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Evaluation of the bioactive constituents and antimicrobial potentials of the ethyl acetate extract of *Costus afer* stem

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Abstract
Costus afer is a perennial plant, commonly called bush sugar cane or monkey sugar cane. It can be found in the forest belts of Senegal, Guinea, Sierra-Leone, Niger, and Nigeria. In Nigeria, it is commonly found in the Western and Eastern parts. The stem of the plant was phytochemical screening for some metabolites and the quantitation of metabolites was carried out using GC-FID machine. The antimicrobial potential of the Ethyl acetate stem extract of the plant was carried out using agar diffusion method. The phytochemicals present in the stem back of the plant include, alkaloids, saponins, flavonoids, tannins, cardiac glycosides and triterpenoids. Quantitation of these phytochemicals showed that flavonoids represent the highest quantity of all. Most of the test organisms were susceptible to the extract with zones of inhibition that ranged between 6 and 26 mm, with *Bacillus cereus* being the most sensitive. *Aspergillus niger* however, showed resistivity to the extracts with an unclear zone of inhibition of 6 mm. *C. afer* contains a good number of important secondary metabolites that could be isolated as drugs, drugs precursors or lead compounds in drug development process. Thus, adding value to the use of this plant in traditional medicine.

Keywords: *Costus afer*, bioactive constituents, antimicrobial potentials

Introduction
Costus afer is a perennial plant, commonly called bush sugar cane or monkey sugar cane. It is a rhizomatous herb that can be found in the forest belts of Senegal, Guinea, Sierra-Leone, Niger, and Nigeria. In Nigeria, it is commonly found in the Western and Eastern parts. It is a flowering plant that bears yellow and white flowers, measuring to about 6 m tall (Anyasor *et al*., 2010) [2]. *C. afer* is an herbaceous plant that is unbranched with creeping rhizomes (Uwah, 2015) [20], (Omokhua, 2011) [10]. It can be vegetatively propagated by cutting and planting the base of mother plant (Omokhua, 2011) [10].

Costus afer is heavily used in Nigerian Traditional medicines to treat several diseases. In the South Eastern parts, it is used to treat diabetes, inflammation and arthritis (Soladoye and Oyesika, 2008) [16]. Ogba people of Rivers state use a combination of leaves and stems of *C. afer* and *Alchornea cordifolia* (crushed and boiled) to treat malaria and hunch back. The Ikwerre ethnic group of Rivers State use the leaves of the plant in combination with of those from mango, pawpaw and orange trees and boil to treat fever and malaria. In Ogoni Land of Rivers State, the leaf juice is squeezed into eye and nose to treat various ailments such as inflammation of the eye, headache and malaria. Dehydrating children chew the leaves to get strengthened.

The stem decoction is used to treat rheumatoid arthritis in parts of the Niger Delta. In Ogbolom community of Bayelsa State, an infusion of the dried aerial parts of the plant is used to treat hypertension. Other traditional medicinal uses of *C. afer* in Nigeria include treatment of urethral discharges, prevention of miscarriages, and treatment of jaundice and venereal diseases. Some ailments like gonorrhoea, toothache, epilepsy, leprosy and stomach disorders etc. are also treated using various parts of *C. afer* (Omokhua, 2011) [10].

Several phytochemical studies have been conducted on different parts of *C. afer* which led to identification of certain classes of compounds and a number of pure compounds were isolated.

A phytochemical screening of the methanol and aqueous stem extract of *C. afer* by Anyasor *et al*. (2010) [2] revealed the presence of phlobatannins, alkaloids, diterpenes, triterpenes and phytosterols. Ukpai *et al*. (2012) [18] used carbon tetrachloride as extraction solvent to conduct both quantitative and qualitative phytochemical analysis of stem of the plant collected from Abia State. Their study revealed the presence of steroids, alkaloids, flavonoids, triterpenes and glycosides in the plant. A diosgenin saponin was isolated from the rhizomes of *C. afer* by Lin *et al*. (1997). Other saponins isolated from the plant include aferosides A-C and parphyllin C. A flavonoid glycoside, kaempferol-3-O-α-L-rhamnopyranoside was also reported to be isolated from the plant (Aweke, 2007). Isolated from the rhizomes also are; oxalic acid, stigmasterol, sitosterol, lanosterol. We therefore, report the phytochemical screening and of aqueous stem extract of *Costus afer* collected from Rivers State Nigeria. Also, based on the ethnopharmacology of *C. afer* in this area, antimicrobial screening of the aqueous stem extract was investigated in this study using some clinical pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*.

Materials and Method

Plant collection/preparation

The stems of *Costus afer* were collected from Bunu town (Tai L.G.A) Rivers State in the month of July. The plant was identified by Dr. David Sarogoro, of the Department of Forestry, Rivers State University, Port-Harcourt. The stems were washed with water and left to dry under shed. The dried sample was ground to powder for extraction and further analyses

Sample Extraction

The powdered sample, 50 g was macerated with 150 mL distilled water for 48 hours. The mixture was then filtered to and allowed to evaporate under room condition to obtain crude extract for the phytochemical screening and antimicrobial studies.

Qualitative Phytochemical Screening

The chemical tests were carried out on the aqueous extract of *Costus afer* using standard procedures to identify the constituents as described by (Sabri *et al.,* 2012; Satheesh *et al*., 2012) [13, 14].

Test for steroids and triterpenoids (Liebermann-Burchard test)

About 3 mg of the extract was mixed with 3 drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red colour in the lower layer would indicate a positive test for steroids and triterpenoids, respectively.

Test for cardiac glycosides (Keller-Killiani Test)

About 3 mg the extract was mixed with 3 drops of conc. glacial acetic acid and diluted ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turn bluish green would indicate a positive test for glycosides.

Test for phenolics and tannins (ferric chloride test)

About 2 mg each of the crude extract was dissolved in 2 mL of solvent of extraction and treated with 4 drops of ferric chloride solution. Formation of bluish black colour would indicate the presence of phenols. Generally, the formation of bluish-black colour would indicate the presence of gallic tannins and bluish-green would indicate the presence of catechic tannins.

Test for flavonoids (alkaline test)

About 5 mg of the extract was added 5 mL of diluted sodium hydroxide solution. The appearance of yellow colour which would become colourless on addition of few drops of dilute hydrochloric acid would indicate the presence of flavonoids.

Test for saponins

The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. Distilled water (5 mL) was added to the extract (5 mg) and strongly shaken in a test tube. Formation of a large amount of froths that would last for about 30 minutes indicated the presence of saponins.

Test for alkaloids

About 3 mL of an extract was mixed with 1 mL of 10% HCl in a test tube and heated for 20 minutes. This was allowed to cool and filtered; 1 mL of the filtrate was treated with few drops of Mayer's reagent. Appearance of creamy precipitate would indicate the presence of alkaloids.

Preparation of Sample for GC-FID Analysis

Approximately 1 g of sample was weighed and transferred in a test tube and 15 mL ethanol and 10 mL of 50 % m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60 ºC for 1 hour. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water and 3 ml of hexane, which were all transferred to the funnel. This extracts were combined and washed three times with 10 ml of 10 % v/v ethanol aqueous solution, the solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 100 μL of pyridine from which 200 μL was transferred to a vial for analysis (Emejulu *et al*., 2017) [5].

Quantification by GC-FID

The quantitative analysis of phytochemicals was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15 m x 250 µm x 0.15 µm) was used. The injector temperature was 280 ºC with splitless injection of 2 μl of sample and a linear velocity of 30 cmS^{-1}. Helium 5.0pa.s was the carrier gas with a flow rate of 40 m/min^{-1}. The oven operated initially at 200 ºC, was heated to 330 ºC at a rate of 3 ºC min^{-1} and was kept at this temperature for 5 min. The detector was operated at a temperature of 320 ºC.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentrations of the different phytochemicals were expressed in µg/g (Emejulu *et al*., 2017) [5].

The Antimicrobial Screening

The antimicrobial activity of the plant extracts was determined using some pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology, University of Ibadan. The extract (0.1 g) was weighed and dissolved in 10 mL of DMSO to obtain a concentration of 10 mg/mL. This served as initial concentration for determination of the antimicrobial activity. Agar diffusion method was used for screening the extracts.

Mueller Hinton Agar (MHA) was medium used for growth of microbes. The media were prepared according to manufacturer instruction, sterilized at 121 °C for 15 minutes, poured into sterile Petri dishes and allowed to cool and solidify. The sterilized media were then seeded with 0.1 mL of the standard inocula of test microbes. Inocula were spread evenly over the media surfaces by the use of sterile swabs.

Using a standard cork borer (6 mm), a well was cut at the center of each inoculated medium. Solution of the extract (0.1 ML) of concentrated 30 mg/mL was then introduced into each well on the medium. The inoculated medium was then incubated at 37 °C for 24 hours for bacteria and at 30 ºC, for 7 days for fungi, after which each plate was observed for inhibition zone of growth. Zone of inhibition was measured with a transparent ruler and the result recorded in millimeters as described by Tor-Anyiin *et al*., (2015) [17].

Result and Discusion

Phytochemical Screening Result

The phytochemical screening of the stem of *Costus afer* was conducted and the result is presented in Table 1. The study showed the presence of saponins, alkaloids, cardiac glycosides, tannins, Flavonoids and triterpenoids. This result can be compared to that published by Anyasor *et al*. (2015) [2]. The presence of these phytochemical could be responsible for activities of the plant extracts against some microorganism. The qualitative analysis of the extract was further confirmed by the quantitation of the metabolites by GC-FID (Table 2).

Table 1: Qualitative Phytochemical Screening

Metabolites	Comment
Alkaloids	+
Flavonoids	+
Tannins	+
Triterpenoids	+
Saponins	+
Cardiac glycosides	+

KEY: += Presence

Table 2: Quantitative Phytochemicals by GC-FID

Metabolites	Quantity (µg/g)
Rutin	0.2208
Quinine	15.1875
Oxalate	6.5670
Naringin	9.5834
Catechin	3.7244
Anthocyanin	22.8654
Lunamarine	6.5460
Sapogenin	3.8757
Epicatechin	12.1823
Ribalinidine	8.5805
Sparteine	11.7328
Kaempferol	3.4412
Phytate	6.2762
Flavones	1.9192
Naringenin	4.6291
Anthocyanidines	2.0047
Tannin	26.4477

Quantitation of Phytochemicals

The quantitative phytochemical analysis of the powdered sample of stem of *Costus afer* (Table 2) showed how much of the metabolites present in the plant. The analysis showed high content of flavonoid combined together in the plant-naringin (9.54 µg/g), anthocyanin (22.8 µg/g) catechin (3.72 µg/g), epicatedun (12. 18 µg/g), Kaempferol (3.44 µg/g) flavones (1.92 µg/g) and anthocyanidins (2.00 µg/g). The meaningful quantities of these flavonoids especially naringin, epicatechin anthocyanin could be responsible for the reported antioxidant activities of this plant.

A review of pharmacological effects of naringin by Rao *et al*. (2017) [11] showed that it has hypocholesterolemic, antiestrogenic, hypolipidemic, antihypertensive and anti-inflammatory activitiews

Alkaloids found in this plant include quinine (15.19 µg/g), ribatinidine (8.58 µg/g). Alkaloids are also present in high quantity especially quinine.

Quinine is an outdated anti-material because of its pharmacological adverse effects. The presence of quinine in high quantity could justify why the plant is used traditionally to treat malaria. Lunamarine, spartein ribalinidine are related alkaloids that are built on the quinine scarfold. These compounds are reported to have antimalerial, antimicrobial and antiprotozoal properties (Franck *et al*., 2004) [6], (Bachiller *et al*., 2010a) [4]. This plant off course, is highly utilized in south Eastern and Western parts of Nigeria to treat material and fever.

Tannins represent the highest content of metabolites in this plant (26.45 $\mu g/g$). They are well known for their antibacterial, antimicrobial, antiviral antitumor and anti-inflammatory activities etc.

The presence of anthocyanins in high quantity (22.87 µg/g) could also justify why this plant is used in tradomedicines to treat inflammation of the eye, cancer and age related problems (Roy *et al*., 2009) [12].

The use of this plant to treat hypertension wounds, diabetes and inflammation could be attributable to the presence of saponin (3.89 $\mu g/g$) in this plant (Okwu and Okwu, 2004) [9].

Phytates have been proven to have antilipidemic and anti-inflammatory properties. Reports have it that it can also serve as antioxidant and metal chelator (Urbano *et al*., 2000) [19], (Gibson *et al*., 2010) [7].

Antimicrobial Result

Due to the presence of many metabolites like alkaloids, Flavonoids and tannins, there was need to test for the antimicrobial activity of the aqueous stem extract of *Costus after*. The zones of inhibitions and sensitivity test were carried out using some selected microorganism and the result is presented in Table 3. From the result the most sensitive microorganism was *Bacillus cereus*. *Bacillus cereus* is a spore forming bacterium that releases toxins that cause vomiting and diarrhea (Senesi and Ghelardi, 2010) [15]. This study has confirmed the use of this plant in traditional medicines to cure diarrhea. The sensitive species of fusarium had a zone of inhibition (24 mm). Fusarium species are a group of fungi that are relatively abundant in soil. Other organisms that showed sensitivity are *Staphylococcus aureus* and *Pseudomonas aeruginosa* with zones of inhibitions, 18 mm, and 12 mm respectively. *Staphylococcus aureus* is known to cause several diseases blood stream infection, skin disease like boils. *Pseudomonas aeruginosa* is a gram negative bacteria that is known to cause several diseases in humans like bacteremia, pneumonia, eye infections, ear infections etc. *Escherichia coli* is a gram negative bacterium that is commonly found in the

gut of warm blooded animals. Some strains of the *E. coli* are harmless to human and other animals. Some strains of *E. coli* can however cause health problems in human such as pneumonia, urinary tract infections and diarrhea etc. *E. coli* was sensitive to test extracts with a clear zone of inhibition of 14 mm. *Aspergillus niger* how ever showed resistance to the extract with an unclear zone of inhibition of 6 mm. The test organisms were all sensitive to amoxicillin with zones of inhibitions that ranged between 19 and 37 mm.

Table 3: Zones of Inhibition and Sensitivity Test

Organisms	Zones of Inhibition (mm)	Sensitivity	Control Amox. (mm)
Bacillus cereus	26	S	37
Staphylococcus aureus	18	S	34
Escherichia coli	14	S	31
Pseudomonas aeruginosa	12	S	29
Aspergillus niger	6	R	20
Fusarium species	24	S	19

KEY: R= Risistant, S= Sensitive

Conclusion

Costus afer contains the classes of metabolites such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides and triterpenes. The quantification of these metabolites showed that tannins are the highest, followed by flavonoids and alkaloids. The quantification also led to identification of the individual members of the classes such as kaempferol (Flavonoids), quinine (Alkaloid) and rutin (flavonoid) etc. The ethyl acetate extract of *C. afer* stem displayed potential antimicrobial activities, thus adding value to the use of this plant in traditional medicine.

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