Phytochemical screening, antioxidant and antibacterial activity of the root extract of *Cyphostemma adenocaule* (Steud. ex A. Rich.) Wild & R.B.Drumm

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ABSTRACT

Plant secondary metabolites had provided important bioactive principles for developing new lead compounds. Within their confinement, they exhibit unique chemical diversity, which influence their diverse biological properties. The *vitaceae* family are known for their 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*, as well as, evaluating their Biological properties; antioxidant activity (DPPH assay) and antibacterial activity (agar well diffusion test). Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, carbohydrates & glycoside, alkaloids, saponins and tannins. The methanol root extract had the highest percentage in the DPPH assay, providing 50% inhibition IC_{50} of 10.87µg/ml, followed by nHexane (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 moderate activity against *Staph. aureus* (12.5±0.18). The nHex extract was completely inactive against the test organism while the methanol extract showed poor activity against the test organism. 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

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INTRODUCTION

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

Morphine from the opium poppy plant is considered the first pharmacologically-active compound isolated by Friedrich Sertürner (Hamilton and Baskett, 2000 and Joo, 2014). Natural products are important for the development of new drugs, and these products have been in constant use. Drug used as anticancer, antihypertensive, and antimigraine medication, have benefited greatly from natural products (Newman, and Cragg, 2003, Joo, 2014).
Plants have been the part of traditional medicine systems, which have been used for thousands of years (Schultes and Raffauf, 1990; Jain, 1991 and Iwu, 1993). 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, 2002).

Cyphostemma adenocaule (Steud. ex A. Rich.) Wild & R.B.Drumm is a climbing, scrambling or trailing herb that belongs to the *Vitaceae* family (Burkill, 1985 and Bello *et al*., 2019) and locally known as yáákùwár fátààkéé (Hausa, Nigeria) (Burkill, 1985). 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 et al (2019).
The effectivity of Plant bioactive compounds against oxidative stress related diseases and as an anti-infective had been well documented. This study entails to investigate the phytochemical, antioxidant and antibacterial properties of the root extracts of *C. adenacaule* 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 antioxidant and antimicrobial agent.

MATERIALS AND METHOD.

Plant Collection and Identification

Fresh root parts of *C. adenocaule* was collected aseptically in July, 2019 from Shuwarin town, Dutse LGA, Jigawa State, Nigeria, and identified by a Medicinal Botanist (Mal Namadi Sanusi) of the Biology department, ABU 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 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 literature (Brain and Turner, 1975; Vishnoi, 1979; Markhan, 1982; Sofowora, 1993; Silver *et al*., 1998; Trease and Evans, 2002).

Biological Activity

Test for Antioxidant Activity: DPPH Assay

The radical scavenging potential was done using DPPH assay (Brand *et al*., 1995). 3mL 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 37^0 centigrade for 30 minutes in dark. Then absorbance was taken at 517nm wavelength in UV spectrophotometer. Negative control contained 100 µL of methanol in place of 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 positive control. Inhibition curves were made and IC50 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 gotten from the Microbiology department, University of Maiduguri and were stored at 2-8^{0}C until required.

Preparation of Extract solutions for Pathogenic Assay

Stock solution of the extracts was prepared by dissolving 10g of extract in 10mL of distilled water, and 1000mg/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 24hrs 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 10^8cells/mL. This was then diluted to produce 10^6cells/mL and used in the experiments. 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 18.5g powder in 500mL of distilled water) and sterilized at 121^{0}C for 15min. The sterilized agar was allowed to cool to 50^{0}C in a water-bath. The test organism (1mL) (10^6cells/mL) was inoculated into pre-labeled petri plates (90mm diameter), then 19mL of the molten agar was added to each petri plate, shacked and allowed to set at room temperature on a flat surface.

Antimicrobial Susceptibility Assay (agar well diffusion method)

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by 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 a 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 h and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extract. After 24h, 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 deviation with observation recorded in triplicates. Analysis of variance for individual parameters was performed on the basis of 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.

RESULT

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. adenocule.*

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

Table 2: Preminilary Phytochemical screening of Methanolic extract of *C. adenocaule*

Phytoconstituent	Test	Result		
		nHex	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: *Invitro* antioxidant activity

Results of the i*nvitro* antioxidant assay (in IC_{50}) of the crude root extracts of *C. adenocaule* and compound CA1 is given in Table 3 below. The MeOH extract showed relative *invitro* DPPH scavenging activity compared to other extracts, while weak activity was shown by compound CA1.

Table 3: *Invitro* antioxidant activity (DPPH assay) of *C. adenocaule* extract.

	Hex	CHCL3	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

Antimicrobial Susceptibility Assay

The $CHCL_3$ showed the highest activity against the test organism .The result of the *invitro* antimicrobial susceptibility assay is given in Table 4.

Table 4: *Invitro* antimicrobial activity of *C. adenocaule* extract.

		Extract			
Concentration (mg/ml)	**Test organism**	**Hex**	**CHCL3**	**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	-

DISCUSSION

Many solvents including Hex, CHCL3 and MeOH had been employed for the extraction of bioactive plant principles. MeOH had shown effective as an extraction solvent in many plant drug analysis especially in the isolation of phenolics and flavonoids content (Do *et al*., 2014 and Troung *et al*., 2018). The percentage yield result from this study shows MeOH to be with the highest extraction yield of 2.97%. (Table 1). This with the results of the phytochemical screening made the MeOH extract, a choice for further analysis.

Pythochmical screening of *C. adenocaule* was positive for the presence of various plant secondary metabolites (Table2). The MeOH extract revealed presence of flavonoids, alkaloids, carbohydrates & glycoside, alakaloids, saponins and tannins (Table 2). Similar results were reported by Feyisayo *et al* on the phytochemical screening of the ethanol root extract *of C. adenocaule* (Feyisayo *et al*., 2015).

DPPH assay remains one of the commonly employed methods for the analysis of antioxidant activity of plant phytochemicals and employs the spectrophotometric application. The ability of a test compound to scavenge DPPH radical is determined on the basis of 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 (Appendix I). In this study, the MeOH extract showed potential scavenging activity (10.87µg/ml) compared to other extract, however, below that of the standard; AA (4.50µg/ml) (Table 3). This activity might be due to the presence of phenolics, 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 and Rivière *et al*., 2012). Feyisao and colleagues, reported a DPPH scavenging activity of the ethanol root extract of *Cissus adenocule* (IC_{50} 38.42µg/ml) with the root total phenolic and flavonoid content to be 182±0.38mg/g TAE and 103±0.42mg/g QE (Feyisayo *et al*., 2015).

The use of plant as an antimicrobial agent in the ethnomedicinal space cannot be overemphasized, as they continued to be use till date. This augments their exploitations for discovery of lead and novel molecules for antimicrobial drug discovery. They provide starting materials and derivatives that are employed as ligands for modification, synthesis, combinatorial and computational application in the drug discovery and development process. This study

explored the antimicrobial activity of the *C. adenocaule* root extracts (Table 4). The nHex extract was completely inactive against the test organism. The CHCL3 extract was active at 100mg/ml and showed a good activity against *E. coli* (24.00±0.15) and moderate activity against *Staph. aureus* (12.5±0.18). The MeOH extract showed poor acivity against the test organism. This results is in consonant with earlier report by Hamil and colleagues on the activity of MeOH root extract of *C. adenocaule* on *E. coli. P. aeruginosa* and *Staph. aurues* (Hamil *et al*., 2013).

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 & glycoside, alkaloids, saponins and tannins where present while anthroquinone where absent. In the assessment of the biological properties, antibacterial sensitivity assay showed $CHCL_3$ to have activity against *E. coli* and moderate against *Staph. aureus* at 100mg/ml respectively, while poor activity was recorded with the MeOH and nHex extract. The DPPH antioxidant assay revealed the free radical scavenging activity, MeOH extract yields the best result with an IC_{50} of 10.87µg/ml. The results from this study adds to the existing literature on *C. adenocaule* with scientific evidence into its biological properties.

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