 |
| 50 | 40.5 | 44.6 | 79.3 | 77.3 |
| 100 | 55.2 | 50.9 | 97.2 | 96.8 |
| $IC_{50}$ | 74.1 | 74.6 | 10.87 | 4.51 |

**Key**: AA: ascorbic acid, $CHCl_3$: Chloroform extract, nHex: n-Hexane extract, MeOH: Methanol extract

Results of the i*n vitro* antioxidant assay (in $IC_{50}$) of the crude root extracts of *C. adenocaule* are given in Table 3 below. The MeOH extract showed relatively *in-vitro* DPPH scavenging activity compared to other extracts.

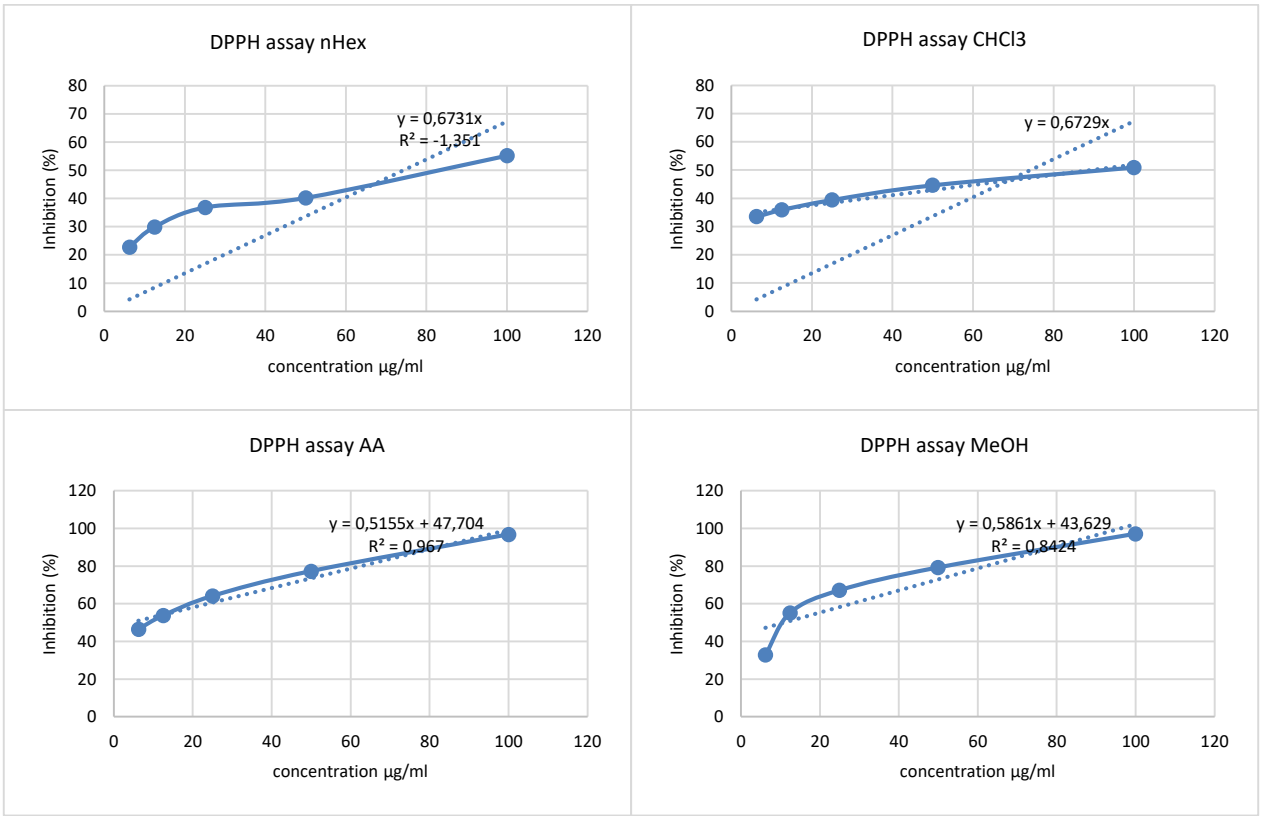


**Figure 1.** DPPH assay; $IC_{50}$ extrapolation graph of nHex, $CHCl_3$, and MeOH extracts of *C. adenocaule.*

## Antimicrobial Susceptibility Assay

The $CHCl_3$ showed the highest activity against the test organism. The result of the *in-vitro* antimicrobial susceptibility assay is given in Table 4.

**Table 4**. *In vitro* antimicrobial activity of *C. adenocaule* extract.

| Concentration (mg/ml) | Test organism | Extracts | | | |
|---|---|---|---|---|---|
| | | **Hex** | **$CHCl_3$** | **MeOH** | **CIP(5mg/ml)** |
| 100 | *E. coli* | 00.00±0.00 | 24.00±0.15 | 0.10±0.02 | 31.00±0.75 |
| | *P. aeruginosa* | 00.00±0.00 | 2.00±0.02 | 2.00±0.00 | 27.30±0.41 |
| | *Stap. aureus* | 00.00±0.00 | 12.50±0.18 | 5.50±0.13 | 29.70±0.17 |
| | *S. epidermis* | 00.00±0.00 | 5.50±0.07 | 1.5.00±0.1 | 25.02±0.84 |
| 75 | *E. coli* | 00.00±0.00 | 12.00±0.15 | 0.10±0.00 | - |
| | *P. aeruginosa* | 00.00±0.00 | 1.00±0.15 | 2.00±0.32 | - |
| | *Stap. aureus* | 00.00±0.00 | 13.50±0.4 | 2.00±0.01 | - |
| | *S. epidermis* | 00.00±0.00 | 7.50±0.12 | 1.0.00±0.00 | - |
| 50 | *E. coli* | 00.00±0.00 | 4.50±0.2 | 0.10±0.00 | - |
| | *P. aeruginosa* | 00.00±0.00 | 0.00±0.00 | 0.00±000 | - |
| | *Stap. aureus* | 00.00±0.00 | 9.10±0.16 | 2.00±0.10 | - |
| | *S. epidermis* | 00.00±0.00 | 2.60±0.05 | 1.70±0.18 | - |
| 25 | *E. coli* | 00.00±0.00 | 3.00±0.21 | 0.10±0.00 | - |
| | *P. aeruginosa* | 00.00±0.00 | 0.00±0.00 | 0.20±0.00 | - |
| | *Stap. aureus* | 00.00±0.00 | 2.50±0.4 | 1.00±0.10 | - |
| | *S. epidermis* | 00.00±0.00 | 1.00±0.00 | 1.50±0.00 | - |

**Key**: CIP: ciprofloxacin

## DISCUSSION

Many solvents including Hex, $CHCl_3$, and MeOH had been employed for the extraction of bioactive plant principles. MeOH had shown effective as an extraction solvent in many plant drug analyses especially in the isolation of phenolics and flavonoids content (Do et al., 2014; Truong et al., 2019). The percentage yield result

from this study shows MeOH to be with the highest extraction yield of 2.97%, followed by $CHCl_3$ (0.83 %) and nHex (0.36 %) respectively. (Table 1).

Phytochemical screening of *C. adenocaule* using standard methods revealed the presence of alkaloids, carbohydrates, saponins, and tannins in the nHex extract. Flavonoids, carbohydrates and glycoside, alkaloids, saponins, terpenoids, and tannins were present in both the $CHCl_3$ and MeOH extracts (Table 4). Similar results were reported by Akinwunmi and colleagues on the phytochemical screening of the ethanol root extract of *C. adenocaule* (Akinwunmi et al., 2015).

DPPH assay remains one of the commonly employed methods for the analysis of the antioxidant activity of plant phytochemicals and employs spectrophotometric application. The ability of a test compound to scavenge DPPH radical is determined based on its concentration providing 50% inhibition ($IC_{50}$), which is the value of the concentration of the sample to cause 50% inhibition and is obtained by the interpolation from the linear regression analysis (Figure 1). In this study, different fractions of the extracts were screened for their antiscavanging activity, and it was observed that the MeOH extract showed the highest potential with an $IC_{50}$ 10.87 µg/mL scavenging activity, followed by nHex (74.1 7 µg/mL) and $CHCl_3$ (74.6 7µg/mL) with the least scavenging activity. Though, the results are below that of the standard; Ascorbic acid AA with $IC_{50}$ 4.50 µg/mL (Table 4). This activity might be due to the presence of phenolics and flavonoids and other secondary metabolites present in the MeOH extract that are known potent antioxidants, and taking into consideration, *C. adenocaule* belong to the *Vitaceae* family which are known for their potent antioxidant principles (Murias et al., 2005; Piotrowska et al., 2012; Rivière et al., 2012). Akinwunmi and colleagues, reported a DPPH scavenging activity of $IC_{50}$ 38.42 µg/mL for the ethanol root extract of *Cissus adenocaule* with the root total phenolic and flavonoid content to be 182±0.38 mg/g TAE (Tannic acid equivalent) and 103±0.42 mg/g QE (Quercetin equivalent) [Akinwunmi et al., 2015].

The use of the plant as an antimicrobial agent in the ethnomedicinal space cannot be overemphasized, as they continued to be used to date. This augments their exploitations for the discovery of lead and novel molecules for antimicrobial drug discovery. They provide starting materials and derivatives that are employed as ligands in the drug discovery and development process. This study explored the antimicrobial activity of the *C. adenocaule* root extracts (Table 4). The n-Hexane extract was completely inactive against the test organism. The $CHCl_3$ extract was active at 100mg/ml and showed good activity against *E. coli* (24.00±0.15) and moderate activity against *Staph. aureus* (12.5±0.18). The MeOH extract showed poor activity against the test organism. This result is inconsonant with an earlier report by Hamil and colleagues on the activity of MeOH root extract of *C. adenocaule* on *E. coli. P. aeruginosa* and *Staph. aureus* (Hamill et al., 2003).

## CONCLUSION

The present study undertook the phytochemical screening, isolation, and characterization of chemical compounds present in the methanol root extract of *C. adenocaule*, as well as, determination of their antioxidant and antibacterial activity. Methanol presents the best extraction solvent in terms of percentage yield. Flavonoids, alkaloids, carbohydrates and glycoside, saponins, and tannins were present while anthraquinone were absent. In the chloroform and hexane extracts; anthraquinones and flavonoids were absent, with carbohydrates also absent in the hexane extracts. In the assessment of the biological properties, the antibacterial sensitivity assay showed Chloroform to have activity against *E. coli* and moderate against *Staph. aureus* at 100mg/ml respectively, while poor activity was recorded with the Methanol and n-Hexane extract. The DPPH antioxidant assay revealed the free radical scavenging activity, Methanol extract yields the best result with an $IC_{50}$ of 10.87µg/ml. The results from this study add to the existing literature on *C. adenocaule* with scientific evidence into its biological properties.

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