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Chemical Composition of the Stem Extract of *Costus afer* (Bush Cane) and Its Antimicrobial Activity

Rosemary I. Uchegbu[1*], Jacinta N. Akalazu[2], Chioma O. Ibe[1], Ahamefula A. Ahuchaogu[1] and Cosmas U. Amadikwa[1]

[1]Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.
[2]Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author RIU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JNA performed the antifungal activity. Author AAA performed the spectroscopy analysis. Authors COI and CUA did the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the chemical composition of the stem extract of *costus afer* and its antimicrobial activity against some human and plant pathogens.
Study Design: The study was designed to identify the phytochemicals present in *Costus afer* and to test the inhibitory ability of the plant extract on human and plant pathogens.
Place and Duration of Study: Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri and Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria, between May to November 2015.
Methodology: The phytochemicals from the stem of *Costus afer* a medicinally important plant of the zingiberaceae family were extracted with ethanol and subjected to GC/MS analysis and the identification of compounds was done by comparing spectrum of the unknown component with the spectrum of the known components stored in the NIST library. The antibacterial activity was

**Corresponding author: E-mail: mrsriu@yahoo.com;*

performed by filter paper disc diffusion technique. The antifungal activities of stem extract of *Costus afer* on mycelial growth evaluation the antifungal activities of stem extract of *Costus afer* along with mancozeb on mycelial growth inhibition of fungi isolated from *Dioscorea rotundata* (poir) (white yam) was also performed by disc diffusion technique.

Results: The analysis revealed that ethanolic extract of *Costus afer* contains ten compounds with n- Hexadecanoic acid forming the bulk of the oil (27.35%), followed by 4–methyl-4-hepten– 3-one (24.27%), Oleic acid (18.79%), Stearic acid (10.27%), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (8.46%). Other compounds present were Furfural (2.46%), 6-Methyl-3(2H)-Pyridazinone (1.75%), etc. The ethanol extract inhibited all the tested organisms *S. aureus, P. mirabilis, K. pneumonia*, *P. aeruginosa and E.coli.* The results revealed that *Costus afer* inhibited the growth of the test organisms *Aspergillus niger*, *Fusarium oxysporium* and *Botryodiploidia theobromae* except *Rhizopus stolonifer. In vivo* results showed that *C. afer* extract was effective in reducing tuber rot which suggested that use of *C. afer* would be helpful in treatment of mycotic infections and in the control of fungal plant disease justifying the use of this plant in the treatment of both human and plant diseases.

Keywords: Phytochemical; tuber rot; bacteria; fungal pathogens.

1. INTRODUCTION

Green plants possess the broadest spectrum of synthetic activity and have been the source of many useful compounds. Nigeria is blessed with most of these green plants which have shown considerable pharmacological activities such as antioxidant, antimicrobial, anti inflammatory, anticancer, antiviral, anti-allergic and vasodilatory properties. For instance, *Costus afer* is a distinct plant with several medicinal applications to cure many ailments. *Costus afer* belongs to the family zingiberaceae. It is a tall perennial herbaceous, unbranched medicinal plant with creeping rhizome. It can be found in shady forests and riverbanks of Senegal, South Africa, Guinea, Nigeria, Ghana and Cameroon. It is also known as Ginger lily or bush cane, Okpete or Opete in Igbo, Kakii-zuwaa in Hausa and Atare tete-egun in Yoruba, all in Nigeria.

An ethnobotanical survey of this plant revealed that the methanolic leaf extract of *Costus afer* contains potent biological active compounds, which have moderate agonistic properties on guinea pig ileum, abortificient property at 3rd trimester of pregnancy and antihyperglycermic property on streptozotocin-induced hyperglycermia [1,2].

The leaf and stem of *C. afer* when cut and crushed into smaller bits, boiled together with the leaf and bark of *Alchornea cordiflora* is used for the treatment of hunch bark, fever and malaria in Rivers state. Also the juice from the stem is used for treatment of cough and measles, the leaf and stem of *C. afer* is used to treat gonorrhoea, reduction of fat and a source of water for grass cutter *(Thynomys swinderianus)* during the dry season in Rivers state. The young and tender leaves when chewed are believed to give strength to the weak and dehydrating patient. An infusion of the inflorescence is taken to treat stomach complaints. A stem decoction mixed with sugarcane juices are taken to treat cough, respiratory problem and sore throat. The leaf sap is used as eye drops to treat eye troubles and as nose drops to treat headache and malaria in Bayelsa State. Also stem sap is applied to treat urethral discharges, venereal diseases, jaundice and to prevent miscarriage in Bayelsa State. A stem decoction is widely used to treat rheumatoid arthritis in parts of the Niger Delta. An infusion of the dried aerial parts is use to treat hypertension. The stem is used in the treatment of worms and hemorrhoids. The pulped stems taken in water are strongly diuretic. A cold water extract of the stem is used to treat epileptic attacks. Rhizome pulp is applied to teeth to cure toothache. The rhizome decoction is taken to treat leprosy and venereal diseases [3,4], in their analysis reported that *C. afer* contains saponins, alkaloids, glycosides, tannins, and steroids, the proximate composition indicated the following; moisture (33.6%), crude fat (2.48%), crude protein (14.02%), carbohydrate (20.14%), crude fiber (15.55%), and ash (14.21%) and Mineral element determination (in mg/100 g) showed the presence of potassium (88.00), sodium (1.94), calcium (200.40), magnesium (191.39), and phosphorus (6.02). [5] who worked on the antibacterial effect of ethanolic leaf extract of *Costus afer* reported that the leaves extract inhibited the tested organisms, *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* but showed no activity against *Klebsiella pneumonia*. [6] in their phytochemical analysis of

C. afer reported the presence of alkaloids, saponins, flavonoids, tannins and phenols in the aqueous stem extract while flavonoids, saponins and phenols were detected in the ethanol extract.

The antimicrobial compounds found in plants are of interest because of the emergence of multi-drug resistant (MDR) bacteria, which is a major cause of treatment failure in many infectious diseases and which is becoming a worldwide public health concern [7,8].

Fungi are reported to be major cause of yam tuber rot in Nigeria and their contamination of food and feedstuff is threat to human and animals [9]. These fungi produce mycotoxins which when consumed cause diseases to man. Synthetic chemicals used in plant disease control have been implicated with environmental pollution, toxicity to human. Thus, naturally occurring antimicrobials are being sought as replacements for synthetic ones. One of the possible strategies towards this objective involves the identification and characterization of bioactive phytochemicals, which have biological activities. Phytochemicals are reported to be ecofriendly, non poisonous to man and therefore a better alternative to plant disease control. Green plants posses the broadest spectrum of synthetic activity and have been the source of many useful compounds.

2. MATERIALS AND METHODS

2.1 Plant Materials

The stems of *Costus afer* were harvested from the field of Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.

2.2 Sample Preparation

The fresh stems were harvested, washed, pounded and soaked in ethanol for 48 hours and filtered. The filtrate was concentrated with rotary evaporator and 3 g was used for GC- MS analysis.

2.3 Gas Chromatography- Mass Spectrum Analysis (GC-MS)

Gas chromatography analysis was performed using GC-MS SHIMADZU QP 2010, JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25 nm x 50 m) and the conditions were as follows: Temperature programming from 80-200℃ held at 80℃ for 1 minute, rate 5℃ / min and at 200℃ for 20 mins. FID temperature 300℃, injection temperature 250℃, carrier gas nitrogen at a flow of 1 ml /min, split ratio 1:75. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 kv and sampling rate of 0.2 sec. The mass spectrum was also equipped Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all analytical grade and were procured from Merck, Germany.

The interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Subsequently, the details about their molecular formula, molecular weight, structure were also obtained.

2.4 Antibacterial Evaluation of the Stem of *Costus afer*

2.4.1 Preparation of extracts

The test solution of each extract was prepared by dissolving 0.1 g of the plant extracts separately in 1.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml.

2.4.2 Microorganisms

The bacteria organisms used were *Staphylococus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and *Escherichia coli.* All the organisms were obtained from the stock culture of the Federal Medical Center, Umuahia. Cultures were brought to laboratory conditions by resuscitating the organisms in peptone water and thereafter subcultured into nutrient agar medium and incubated at 37℃ for 24 hours.

2.4.3 Antibacterial assay

The antimicrobial susceptibility test against the isolates was performed by filter paper disc diffusion method. Filter paper disc (whatman No1, 6 mm diameter) were placed in glass petri dishes and sterilized in hot air oven [10]. The media (10 g nutrient agar in 200 ml distilled water, auto-claved at 115℃ for 30 minutes) was cooled to 50℃. The sterile nutrient agar media were poured into the sterile petri dishes and allowed to solidify. The bacteria were swabbed

with a sterile wire loop. Each disc was impregnated with 0.2 ml of plant extracts. Discs with DMSO (100 mg/ml) served as a control.

The discs were used after drying them in an incubator at 40℃ to remove any trace of solvent [11]. Discs were introduced onto the surface of the medium. The plates were incubated at 37℃ for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and the average of these values were tabulated in Table 2.

2.5 Antifungal Evaluation of the Stem of *Costus afer*

Three pieces of infected, dried and sterilized yam tuber discs (2 mm) were plated on potato dextrose agar (PDA) and in cubated at 27℃. Dried stem extract of *C. afer* was prepared by grinding to powder. Following the procedures of [12], about 4 kg of powdered sample was soaked in 98% ethanol for 4 days. Then 20 g of the ethanolic extract each was soaked separately in 200 ml methanol and 200 ml petroleum ether for 48 hours and filtered through what man filter paper. Each filtrate was concentrated with rotary evaporator at 40℃ to a dark brown extract. Sterile filter paper discs (6 mm) impregnated with the methanol and petroleum ether extract separately were carefully and firmly placed on PDA plates earlier seeded with each fungal pathogen.

For *in vivo* study, yam discs (1 cm thick) were removed from the tubers, then 1 ml of each methanol and petroleum ether extract was separately dispersed into each hole. In control 1 ml sterile water was dispersed into the holes. The holes were replaced with yam discs initially removed and then sealed with Vaseline.

2.6 Statistical Analysis

All values are expressed as mean ± S.D. Statistical analysis were performed by Student's *t*-test. The values of p lower than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

The ethanol extracts of *Costus afer* stem contains rich phytochemical constituents. Ten compounds were identified in the GC/MS analysis. The individual names of compounds identified with respect to their individual peak number, retention time, area %, height % were shown in Table 1. Among the compounds identified, n- Hexadecanoic acid was present in 27.35% forming one of the major constituents in the extracts followed by 4 – methyl -4-hepten – 3-one (24.27%), Oleic acid (18.79%), Stearic acid (10.27%), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (8.46%). Other compounds present were Furfural or 2-Furancarboxaldehyde (2.46%), 6-Methyl-3(2H)-Pyridazinone (1.75%), 1,6- Anhydro-beta-D-glucopyranose (1.65%), 4- Methyl 2,3- hexadien -1-ol (1.60%) and n- Undecanoic acid (3.39%).

Oleic acid is a mono-unsaturated fatty acid found in animal and vegetable oils. High concentrations of oleic acid can lower blood levels of cholesterol and lower the risk of heart problems [13]. Oleic acid also synergistically enhances cancer drug effectiveness [14]. For example oleic acid improved the effectiveness of herceptin, a breast cancer drug. It lowers heart attack risk and artherosclerosis, and aids in cancer prevention. It is used in the food industry to make synthetic butters and cheeses. It is also used to flavor baked goods, candy, ice cream and sodas [13]. Furfural also found in *C. afer* is occasionally used in perfumes. Furfural is an important renewable, non-petroleum based, chemical feedstock. Hydrogenation of furfural provides furfuryl alcohol (FA), which is a useful chemic al intermediate and which may be further hydrogenated to tetrahydrofurfuryl alcohol (THFA). THFA is used as a nonhazardous solvent in agricultural formulations and as an adjuvant to help herbicides penetrate the leaf structure [15]. 6-Methyl-3(2H)- Pyridazinone is another compound identified in the stem of *C. afer.* In recent years a substantial number of pyridazines and pyridazinones have been reported to possess various biological activities such as antimicrobial, antitubercular, antifungal, analgesic and anti-inflammatory. They also act as phosphodiesterase (PDE) inhibitors, cyclooxygenase (COX) inhibitors, antipyretic, antidiabetic, antifeedant, insecticidal, antihypertensive, antiplatelet, anticancer, anticovulsant, anti-HIV, antiasthma & antiallergic, neurological activity, like anti anxiety and depressant, and intermediates for drugs synthesis, agrochemicals and other anticipated biological properties. In particular, a large number of pyridazinone derivatives are well known as therapeutic agents. For instance, Pyridazin-3(2H)-one derivatives represent one of the most active class of compounds possessing a wide spectrum of pharmacological activities ranging from cardiovascular properties, anti-inflammatory, antidiabetic, analgesic, anti-AIDs,

anticancer and anticonvulsant activities [16,17]. Pyridazinone derivatives also possess affinity for benzodiazepine receptors and the ability to inhibit the human matrix metalloproteinase and aldose reductase enzymes [18]. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one has been reported to possess antimicrobial, anti-inflammatory and antiproliferative activities [19].

Ethanolic extracts of the stem of *Costus afer* exhibited antibacterial activities on the pathogens tested as shown in Table 2. The extracts inhibited all the tested organisms *S. aureus P. mirabilis, K. pneumonia*, *P. aeruginosa* and *E. coli*. The organisms *P. mirabilis* and *E. coli* are the common cause of urinary tract infection and traveler's diarrhea [20]. Severe eye infections such as blepharo conjuctivities, corneal ulcers, abscesses, styes, dacryocystitis, orbitalcellutitis and blebs are mainly caused by *Staphylococus aureus* and *Pseudomonas aeruginosa* [21]. It was reported that the leaf sap of *Costus afer* is used as eye drops to treat eye troubles and as nose drops by the natives [3]. The present result also shows that stem of *Costus afer* may be used to treat eye and nose problems. Natural antibiotics are preferred recently since the use of synthetic antibiotics have been reported to have side effects like hypersensitivity reactions, gastric disturbances, nephrotoxicity, etc. [22].

The results of diameters of inhibition of yam rot pathogens (mm) using *C. afer* stem extract and *Invivo* evaluations of yam tuber rot reduction using *C. afer* stem extract are shown in Tables 3 and 4. The result of the antifungal activities of stem extract of *Costus afer* on mycelial growth isolated from *Dioscorea rotundata* (poir) (white yam) showed that *A. niger, R. stolonifer, Fusarium oxysporium and B. theobromae* were fungi associated with yam tuber rot. The results of isolated fungi is similar to that of [23,24] who reported that *F. Oxysporium*, *Penicillium* spp*, Rhizopus* spp have been associated with post harvest rot of yam tubers, tomato, orange and carrot.

Table 1. Chemical constituents of ethanol extract of the stem of *Costus afer*

Peak	Retention time	Area %	Height %	Name of compound
1	3.278	1.53	2.46	Furfural
2	4.90	0.96	1.75	2- Methyl-5- formylfuran
3	8.312	5.81	8.46	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one
4	10.122	20.96	24.2	4-Methyl-4-hepten-3-one
5	14.555	1.66	1.65	1,6-Anhydro-beta-D-Glucopyranose
6	15.375	1.18	1.60	4-Methyl-2,3-hexadien-1-ol
7	16.939	1.59	3.39	n- Hexadecanoic acid
8	20.930	37.87	27.35	n-Hexadecanoic acid
9	23.584	22.60	18.79	Oleic acid or 9-Octadecenoic acid
10	23.813	5.84	10.27	Stearic acid or Octadecanoic acid

Table 2. Inhibition Zone Diameter (IZD) (mm) of *Costus afer* on the human pathogens

Pathogens	*Costus afer*
Proteus mirabilis	8.00±0.03
Klebsiella pneumonia	10.00±0.20
Staphylococus aureus	12.00±0.08
Pseudomonas aeruginosa	11.00±0.25
Escherichia coli	13.50±0.01

Values are mean of triplicate determination ± standard error

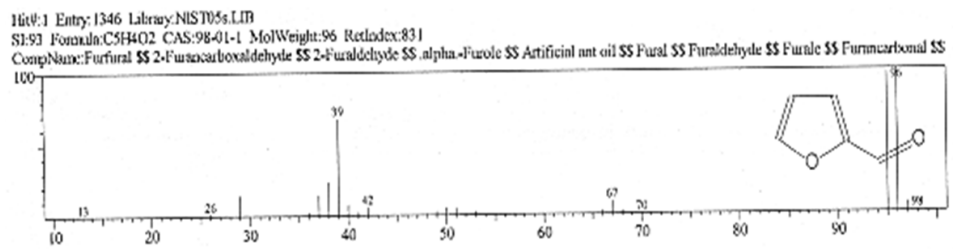


Fig. 1.

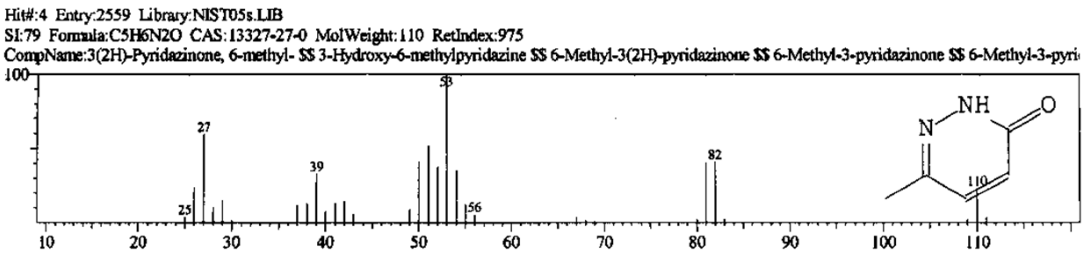
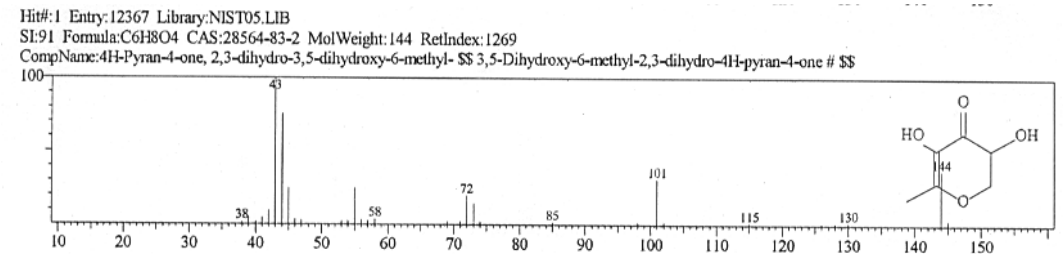


Fig. 2.



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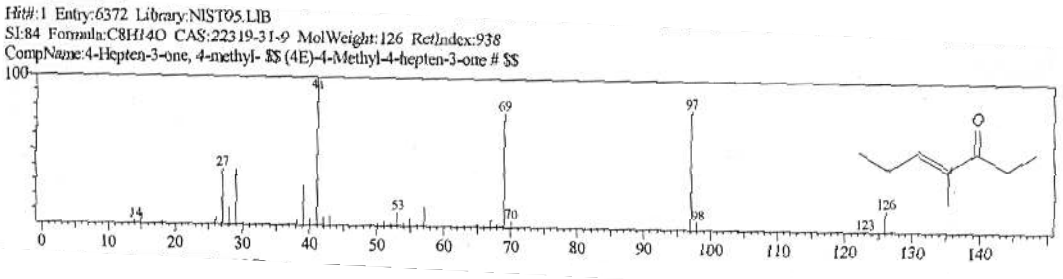

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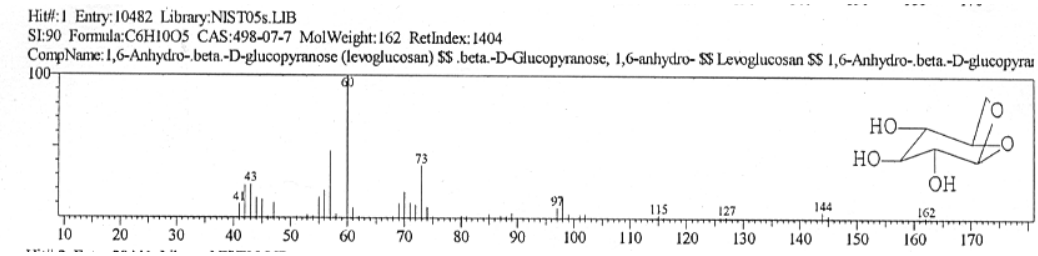

Fig. 5

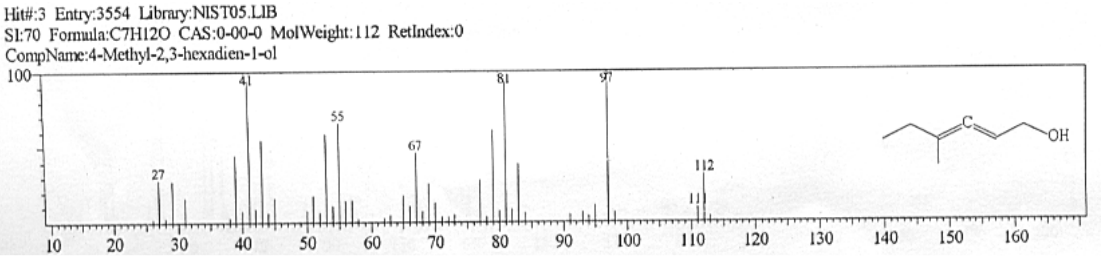

Fig. 6

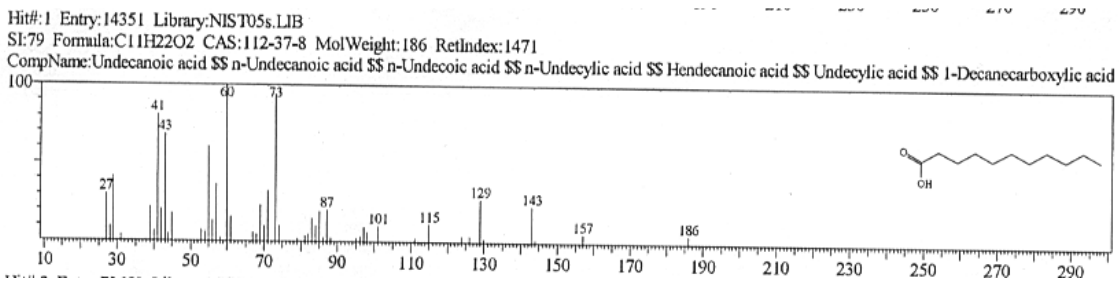

Fig. 7

Figs. 1-7. The mass spectrum, molecular formula, molecular weight, structure of some of the phytochemicals identified in ethanol extract of *Costus afer* stem

Table 3. Diameters of inhibition of yam rot pathogens (mm) using *C. afer* stem extract

Pathogens	Petroleum ether (30 mg/l)	Methanol 30 mg/l	Mancozed 0.30 g/ml
Aspergillus niger	15.20±0.09	17.37±0.50	6.00
Fusarium oxysporium	16.83±0.72	17.10±0.52	6.00
Botryodiplodia theobromae	17.30±0.52	17.20±0.52	6.00
Rhizopus stolonifer	7.90±0.17	7.90±0.06	6.00

N.B All dimensions are in millimeter and include the diameter of the discs (6 mm)

Table 4. *In vivo* evaluations of yam tuber rot reduction using *C. afer* stem extract

	Dimensions of rot reduction (cm^2)			
Treatments	***A. niger***	***F. oxysporium***	***B. theobromae***	***R. stolonifer***
Petroleum ether	4.00	4.50	6.30	0.60
Methanol	4.30	4.00	3.60	1.20
Mancozeb (0.3 g/ml)	0.30	0.00	0.40	0.00
Water (control)	7.50	6.00	9.50	5.50

Antifungal potency of *C. afer* against the isolated fungi showed that the stem extract inhibited the growth of these fungi except *Rhizopus stolonifer*. Petroleum ether and methanol were effective in extracting the antifungal compounds present in the stem of *C. afer*. This result agrees with the reports of [25] who reported that *C. afer* inhibited the growth of *Aspergillus spp*, and *Botryodiplodia spp*. *Costus afer* was also reported to have inhibited the growth of *Pythium aphanidermatum* [26]. Similarly *C. afer* leave was reported to inhibit the growth of *Staphylococcus pneumonae*, *E. coli, P. aeruginosa* and *K. pneumoniae* at very high concentration [5].

4. CONCLUSION

The phytochemical constituents of *Costus afer* revealed the rich pharmacological potential of this medicinal plant which deserves further scientific experiments to unveil some of the novel pharmacophores that might exist in this plant parts. The results of the analysis justify the use of the stem of *Costus afer* as bactericidal agent for the treatment of venereal diseases, jaundice, etc by the herbalists and as antifungal agent in treatment of mycotic infections and in the control of fungal plant disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anaga AO, Njoku CJ, Ekejiuba ES, Esiaka MN, Asuzu IU. Investigation of the methanolic leaf extract of *Costus afer* Ker

for pharmacological activities in vitro and in vivo. Phytomedicine. 2004;11:242-248.

2. Guzman AI, Guerrero RO. Efecto hipoglycemiante de Costus specious (Zingiberacear), en rates. Vitea. Revista de la F aculdad de Quimica Farmaceutica. 2002;9:51-57.
3. Omokhua GE. Medicinal and socio-cultural importance of *Costus Afer (*Ker Grawl) in Nigeria. International Multidisciplinary Journal, Ethiopia. 2011;5(5):282-287.
4. Ukana D Akpabio, Uwemedimo E Udo, Aniekan E Akpakpan. Evaluation of phytochemical, proximate and mineral element composition of stem of *Costus afer* (Bush cane). Asian Journal of Plant Science and Research. 2012;2(5):607-612.
5. Akpan MM, Odeomena CS, Nwachukwu CN, Danladi B. Antimicrobial assessment of ethanolic extract of *Costus afer* leaves. Asian Journal of Plant Science and Research. 2012;2(3):335-341.
6. Ukpabi Chibueze F, Agbafor Kingsley N, Ndukwe Okorie K, Agwu Akuagwu, Nwachukwu Success N. Phytochemical composition of *Costus Afer* extract and its alleviation of carbon tetrachloride – Induced hepatic oxidative stress and toxicity. International Journal of Modern Botany. 2012;2(5):120-126.
7. Mora A, Blanco JE, Blanco M, Alonso MP, Dhabi G, Echeita A, et al. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol. 2005;156:793-806.
8. Navon-Venezia S, Ben-Ami R, Carmeli Y. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. Curr. Opin. Infect. Dis. 2005;18:306-313.
9. Agwu AE, Alu JI. Farmers perceived constraints to yam production in Benue State, Nigeria. Proceedings of the 39th annual conference of the agricultural society of Nigeria. 2005;347-350.
10. Ekundayo EO, Ezeogu LI. Evaluation of antimicrobial activities of extracts of five plants used in traditional medicine in Nigeria. International J. of Tropical Medicine. 2006;93-96.
11. Anishmon VS, Toji T. In vitro antibacterial activity of *Lygodium flexuosum* Nig. J. of Natural Products and Medicine. 2005;9:47.
12. Okigbo RN, Omodamiro OD. Antimicrobial effect of leaf extracts of pigean pea (*Cajanus cajan* (L)) on some human pathogens. Journal of Herbs, Spices and Medicinal Plant. 2006;12:117-121.
13. David TW. Oleic acid – The anti-breast cancer component in olive oil AU J.T. 2005;9(2):75-78.
14. Mendez JA, Vellon L, Colomer R, Lupu R. Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erbB-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin™) in breast cancer cells with Her-2/neu oncogene amplification. European Society for Medical Oncology; 2005.
Available:http://www.annonc.oupjournals.org/cgi/content/ abstract/mdi090v1
15. Harrison RJ, Moyle M. Organic syntheses. Coll. 1963;4:493.
16. Nkol TO¨, Dogruer D, Ito S, Sahin M. "Synthesis and antinociceptive activity of (5-chloro-2-benzothiazolinon-3-yl) acetamide derivatives. Archiv der Pharmazie. 2000;333(10):337–340.
17. Asif M. The pharmacological importance of some diazine containing drug molecules. Sop Transactions on Organic Chemistry. 2014;1(1):1-16.
18. Rafia B, Shafiya Y, Syed O, Shamim A, Hinna H, Alam MS, Mohammad S, Kalim J. Synthesis and biological evaluation of some novel sulfamoylphenyl-pyridazinone as anti-inflammatory agents (Part-II*). Journal of Enzyme Inhibition and Medicinal Chemistry. 2012;1–5.
19. Rane Z, Anish Kumar P, Anusha B. Phytochemical evaluation by GC-MS and *in vitro* antioxidant activity of *Punica granatum* fruit rind extract. Journal of Chemical and Pharmaceutical Research. 2012;4(6):2869-2873.
20. Uchegbu RI, Mbadiugha CN, Ibe CO, Achinihu IO, Sokwaibe CE. Antioxidant, anti-inflammatory and antibacterial activities of the seeds of *Mucuna flagellipes*. American Journal of Chemistry and Applications. 2015;2(5):114-117.
21. Singh S, Bhawani Rajesh BS, Sahoo K, Subudhi E, Nayak S. Chemical composition of turmeric oil (*Curcuma longa* L.) and its antimicrobial activity against eye infecting pathogens. Journal of Essential Oil Research. 2013;23:11-18.
22. Cosgrove SE, Vigliani GA, Campion M. Initial low dose gentamicin for *Staphyloccocus aureus* bacteremia and

endocarditis is nephrotoxic. Clin Infect Dis. 2009;48(6):713-21.
23. Adeogun OO, Adekunle AA, Samuel OT, Ebabhi AM. Studies of fungi isolated from *D. rotundata* collected from open markets in Lagos Nigeria. International Journal of Phytopathology. 2014;3(3):139-147.
24. Okigbo RN, Ogbonnaya UO. Antifungal effects of *Ocimum gratissimum* and *Aframomum melegeta* on post harvest yam (*Dioscorea spp*) rot. African Journal of Biotechnology. 2006;5(9):727-731.
25. Opara EU, Nwokocha NJ. Antimicrobial activities of some local medicinal plants against post harvest yam rot pathogens in numid South Eastern Nigeria. Journal of Microbiology Research and Reviews. 2015;3(1):1-9.
26. Abirami K, Baskaran V, Singh DR, Gopinath P, Sakthivel V, Dam Roy S. Phytochemical profile and antifungal activity of *Costus spp* of Bay Islands. Journal of the Andaman Science Association. 2014;19(1):45-59.

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