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PHYTOCHEMICALS AND TRACE ELEMENTS COMPOSITION OF LEAF, STEM AND *RHIZOME* OF *Costus afer* Ker Gawl.

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Abstract
Phytochemicals and nutrients are indicators of the therapeutic potentials and values of medicinal plants. The discovery of different types of phytochemicals, macro and micro nutrients in plants through scientific investigations continues to provide insights into their therapeutic properties. Costus afer Ker Gawl is ethno-medicinally valued for its anti-diabetetic, anti-hypertensive, antimalarial, anti-hemorrhagic and antimicrobial properties in addition to the several other importance of the plant. This study was designed to assess the phytochemicals and trace elements constituents of different parts of Costus afer. Plant samples collected were subjected to qualitative phytochemical screening and the presence of trace elements was detected using bulk Atomic Absorption Spectrometry. Data were analyzed using One-way Analysis of Variance. The results obtained showed the presence of saponins, tannins, alkaloids and cardiac glycosides in all plant parts studied. However, anthraquinones were not detected in any of the plant parts. Manganese concentration was highest in the leaf (0.165 ± 0.001 mg/g), while the highest concentration of iron (0.136 ± 0.001 mg/g) was detected in the rhizome. Also, the rhizome had the highest concentration of zinc (0.044± 0.001 mg/g).Copper and cadmium were however detected in negligible amounts in the leaf and stem samples. The use of Costus afer in traditional medicine,as well as the preference of a plant part over others can be scientifically attributed to the presence of phytochemicals and other nutrients in the various parts used. However, the mechanisms of action of these metabolites and the biochemical roles that these chemical elements play in disease management involving the use of Costus afer have not been scientifically explained. Consequently, pharmacological investigations that will focus on these aspects of disease management and drug discovery are recommended.

Keywords: *Costus afer, Phytochemicals, Trace elements, Bioactive Compounds, Traditional Medicine.*

Introduction

The use of plants as herbal remedies for various infections and disease conditions dates back to the existence of man. Secondary metabolites also known as phytochemicals and elements inherent in plants play vital roles in the prevention, treatment and management of several chronic disease conditions and infections. Plants remain the natural reservoir of bioactive compounds from which drug leads are extracted for therapeutic purposes (Larayetan *et al.,* 2019). Phytochemicals such as saponins, tannins, alkaloids, flavonoids and cardiac glycosides are natural products that have been shown to possess notable therapeutic properties which include antimicrobial, antiseptic, anti-inflammatory, antioxidant, hypotensive, antimalarial, anti-carcinogenic and anti-mutagenic properties(Sahare and Moon, 2015; Shen *et al.,* 2017). Elements, categorized as essential and non-essential, play important roles in many biochemical functions including normal enzyme activities and also in defense system (Beck and Namdeo, 2017) while deficiencies of such elements are characterized by metabolic disorders, bone diseases as well as nervous system disorders. Trace elements are essential and needed for biological, chemical and molecular functions as they act as co-factors for various enzymes and proteins, and are also important for the stabilization of cellular structures (Prashanth *et al.,* 2015). However, deficiency of these trace elements may result in disease states including poor metabolism, obesity, and electrolyte imbalance among others. Edeoga and Okoli (2000) reported that medicinal plants are natural sources of bioactive compounds and nutrients which make them effective remedies for various ailments.

Costus afer commonly called spiral ginger, ginger lily or bush sugar cane is a medicinal plant that is mostly found in moist and shady forests of Western and Tropical Africa. It is one of about 150 perennial, rhizomatous herbs of the Costaceae family. The plant is endemic in Nigeria but is mainly grown in home gardens for medicinal purposes. In traditional medicine, its leaves, fruits, stem and rhizomes are harvested for the treatment of various ailments including sore throat, cough, diarrhea and hemorrhage (Okwu and Okwu, 2004); diabetes and arthritis (Soladoye and Oyesika, 2008); fever, venereal diseases, eye inflammation, jaundice, hemorrhoids, hypertension, leprosy, toothache, measles and malaria (Omokhua, 2011);

stomachache and sleeping sickness (Iwu, 1993). According to Zafei and Ullah (2010), it is of great importance to screen plants and plants materials for bioactive compounds and also assess their inherent therapeutic values. Previous studies by Anyasor *et al.*(2010); Momoh *et al.*(2011); Akpan*et al.*(2012); Ukpabi *et al.*(2012); Ezejofor *et al.*(2013); Tshamgoue *et al.*(2015) and Udoh *et al.*(2018) focused on the leaf or stem or both parts or stem juice extract of the plant. However, there is insufficient information on comparative phytochemical and elemental composition of leaf, stem and rhizome of *Costu safer*. The aim of this study therefore was to determine the phytochemicals and nutrients composition of the leaf, stem and rhizome of *C. afer* and their potentials in the treatment and management of diseases and infections.

Materials and Method

Sample Collection

Costus afer plants were collected from the wild around Ogbe riverbank, Togunmaga in Ijebu East local government area of Ogun State, Nigeria (fig. 1). The plant was identified at the Taxonomy Section of the Forestry Research Institute of Nigeria, Ibadan.

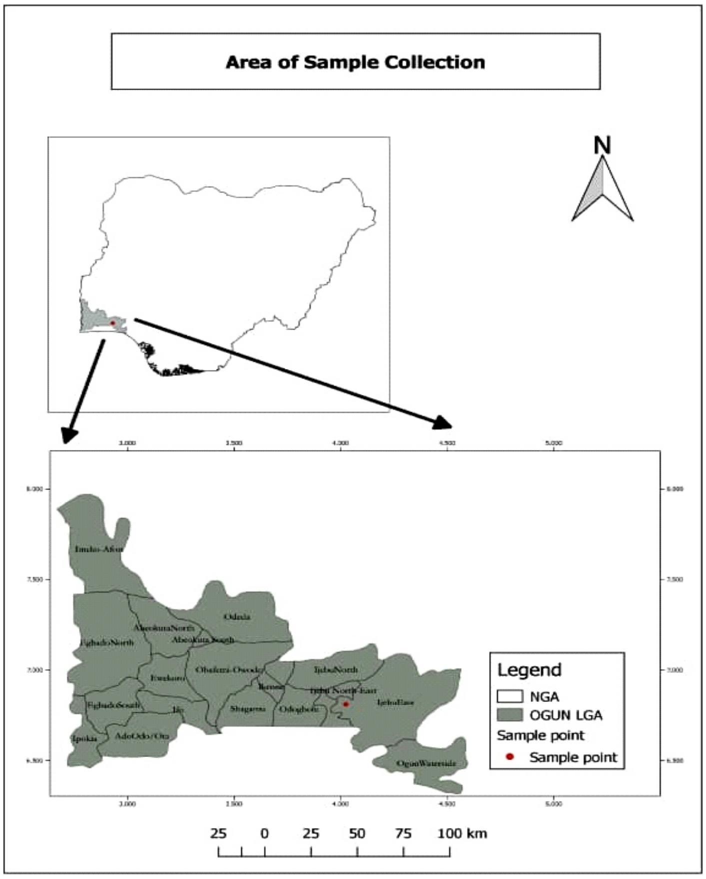

Figure 1. The map showing the location of sample collection.

Sample Preparation

The plant samples were separated into leaf, stem and rhizome, were thoroughly washed with clean water, air-dried, milled into powder and stored in air-tight containers for laboratory analyses.

Phytochemical Screening

Phytochemical analysis of powdered leaf, stem and rhizome of samples was carried out at the Biomedicinal Research Centre Laboratory, FRIN and was conducted according to the standard methods of Trease and Evans (1989) and adopted by Sofowora (1993).

Test for saponins: Frothing Test

One gramme of the powdered sample was boiled with 10 ml of distilled water for 10 minutes, filtered while hot and the filtrate was allowed to cool. 1ml of the filtrate was diluted to 10 ml with distilled water and hand-shaken vigorously for 20 minutes. The formation of persistent foam indicated the presence of saponins.

Test for Alkaloids

One gramme of the powdered sample was stirred in 10 ml of 10% (v/v) HCl on a steam bath followed by filtration. Four drops each of Meyer's, Wagner's and Dragendorff's reagents were added to 1 ml of cooled filtrate in separate test tubes. The formation of precipitate or turbidity indicated the presence of alkaloids.

Test for Tannins
A gramme of powdered sample was boiled in 10 ml distilled water, filtered while hot and cooled. The filtrate was adjusted to 10ml with distilled water. Four drops of 1% ferric chloride were then added to 1ml of the filtrate. The formation of blue, dark brown, blue black, green or green-black solution or precipitate indicated the presence of tannins.

Test for Favonoids
One gramme of the powdered sample was boiled in 10 ml of ethanol, filtered while hot and the filtrate was allowed to cool. Two drops of 1% $FeCl_3$ was added to 2 ml of the filtrate. . The formation of a dusty green solution indicated the presence of flavonoids. A little quantity of dilute NaOH was added to another 2 ml of the filtrate followed by gentle addition of conc. HCl down the side of the tube. The formation of red solution indicated the presence of flavonoids.

***Test for Free Anthraquinones*:**
0.5 g of the powdered sample was shaken with 5ml of chloroform for 10 minutes and filtered. 5 ml of 10% ammonia solution was added to the filtrate and shaken. The formation of a pink, red or violet colour in the ammonia phase indicated the presence of free anthraquinones.

***Combined Anthraquinones*:**
One gramme of the powdered sample was boiled with 5 ml of 10 % HCl for 5 minutes and filtered while hot. Cooled filtrate was partitioned against equal volumes of chloroform avoiding vigorous shaking. Chloroform layer was carefully transferred to a clean tube and equal volume of 10 % ammonia was added. A pink, red or violet colour in the aqueous layer indicated the presence of combined anthraquinones.

Test for Cardiac Glycosides: Keller-Killiani Test
1 g of powdered sample was extracted with 10 ml of 80 % ethanol for 5 minutes on a water bath and filtered. Cooled filtrate was diluted with equal volumes of distilled water. A few drops of lead acetate solution was added, shaken and filtered after standing for few minutes. The filtrate was then extracted with aliquots of chloroform and evaporated to dryness on steam bath. Dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of 1% $FeCl_3$ solution in a clean test tube. 2 ml of conc. H_2SO_4 was then poured down the side of the tube so as to form a layer below the acetic acid. The formation of a purple, reddish-brown or brown ring at the interface or a green colour in the acetic layer indicated the presence of cardiac glycosides.

Elemental Analyses
Elemental analysis of leaf, stem and rhizome of *C. afer* was carried out at the Soils and Tree Nutrition laboratory, FRIN, using Bulk Atomic Absorption Spectrophotometer (AAS), according to standard method of AOAC (1990).

Statistical Analysis
Data obtained were subjected to One-Way Analysis of Variance and mean separation was carried out with Duncan Multiple Range test where p values less than 0.05 were taken to be statistically significant.

Results
The results of the screening of *Costus afer* for secondary metabolites showed that the leaf, stem and rhizome of the plant have saponins, tannins, alkaloids and cardiac glycosides as shown in Table 1. The presence of flavonoids was detected in leaf and rhizome only, while anthraquinones were not detected in any of the plant parts. Other phytochemical constituents of the leaf, stem and rhizome of *C. afer* as recorded in this study are in agreement with previous reports of Anyasor *et al.* (2010), Momoh *et al.* (2011).

Table 1: Phytochemical composition of leaf, stem and rhizome of *Costus afer*

Phytochemical	Leaf	Stem	Rhizome
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Flavonoids	+	-	+
Cardiac glycosides	+	+	+
Anthraquinones	-	-	-

+ indicates present; - indicates absent

The results of the chemical element analyses of the different parts of the plant species showed that manganese, iron, zinc, copper, lead and cadmium were detected in all the plant parts. However, there were significant differences in the concentrations of manganese, iron and zinc among the plant parts with the leaf having the highest concentration of manganese (0.165±0.001 mg/g) while the rhizome had the highest concentrations of iron (0.136±0.001 mg/g) as depicted in table 2. Zinc concentrations in the leaf, stem and rhizome were 0.028±0.000, 0.018±0.001 and 0.044±0.001 mg/g respectively. The rhizome also had the highest concentration of copper while there was no significant difference in copper concentrations of the leaf and stem. Lead and cadmium, though detected in trace amounts, were found to be highest in the leaf and stem of *C. afer* respectively. The difference in the concentrations of trace elements in the different parts of the plant were found to be statistically significant as shown in table 3.

Table 2: Chemical element composition of leaf, stem and rhizome of *Costus afer*

Element	**Plant part**		
Concentration (mg/g)	**Leaf**	**Stem**	**Rhizome**
Manganese	0.165 ± 0.001^{c}	0.042 ± 0.001^{a}	0.054 ± 0.001^{b}
Iron	0.050 ± 0.006^{b}	0.024 ± 0.000^{a}	0.136 ± 0.001^{c}
Zinc	0.028 ± 0.000^{b}	0.018 ± 0.001^{a}	0.044 ± 0.001^{c}
Copper	0.003 ± 0.000^{a}	0.002 ± 0.000^{a}	0.012 ± 0.001^{b}
Lead	0.023 ± 0.002^{b}	0.018 ± 0.001^{a}	0.016 ± 0.000^{a}
Cadmium	0.004 ± 0.000^{a}	0.005 ± 0.000^{a}	0.003 ± 0.001^{b}

Data are presented as mean±standard error of mean (SEM) of three replicates
Superscripts across row indicate significant difference $P<0.05$

Table 3. One-way **ANOVA** of chemical elements composition of *Costus afer.*

		Sum of Squares	Df	Mean Square	F	Sig.
MN	Between Groups	.028	2	.014	6898.500	.000
	Within Groups	.000	6	.000		
	Total	.028	8			
FE	Between Groups	.020	2	.010	293.764	.000
	Within Groups	.000	6	.000		
	Total	.021	8			
ZN	Between Groups	.001	2	.001	118.318	.000
	Within Groups	.000	6	.000		
	Total	.001	8			
CU	Between Groups	.000	2	.000	68.630	.000
	Within Groups	.000	6	.000		
	Total	.000	8			
PB	Between Groups	.000	2	.000	10.906	.010
	Within Groups	.000	6	.000		
	Total	.000	8			
CD	Between Groups	.000	2	.000	15.606	.004
	Within Groups	.000	6	.000		
	Total	.000	8			

Discussion

Phytochemicals also known as secondary metabolites, confer therapeutic potentials on medicinal plants. Saponins have been shown to possess a wide range of biological activities including anti-diarrheal, anti-helminthic, anti-microbial (Kaya and Incekara, 2000) as well as anti-tumorigenic properties (Man *et al.,* 2010). Akpan*et al.* (2012) and Uchegbu *et al.* (2016) have reported the antimicrobial properties of *C. afer.* Studies have shown that tannins possess anti-carcinogenic and anti-mutagenic properties (Chung *et al.,* 1998), antimicrobial (Haars *et al.,* 1981), antioxidant (Sherwin, 1990), antiviral as well as anti-hemorrhagic properties (Chung *et al.,* 1998). The presence of tannins in *C. afer* may account for its traditional use in the treatment of hemorrhage and microbial infections including sore throat, cough and diarrhea (Omokhua, 2011). Also, alkaloids are known to possess biological activities ranging from antimicrobial, insecticidal, hypotensive, antiseptic, anti-inflammatory and antimalarial properties (Bribi, 2018). The ethno-medicinal uses of *Costus afer* against microbial infections, malaria and hypertension (Omokhua, 2011) could be attributed to the presence of alkaloids in it. Flavonoids are polyphenolic compounds with proven antioxidant activities both in-vitro and in-vivo. They are known to have protective effects against organ damage, cancer, infectious, cardiovascular as well as neurodegenerative diseases (Shashank and Abhay, 2013). Antimicrobial, anti-inflammatory, anti-diarrheal, as well as anti-hypertensive and anti-obesity effects of flavonoids have also been reported (Cassidy *et al.,* 2011). These properties tend to justify the traditional uses of *C. afer* in the treatment of arthritis, inflammatory and oxidative stress-related disorders (Anyasor *et al.,* 2010; 2014b).Ezejeofor *et al.* (2013) reported the hepato-protective and nephron-protective effects of aqueous leaf extract of *C. afer*. Furthermore, studies carried out by Atere *et al.*(2018); Anyasor *et al.*(2014b); Anyasor *et al.*(2015a); Anyasor *et al.*(2015b); Uboh *et al.* (2014) and Anyasor *et al.*(2017) have showed the antioxidant, lipid-lowering and anti-inflammatory as well as organ-protective effects of *Costus afer* both in-vitro and in vivo. Cardiac glycosides are effective in the treatment of heart failure and maintenance of normal cardiac rhythm (Bohm, 1997). The presence of cardiac glycosides in *C. afer* may be responsible for its effectiveness in the traditional treatment of hypertension. Cardiac glycosides have also been shown to selectively sensitize cancer cells to apoptosis during radiation therapy (Lawrence, 1998).

Health benefits of macro and micro elements found in plants include normal growth and development, metabolism and proper functioning of various enzymes among others. Manganese (Mn) is a micro element that is important for insulin secretion as well as carbohydrate and fat metabolism and calcium absorption (Soetan *et al.,* 2010). Mn is also a vital activator of some enzymes notably, superoxide dismutase (SOD), an antioxidant enzyme that is responsible for scavenging free radicals (reactive oxygen species) generated as a result of oxidative stress in cells (Li and Yang, 2018). The important roles played by manganese in metabolism may account for the traditional use of *C. afer* leaves in the management of diabetes, arthritis and hypertension (Omokhua, 2011). Iron (Fe), a micro element constitutes an essential component of all plants principally needed for electron transport and is also an integral part of hemoglobin for the transport of oxygen in red blood cells (Kaya and Incekara, 2000). The folkloric use of *C. afer* in the treatment of hemorrhage (Omokhua, 2011) may be due to its high iron content. Zinc (Zn) plays various vital roles in human body which include tissue repair and wound healing, digestion and protein metabolism and normal insulin function (Malhotra, 1998). The wound healing properties of *C. afer* has been reported by Udoh *et al.* (2018). Copper (Cu) is essential for normal neurologic and hematologic functions. It also helps in the absorption of iron from the gastrointestinal tract (Tan *et al.,* 2006). Lead is a micro, non-essential element with no beneficial functions in both humans and plants. However, increased intake and accumulation of this heavy metal in various tissues and organs of the body have deleterious effects on the kidney, liver, vascular, immune and nervous systems (Tchounwou *et al.,* 2014). The concentrations of lead recorded in this study are a bit higher compared to the results of Anyasor *et al.* (2014a) who reported negligible amounts in the leaf (0.01 ± 0.00 mg/kg) and stem (0.02 ± 0.00 mg/kg), and also did not detect cadmium in the leaf and stem of *C. afer*. The difference observed might be due to difference in sample collection sites and the type of commercial activities around the sites. Cadmium is a heavy metal without specific health benefits. It is readily absorbed and distributed to tissues following exposure thereby inhibiting antioxidant enzymes and causing increased levels of free radicals, which are markers of oxidative stress which further results in loss of membrane-bound enzymes and normal membrane function (Tchounwou *et al.,* 2014).

Conclusion and Recommendations

Costus afer Ker Gawl. is a medicinal plant with diverse therapeutic potentials. The findings of this study showed that *C. afer* is a good source of phytochemicals and essential elements necessary for normal body function and defense system. However, scientific validation of folkloric uses of this plant in the treatment of malaria and its potential as an anti-cancer agent need to be considered. Furthermore, elucidative studies on the mechanism of action of *C. afer* that will explain the therapeutic roles of phytochemicals and trace elements of *C. afer* in the treatment and management of arrays of diseases and infections are also needed.

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