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Phytochemical Screening, Proximate, Mineral, and Heavy Metal Composition of Stem Bark of *Cylicodiscus gabunensis* Harms (*Leguminosae*)

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ABSTRACT
The trade in herbal medicines such as supplements, cosmetics, and additives has witnessed an exponential increase in recent times. Therefore authentication of herbal plant materials is important in establishing the quality of herbal medicines in a bourgeoning market. This study aims to determine the phytochemical constituents, proximate parameters, mineral composition, and levels of toxic metals in the stem bark of *Cylicodiscus gabunensis* to establish its safety. The phytochemical and proximate analysis of the plant sample was carried out using standard procedures. The mineral elements and heavy metals were determined using a flame photometer and AAS. The phytochemical analysis revealed the presence of alkaloids, tannins, phenols, flavonoids, saponins, steroids, deoxysugars, and carbohydrates. The proximate analysis showed the moisture content, total ash value, acid insoluble ash, water-soluble ash, alcohol extractive value, and water extractive value to be 10.91%, 5.62%, 1.83%, 0.72%, value 11.74%, and 10.90%, respectively. The elemental analysis gives the content of calcium, zinc, iron, magnesium, copper, manganese, sodium, and potassium at 4,100 ppm, 2.5 ppm, 144 ppm, 169 ppm, 2 ppm, 13.5 ppm, 75 ppm, and 4,200 ppm, respectively. The concentrations of the heavy metals were found to be 3.5 ppm, 2.5 ppm, and 1.5 ppm for nickel, lead, and cadmium, respectively. It thus reveals that the wide range of secondary metabolites in the plant sample may be responsible for its pharmacological activity and use in ethnomedicine. The proximate parameters showed that the crude powdered plant is a rich source of vital minerals, could withstand longer shelf storage, and is free from inorganic materials contaminants. However, the levels of cadmium and lead are above the permissible limit for plants samples.

Keywords: *Cylicodiscus gabunensis*, Heavy metals, Phytochemicals, Proximate analysis

INTRODUCTION
Since antiquities, humans have been using plants as a source of healthcare. Authentication of herbal plant materials is important in establishing the quality of herbal medicines in a bourgeoning market. The growing use of herbal products worldwide is due to their claimed safety. However, studies have shown that herbal medicines can be potentially toxic to human health, mutagenic and may cause unknown effects and certain herb-drug interactions (Matthews *et al*., 2003; Ferreira-Machado *et al*., 2004). Also, variations in the chemical constituents of herbal products make it difficult to establish quality control parameters, not forgetting their adulteration/substitution, which in some cases may be intentional (Khan et al., 2011). All of the above have given rise to poor quality herbal formulations and necessitate an urgent need to authenticate the safety and purity of these plants' products.

Cylicodiscus gabunensis Harms has been used for centuries by the natives for treating different ailments and for domestic applications as a source of wood. In traditional medicine, it has been used to treat venereal diseases, malaria, psoriasis and rheumatism (Ayarkwa and Owusu, 2008). It is also used to relieve stomach aches, migraine and vomiting. Pharmacological investigations of the stem bark extract of the plant revealed its effects as antimalarial, antimicrobial, antidiabetic, anti-inflammatory, antioxidant, and anti-diarrhoea. (Aldulaimi *et al*., 2019; Oboh et al., 2018; Gyamfi et al., 1999; Okokon et al., 2006; Okokon et al., 2016).

This study aimed at identifying the secondary metabolites, mineral and heavy metal composition of *C. gabunensis*.

MATERIALS AND METHODS
Plant materials and preparation
The fresh stem bark of *Cylicodiscus gabunensis* was collected from Benin City, Edo State, Nigeria, in January 2021. It was identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, where a voucher number of UBF-V261 was assigned. The stem bark was air-

dried, ground to powder with a mechanical blender, weighed and stored until further use.

Phytochemical screening

Qualitative phytochemical screening was carried out on the aqueous extract of the powdered material to determine the presence of alkaloids, tannins, phenols, flavonoids, saponins, steroids, deoxysugars, and carbohydrates following the standard procedure (Yadav and Agarwala, 2011).

Proximate Analysis

The proximate evaluation for total ash, acid-insoluble ash, water-soluble ash, and moisture content was performed according to the AOAC and African Pharmacopoeia methods (AOAC, 1984; African Pharmacopoeia, 1986). Water-soluble extractive and alcohol soluble extractive values were determined using the BP standards (BP, 1998).

a) Moisture content

2 g of the powdered material was weighed into a clean, dry crucible of known weight. The crucible with its content was oven-dried at 105^{o}C until a constant weight was reached. The moisture content (average percentage weight loss) was determined for three replicates with reference to the air-dried powdered sample from the formula below:

$$\text{Moisture content (\%)} = \frac{Weight\ loss}{Initial\ weight\ of\ sample}\ X\ 100$$

b) Total ash

Six crucibles were washed thoroughly and dried in a hot oven at 100^{o}C. While still warm, they were marked numbers 1 to 6 and subsequently cooled in a desiccator and weighed. 2 g of the powdered sample were weighed into each crucible. The crucibles with their contents were ashed in a furnace at 600^{o}C for 6 hours. After which, the furnace was switched off, and the temperature was allowed to drop. The crucibles were then removed, cooled in a desiccator and reweighed. The percentage of ash was calculated for each sample using the equation below.

$$\text{Percentage Total ash (\%)} = \frac{W-Z}{N}\ X\ 100$$

Where W = weight of the crucible and ash
Z = weight of the empty crucible
N = weight of the sample

c) Acid insoluble ash

Three crucibles from the total ash experiment above were transferred into three beakers containing 25 mL of 0.1M HCl. The beakers' content was boiled for 5 minutes and filtered through an ashless filter paper. The filter paper with the residue was folded into a small cone, transferred into the crucible, and heated in the furnace until the filter paper was completely ashed. The weight of the residue was determined, and the percentage of acid insoluble ash was calculated based on the initial weight of the dried powdered sample. The percentage of acid insoluble ash was calculated from the formula below for each sample.

$$\text{Acid Insoluble Ash Value (\%)} = \frac{Weight\ of\ Residue}{Initial\ weight\ of\ sample}\ X100$$

d) Water-soluble extractive value

The powdered sample (5 g) was weighed into a 250 mL conical flask and was macerated with 50 mL of water for 6 hours. The extract was filtered using a Whatman No.1 filter paper. The filtrate (20 mL) was measured into a clean, dried and weighed crucible. It was then evaporated to dryness. The residue was dried to a constant weight, and the final weight was recorded. The water-soluble extractive value was then calculated for three replicates with reference to the initial weight of the powdered sample expressed as a percentage.

$$\text{Water-soluble Extractive Value (\%)} = \frac{Weight\ of\ Residue}{Initial\ weight\ of\ sample}X100$$

e) Alcohol soluble extractive value

This followed the same procedure as in d above, except the plant sample was macerated with 50 mL of 95% ethanol for 6 hours. Alcohol soluble extractive value was then calculated for three replicates with reference to the initial weight of the powdered sample expressed as a percentage.

$$\text{Alcohol soluble Extractive Value (\%)} = \frac{Weight\ of\ Residue}{Initial\ weight\ of\ sample}X100$$

Determination of Mineral and Heavy Metal Contents

Sample Digestion

One gram (1 g) of the powdered sample was weighed into a measuring cylinder. A mixture of nitric acid and perchloric acid (3:1) was added into the sample, mixed and heated in the fume cupboard until the brown fumes became clear. The digested sample was then filtered into a 100 mL volumetric flask, and the mixture was made up to the 100 mL mark with distilled water. Analysis of the sample for calcium, iron, zinc, magnesium, lead, manganese, cadmium, chromium, nickel, and copper was carried out in duplicates with an Atomic Absorption Spectrophotometer (Model-Solaar 969 UNICAM Series). The elements, sodium and potassium, were determined in duplicates with a flame photometer (Model 410, Sherwood Scientific, UK), and their results were noted.

Statistical Analysis

The results were expressed as mean ± standard error of the mean using Microsoft office Excel software 2019.

RESULTS AND DISCUSSION

The phytochemical screening of the stem bark of *Cylicodiscus gabunensis* revealed the presence of alkaloids, tannins, phenols, flavonoids, saponins, steroids, carbohydrates, reducing sugars, and deoxysugars (Table 1), which may be responsible for the medicinal properties of the plant. According to a study, phenols possess antimicrobial and antioxidant properties (Egbuna *et al*., 2018). In another study, the antimicrobial property of *Cylicodiscus gabunensis* was evaluated using the aqueous extract of the stem bark on two test organisms; *Salmonella typhi* and *Staphylococcus aureus* using Streptomycin as the standard drug. Compared to the standard, there was appreciable inhibition of the organisms (Ayuk *et al.*, 2015). Tannins have been known to exert anti-diarrhoeal activity (Hussein and El-Anssary, 2017), supporting the claim of *Cylicodiscus gabunensis* in treating diarrhoea. This claim was also confirmed in a study using an organic solvent for extraction. It was observed that single oral doses (375 and 750 mg/kg body weight) of *Cylicodiscus gabunensis* ethyl acetate extract produced a significant (79.22% and 65.58%, respectively) decrease in the severity of diarrhoea compared to Loperamide 70.38% at a dose of 3 mg/kg body weight (Kouitcheu *et al.*, 2006). Tannins also possess wound-healing and antimicrobial properties, further supporting the antimicrobial activity of the stem bark of *Cylicodiscus gabunensis* (Egbuna *et al*., 2018). Alkaloids possess several pharmacological actions, including analgesic, muscle relaxation and treatment of migraines (Hussein and El-Anssary, 2017). Studies have confirmed the antiplasmodial action of flavonoids. Therefore, it is no wonder that plant extract has been used in ethnomedicine to treat malaria and fevers. (Marliana *et al*., 2018). In another study, flavonoid compounds were isolated from the plant's stem bark and tested against a *Plasmodium falciparum* strain. There was significant activity against the parasite (Zelefack *et al.*, 2012). Steroids have anti-tumour and immunosuppressive properties (Patel and Savjani, 2015). Saponins possess analgesic properties and anti-tumour properties.

Table 1: Phytochemical constituents of *Cylicodiscus gabunensis* stem bark

Phytochemicals	Inference
Alkaloids	+
Phenols	+
Flavonoids	+
Proteins	-
Reducing sugars	+
Saponins	+
Tannins	+
Carbohydrates	+
Steroids	+
Deoxysugars	+

The proximate analysis of the powdered stem bark of *Cylicodiscus gabunensis* established important parameter values, including parameters against adulteration (Table 2). The total ash was confirmed to be 5.623± 0.103 %, acid-insoluble ash 1.83 ± 0.133 %, and water-soluble ash 0.724 ± 0.237. The alcohol extractive value was higher than the water extractive value, indicating that alcohol may be a more efficient solvent for extraction than water. Also, the moisture content was 10.913 ± 0.060, which is within the African pharmacopoeia limit for vegetable drugs (8-14%) (African Pharmacopoeia, 1986). The moisture content is very significant in terms of prolonged shelf life and resistance to microbial degradation of the crude drug.

Table 2: Proximate composition of *Cylicodiscus gabunensis* stem bark

Parameter	Value ± SEM (%)
Moisture content	10.91 ± 0.060
Total ash	5.62± 0.103
Acid insoluble ash value	1.83 ± 0.133
Water soluble ash value	0.72 ± 0.237
Alcohol extractive value	11.74 ± 0.280
Water extractive value	10.90 ± 1.346

The mineral analysis (Table 3) of the powdered stem bark of *Cylicodiscus gabunensis* showed the presence of calcium, iron, zinc, magnesium, lead, manganese, cadmium, nickel, copper, sodium and potassium. Chromium was undetected. It was found that the mineral elements were below the upper level of intake and within the recommended daily intake in healthy individuals based on a reference established by a study (Capra, 2006).

These mineral elements have important physiological actions in the body. They can be toxic if present in higher amounts, but they are needed for survival (Gupta, 2018). Sodium and potassium maintain osmotic balance and are involved in action potentials and muscle contraction. Sodium regulates the volume of plasma as it is the major intracellular ion. The sodium deficiency could result in hypotension and paresthesias (Soetan *et al*., 2010). Potassium is also essential in glycogenesis. Potassium deficiency affects tubules of the kidney. Calcium is essential for strong bones and teeth and blood clotting (converting prothrombin to thrombin) (Soetan *et al*., 2010). Several enzymes rely on calcium, including adenosine triphosphate. Reduced extracellular blood calcium increases the irritability of nerve tissue. Magnesium is needed for the proper functioning of many enzymes in which thymine pyrophosphate is a cofactor (Soetan *et al*., 2010). Oxidative phosphorylation is reduced greatly in the absence of magnesium (Cooper, 2000). Magnesium deficiency could result in hyperemia and erythema (Soetan *et al*., 2010). Zinc boosts the immune system and is a constituent of many enzymes like lactate dehydrogenase and alcohol dehydrogenase. Copper plays a role in iron absorption and is an essential micronutrient necessary for hematologic and neurologic systems. Copper deficiency could cause impaired growth, reproductive performance and anaemia. Iron is essential for the transfer of oxygen between tissues of the body. Iron is a cofactor required by some enzymes involved in neurotransmitter synthesis. Iron deficiency results in anaemia. Manganese serves as a cofactor of hydrolase, decarboxylase and transferase enzymes. It is also involved in proteoglycan synthesis (Soetan *et al*., 2010).

Three heavy metals, namely; lead, nickel and cadmium, were analyzed (Table 4). The level of nickel was found to be below the intervention value of soil and permissible value in plants based on references from previous studies (Denneman and Robberse, 1990; Permissible limits of heavy metals, 1996). The lead level was below the intervention value of soil but higher by 0.5 mg/kg than the permissible value in plants. Cadmium was also present at a level lower than the intervention value of soil but higher than the permissible value in plants by 1.48 mg/kg. Lead is a toxic metal that could result in teratogenic effects. Lead has also been implicated in poor development of the brain's grey matter, resulting in children's poor intelligence quotient (Engwa *et al*., 2019). Cadmium is not considered to be beneficial in normal biological function. However, its presence could result in skeletal damage, resulting from the direct action of cadmium on bone cells or secondary response to kidney damage (Mahurparwar, 2015). Cadmium has been implicated in promoting apoptosis, oxidative stress, DNA methylation and damage (Engwa *et al*., 2019). The contamination of the plant with these toxic metals could result from indiscriminate dumping of chemical wastes from mining sites, car battery chargers and wastes from car mechanics and welders' workshops. The toxic metals may be transported through streams and stored in river bed sediments (Duruibe *et al*., 2007).

Table 3: Mineral contents of the stem bark of *Cylicodiscus gabunensis*

Metals	**Content (mg/g) ± SEM**	***Recommended Daily Intake (RDI) for a healthy male aged 19-30 years (mg/day)**	***Upper Level of Intake (mg/day)**
Calcium	4.1 ± 0.05	1000	2500
Zinc	0.0025 ± 0.0003	14	40
Iron	0.144 ± 0.034	8	45
Magnesium	0.169 ± 0.013	400	350
Copper	0.002 ± 0	1.7	10
Manganese	0.0135 ± 0.0005	5.5	-
Sodium	0.075 ± 0.005	460-920	-
Potassium	4.20 ± 0.1	3800	-

*Source: (Capra, 2006)

Table 4: Heavy metal Content

Heavy Metal	Content (mg/kg) ± SEM	*Intervention values of soil (mg/kg)	**Permissible value of plants (mg/kg)
Nickel	3.5 ± 1.5	210	10
Lead	2.5 ± 0.5	530	2
Cadmium	1.5 ± 0.5	12	0.02

* Source: (Denneman and Robberse 1990)

** Source: (WHO, 1996. Permissible limits of heavy metals in soil and plants)

CONCLUSION

The presence of alkaloids, tannins, phenols, flavonoids, saponins, steroids, carbohydrates, reducing sugars, and deoxysugars in the plant sample was established, which may be responsible for its biological activities. The proximate parameters have been established to detect adulteration. Although the stem bark of *Cylicodiscus gabunensis* could serve as a source of mineral elements in quantities within recommended limits, amounts of cadmium and lead in the stem bark sample of the plant are above allowable limits for plants materials. These limits may be due to the source of collection of the plant material.

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