International Journal of Basic and Applied Chemical Sciences ISSN: 2277-2073 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jcs.htm
2016 Vol. 6(2) April-June, pp.79-84/Otuokere et al.
Research Article

CHARACTERIZATION OF *COSTUS AFER* STEM ETHANOL EXTRACT BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

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ABSTRACT
Costus afer is a stout perennial rhizomatous herb that belongs to the genius *Costus. Costus afer* is used for ceremony, religious rites and as a traditional medicine. Gas chromatography – mass spectrometry analysis was carried out on the stem ethanol extract of *Costus afer*. The gas chromatogram showed the presence of five compounds. The molecular mass of the compound were established based on the molecular ion in the mass spectra. The compounds were proposed based on comparison with National Institute of Standards and Technology (NIST) database. The suggested compounds are hexadecanoic acid, methyl ester (9.11%), alpha-tocopherol (44.54%), 10, 12-nonacosadiynoic acid (29.45%), D-alanine, N-(4-ethylbenzoyl)-pentadecyl ester (18.77%) and 9,12,15-octadecatrien-1-ol 2.69%). The suggested compounds exhibit the following bioactivities: catechol-o-methyl-transferase-inhibitor, 5-alpha-reductase-inhibitor, acidifier, acidulant, arachidonic acid-inhibitor, inhibit production of uric Acid, down regulation of cytosol androgen, anaphylactic (antidote) and oligosaccharide provider. We concluded that *Costus afer* contains pharmacological active compounds that may enhance its use as a traditional drug. Isolation and synthesis of these bioactive compounds is recommended.

Keywords: *Costus afer, Gas Chromatography, Mass Spectrometry, Extract, Bioactivity*

INTRODUCTION
Costus afer is a stout perennial rhizomatous herb that belongs to the genius *Costus* (Edeoga and Okoli, 2000). It is naturalized in Nigeria, Ghana, Niger, Guinea, South Africa and Senegal (Oliver, 1960). The Igbos in Nigeria call it "Okpete" or "Okpoto". It is called "Kakizawaa" by the Hausas in Nigeria, "tete-gun" in Yoruba language and "Mbritem" by Nigerian Efiks. Camerounian Anglophones call it "Monkey sugar cane" (Ezejiofor *et al*., 2013). It grows up to 4 metres tall (Protabase, 2016). The leaves are spirally arranged, tubular sheath with green-purple blotches. The spikes are conical with a length of 2.5 -7.5 cm long (Aweke, 2007). The flowers are bisexual and the corolla tubes are 2 cm long. *Costus afer* flowers and fruits vigorously throughout the year, depending on soil humidity (Aweke, 2007). In Southern Nigeria, it is regarded as a weed in rice plantation (Aweke, 2007). The pictorial view of *Costus afer* is presented in Figure 1.

Figure 1: Pictorial View of *Costus afer*

Costus afer is used for ceremony, religious rites and as a traditional medicine (Protabase, 2016). The seed, stem, and rhizome contains diosgenin and other steroidal sapogemins (Protabase, 2016). 0.05% diosgenin has been reported to be present in the rhizome (Protabase, 2016).

Diosgenin is an important precursor for the synthesis of oral contraceptives, anabolics, sex hormones, and corticosteroids (Protabase, 2016).

The rhizomes also contain a papaverine-like alkaloid which helps in smooth muscle relaxation, anti-spasmodic, central nervous system depressant and diuretic (Protabase, 2016). Anti-inflammatory activity of methanol extract of *Costus afer* has been reported (Protabase, 2016).

Extract of the plant is used in the management of rheumatism, arthritis, inflammation and cough (Awouters, 1978).

It is also used as a purgative and diuretic (Awouters, 1978). The boiled root is used in the treatment of cuts and sores (Awouters, 1978).

In spite of the numerous studies on *Costus afer* plant, there is limited information on its GC-MS characterization.

The present study was designed to investigate the bioactive constituents of *Costus afer* stem ethanol extract by GC-MS analysis.

MATERIALS AND METHODS

Plant Materials

Fresh stems of *Costus afer* were harvested from natural habitat, in Ogbuebulle, Ikwuano, Abia State, Nigeria. The plants were identified at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of Plant Extract

The plants were washed thoroughly in running water. They were dried for 15 days at room temperature and pulverized to powder using electrical grinder. The stem of *Costus afer* was prepared according research reports found in literature (Janakiraman *et al*., 2012; Gopalakrishnan and Udayakumar, 2014). 20g stem powder of *Costus afer was* soaked in 200ml of ethanol and agitated in an orbit shaker for 48 hours.

After 48 hours, the mixture was filtered using Whatman no 1 filter paper and then concentrated at 40ºC using hot air oven. The concentrated ethanol extract was used for GC-MS analysis.

GC-MS Analysis of Costus Afer

Characterization of the phytochemicals in *Costus afer* was done using GC-MS QP2010 Plus (Shimadzu, Japan) with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV.

Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60m, XTI-5).

The initial column temperature was 80ºC for 1min, and then increased linearly at 70ºC min-1 to 220ºC, held for 3 min followed by linear increased temperature 10ºC min-1 to 290ºC for 10 min.

The temperature of the injection port was 290ºC and the GC-MS interface was maintained at 290ºC. The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min-1.

Identification of Phytocompoments in Costus Afer

The retention indices, peak area percentage and mass spectra molecular ion ethanol extract of *Costus afer* stem were compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB (Stein, 1990). The name, molecular weight, formula, structure and bioactivities of the compounds were proposed.

RESULTS AND DISCUSSION

Gas chromatogram and mass spectra of stem ethanol extract of *Costus afer* are presented in Figures 2 and 3 respectively.

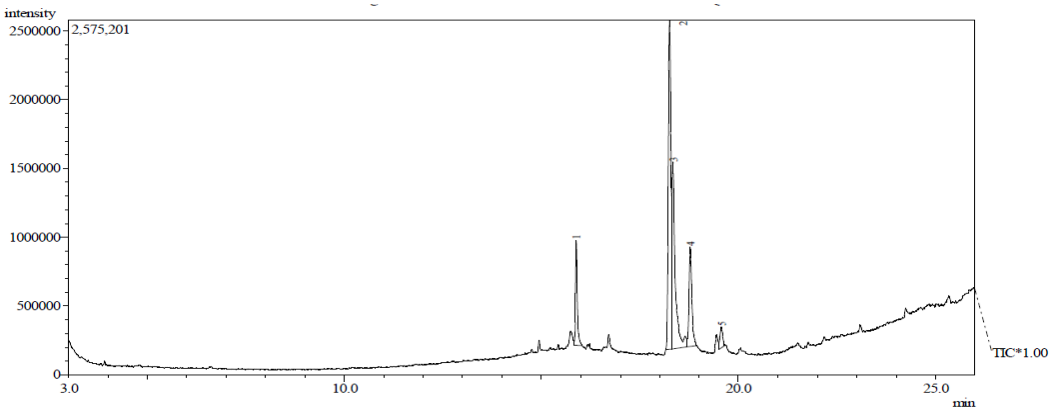

Figure 2: Gas Chromatogram of Costus *Afer* Stem Ethanol Extract

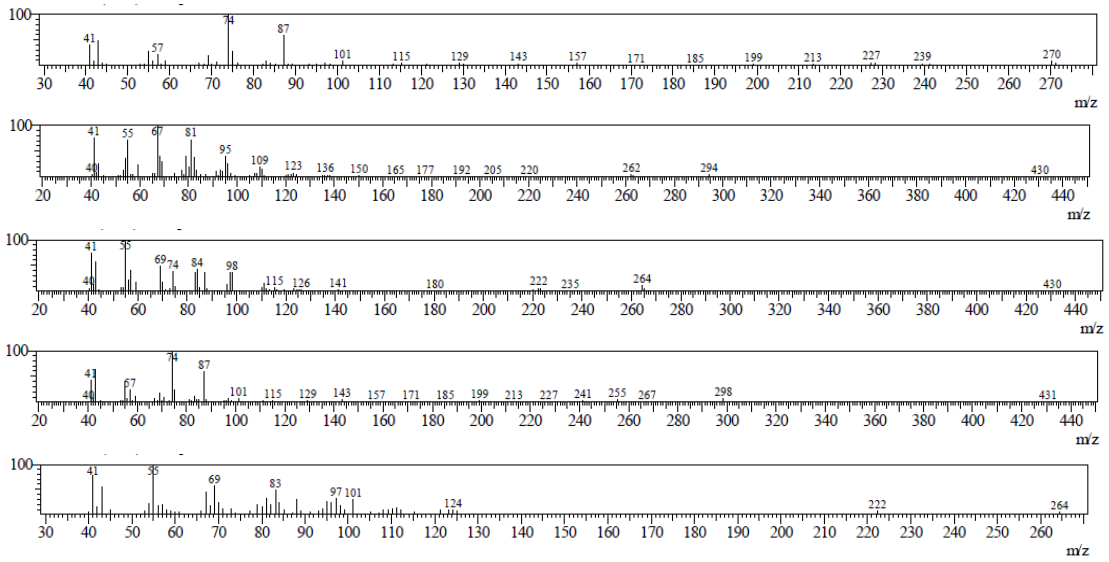

Figure 3: Mass Spectra of *Costus afer* Stem Ethanol Extract

Table 1: Proposed Compounds, Retention Time, Peak Area, Molecular Weight, Molecular Formula and Bioactivity of *Costus afer* Stem Ethanol Extract

S/No	Name of Compound	Retention Time	Peak Area %	Molecular Weight	Molecular Formula	Bioactivity
1	Hexadecanoic acid, methyl ester	15.886	9.11	270.45	$C_{17}H_{34}O_2$	Catechol-O-methyl-transferase-inhibitor
2	Alpha-tocopherol	18.252	44.54	$C_{29}H_{50}O_2$	430.70	5-alpha-reductase-inhibitor,
3	10,12-Nonacosadiynoic acid	18.339	29.45	$C_{29}H_{50}O_2$	430.70	Acidifier, acidulant, arachidonic acid-inhibitor, inhibit production of uric Acid,
4	D-Alanine,N-(4-ethylbenzoyl)-pentadecyl ester	18.777	14.21	$C_{27}H_{45}NO_3$	431.65	Down regulation of cytosol androgen, anaphylactic (antidote),
5	9,12,15-Octadecatrien-1-ol	19.564	2.69	264.44	$C_{18}H_{32}O$	Oligosaccharide provider

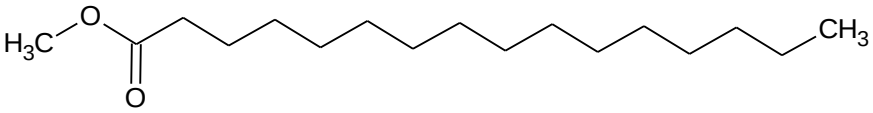

Figure 4: Hexadecanoic Acid, Methyl Ester

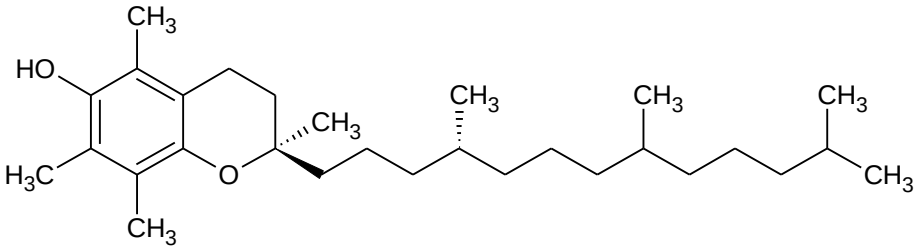

Figure 5: Alpha-Tocopherol

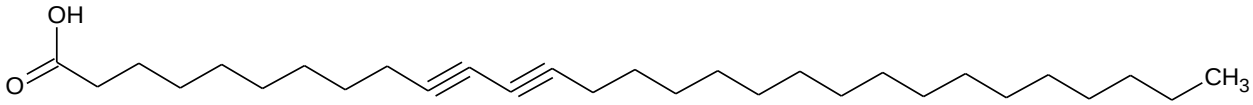

Figure 6: 10, 12-Nonacosadiynoic Acid

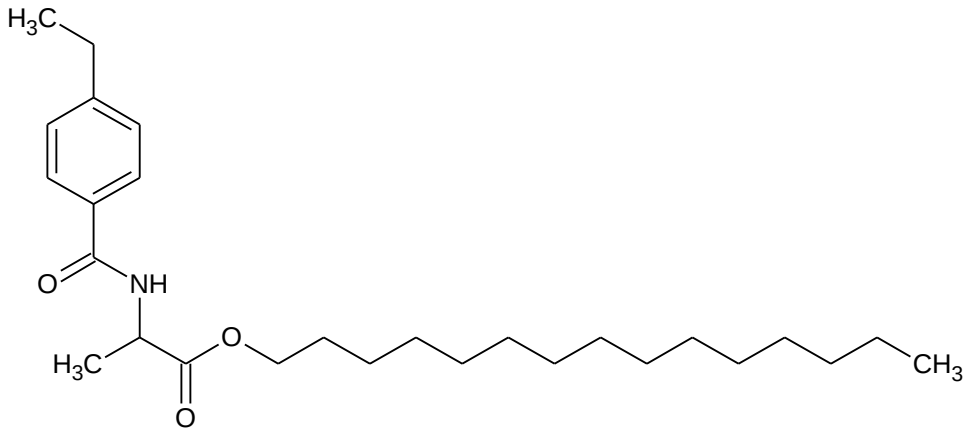

Figure 7: D-Alanine, N-(4-Ethylbenzoyl)-Pentadecyl Ester

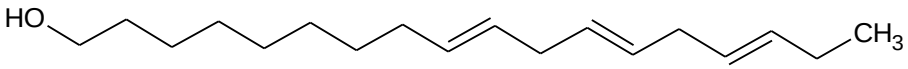

Figure 8: 9, 12, 15-Octadecatrien-1-ol

Five peaks were shown in the gas chromatogram (Figure 2). This suggests the presence of five phytocompounds in the stem ethanol extract of *Costus afer*. The proposed compounds, retention time (RT), peak area percentage, molecular weight, molecular formula and bioactivities *are* discussed in Table 1. The structures are shown in Figures 4 -8 respectively.

Hexadecanoic acid, methyl ester is an inhibitor of catechol-O-methyl-transferase (Duke, 1992-1996). Catechol-O-methyl-transferase is involved in the degradation of neurotransmitters. Catechol-o-methyl-transferase-inhibitors opposes the degradation of neurotransmitters. Chronic infections like Parkinson's disease is treatable with a catechol-O-methyl-transferase-inhibitors.

Clinically, 5α-reductase inhibitors are used in the treatment of conditions that are exacerbated by dihydrotestosterone (DHT) (Rossi, 2004). It has been reported that Alpha-tocopherol is a 5α-reductase inhibitor (5-ARI) (Duke, 1992-1996). They are primarily used in the management of benign prostatic hyperplasia (BPH) and androgenic alopecia because of their antiandrogen effects. Metabolic

transformation of these endogenous steroids is possible since these compounds inhibit the enzyme 5α-reductase. The major role of 5α-reductase inhibitors is the inhibition of testosterone to dihydrotestosterone (DHT).

10,12-Nonacosadiynoic acid have been reported to be an acidifier, acidulant and arachidonic acid inhibitor (Duke, 1992-1996). Acidifiers are chemicals that reduce the pH of the body. Acidifiers are needed for food digestion especially in patients suffering from achlorhydria. These patients are not able to secret HCl for food digestion. 10, 12-Nonacosadiynoic acid will be beneficial since it increases gastric acid when ingested. Arachidonic acid is present in the brain, muscles, and liver (Smith *et al*., 2011). Arachidonic acid is a fatty acid that is polyunsaturated in nature and responsible for the repair and growth of skeletal body tissue (Trappe *et al*., 2001). Arachidonic acid does not cause cancer but studies have proven that it might be a major cause of inflammation (Schuurman *et al*., 1999; Leitzmann *et al*., 2004; Astorg, 2005; Whelan and McEntee, 2004). Additives that reduce the pH of food in order to add a tart taste and characteristic tang are called acidulants. They also have preservative and antioxidative properties (Faia, 2016).

9,12,15-Octadecatrien-1-ol, a suggested phytocompouns in *Costus afer* stem ethanol extract is responsible for the provision of oligosaccharide (Duke, 1992-1996). It helps in cell division and cell binding. It also improves gastrointestinal health, energy levels and performance. Oligosaccharide provider simply means little or few sugar. The breaking down of purine nucleotides leads to the formation of uric acid. High concentration of uric acid in the blood can lead to gout, diabetes and formation of ammonium acid urate kidney stones. *Costus afer* stem ethanol extract may help in the inhibition of uric acid because 10,12-Nonacosadiynoic acid is an inhibitor of uric acid (Duke, 1992-1996). Research has proven that D-Alanine, N-(4-ethylbenzoyl)-pentadecyl ester is an anaphylactic (antidote) (Duke, 1992-1996). Anaphylaxis is a serious infection that might lead to death if not checked because its duration is within minutes to hours. This allergic reaction is triggered by insect bites, medication, food or any foreign substance (Lee and Vadas, 2011; Worm, 2010). It is an allergic reaction that is accompanied with low blood pressure, itching, swelling of the tongue, shortness of breath, vomiting, and lightheadedness (Simons, 2010; Boden and Wesley, 2011). D-Alanine, N-(4-ethylbenzoyl)-pentadecyl ester helps down regulation of cytosol androgen (Duke, 1992-1996). Prostate epithelial cells require androgen for growth (Hein, 2000). Androgen binds to androgen receptors, which further binds to androgen-responsive elements for the promotion of androgen-regulated genes such as prostate-specific antigen (PSA). The down regulation of cytosol androgen provides an important mechanism in prostatic cancer chemoprevention (Isaacs, 1994).

Conclusion

Five compounds have been identified in the ethanol extract of *Costus afer* stem using GC-MS analysis. The bioactivities of these compounds have been discussed. These identified phytocompounds will enhance the development of drugs from this plant. These phytocompounds needs further pharmacological investigation in order to develop new drugs for the treatment of specific diseases. We concluded that *Costus afer* contains pharmacological active compounds that may enhance its use as a traditional drug.

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