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Phytochemical Constituents in Hexane Fraction of *Costus afer* Ker Gawl. Stem

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ABSTRACT

In view of the high demands for alternative medicine in developing countries and the dearth of valid scientific data on herbal remedies used in these countries. This study was designed to identify the bioactive compounds present in hexane fraction of *Costus afer* ker Gawl stem, an indigenous medicinal African plant used as therapy in the treatment of chronic inflammatory disease such as rheumatoid arthritis. Phytochemical evaluation and gas chromatography-mass spectrometry (GC/MS) analytical methods were carried out. The mass spectra of the identified compounds were compared with those of the National Institute of Standards and Technology database library. Results showed the presence of alkaloids, diterpenes, triterpenes, phytosterol, phenol, phlobatannins and tannins while GC/MS data detected 16 compounds including benzofuran 2,3-dihydro and oleic acid. These identified compounds in hexane fractions of C. *afer* stem may explain the folkloric use of C. *afer* plant stem extract in the treatment of chronic inflammatory diseases.

Keywords: *Costus afer, GC/MS, herbal, stem, phytochemicals*

Introduction

In developing countries, herbal medicine remains the main stay of the people both for prophylactic as well as therapeutic purposes, especially in the area of primary health care delivery [1]. The use of herbal medicines in the world far exceeds that of the conventional drugs by the ratio of 2 to 3 [2]. Traditional medical practitioners generally use unpurified plant extracts either from the stem, leaves, roots, rhizomes or the whole plant in treatment of various ailments [3]. It is generally claimed that the chemical constituents present in these extracts elicit their biological effect synergistically, so that the effect of the whole herb is greater than the effect of the individual compounds [4]. However, many of these chemical constituents as well as their mode of action have not yet been well documented to enhance their acceptability in main stream medicine [4].

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Costus afer Ker Gawl. (Costaceae) is an indigenous West African medicinal plant which belongs to one of 150 species of stout, perennial, and rhizomatous herbs. It grows in moist or shady farm lands and river banks [5]. C. *afer* is commonly called gingerlily or bush cane and in Nigeria, it is called "Ireke omode" in Yoruba, while it is called "Okpete" in Igbo, "Kakizawa" in Hausa, "Mbritem" in Efik. Ghanaian calls it "Akan asante" while Anglophone Cameroon calls it "Monkey sugar cane" [6]. C. *afer* stem extract has been used as medicinal herb especially in the treatment of inflammation, rheumatoid arthritis, cough, sore throat, colic, hemorrhoids and epileptic attack. It can also serve as laxative, diuretic, and an antidote for snake poison [5-7]. Aqueous and methanol extracts of C. *afer* stem exhibited antioxidant activity *in vitro* [8].

The majority of the chemical compounds identified in *C. afer* plant are from the rhizome, which have been reported to contain saponins aferosides A – C, dioscin and paryphyllin C, and it also contains flavonoid glycoside kaempferol 3-O-α-L-rhamnopyranoside [9]. Sesquilavandulyl acetate, β-carophyllene, Z, E-farnesol have also been identified in the essential oil of *C. afer* leaves [10]. To the best of our knowledge no attempts have been made to identify the chemical compounds present in hexane fractions of *C. afer* stem using gas chromatography-mass spectrometry methods. Therefore, this study was aimed to identify the bioactive compounds present in hexane fractions of *C. afer* stem with the objective to proffer scientific rationale for the ethno-medical use of *C. afer* stem in the treatment of inflammatory diseases.

Materials and Methods

Collection of Plant Materials

C. afer plant was obtained from a farm land at Irolu in Ikenne Local Government Area, Ogun State, Nigeria. The plant was identified and authenticated by Professor A.O. Denton, a crop scientist in the Department of Agronomy and Landscape Design, School of Agriculture and Industrial Technology, Babcock University, Ilisan-Remo, Ogun State, Nigeria. A voucher sample with number of FHI-108001 has been deposited at Forestry Herbarium Ibadan (FHI).

Plant Processing, Extraction and Solvent Partitioning

The leaves and roots were plucked out of the stem and discarded. The chopped stem pitches were air-dried under room temperature and pulverized using mechanical grinder. Three hundred grams of powdered stem samples were extracted using 1800 ml of 70% methanol with intermittent shaking for 48 h. The extract was filtered using Whatman No.1 filter paper and the filtrate was subsequently concentrated using rotary evaporator at 30 °C (Buchi Rotavapor RE; Switzerland). The concentrates were reconstituted with distilled water in a ratio of 1:2 (concentrate: distilled water) and partitioned using *n*-hexane. The hexane stem fraction obtained was concentrated in a rotary evaporator at 30 °C. The hexane stem fraction was then subjected to phytochemical evaluation and gas chromatography-mass spectrometry analytical methods.

Phytochemical Evaluation

Phytochemical evaluation was performed on the isolated hexane fraction of *C. afer* stem using standard procedures to identify chemical constituents as described by Trease and Evans [11], Harborne [12] and Sofowora [13]. The procedures were as follows:

Screening for Alkaloids

Hexane stem fraction of *C. afer* was dissolved individually in 1% HCl on steam bath and filtered while hot. The filtrate was used to test for the presence of alkaloids according to:

Mayer's Test

Filtrate obtained was treated with Mayer's reagent (potassium mercuric iodide). The formation of cream colored precipitate indicated the presence of alkaloids.

Wagner's Test

Filtrate was treated with Wagner's reagent (Iodine in potassium iodide). The formation of brown / reddish brown precipitate indicated the presence of alkaloids.

Screening for Glycosides

Hexane stem fraction was hydrolyzed with 1% HCl and then subjected to test for glycosides using:

Modified Borntrager's Test

Hydrolysed fraction was treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. The formation of rose-pink color in the ammoniacal layer indicated the presence of anthranol glycosides.

Legal Test

Hydrolysed fraction was treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of pink to blood red color indicated the presence of cardiac glycosides.

Liebermann Burchard's Test

Hydrolysed fraction was treated with chloroform and a few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added carefully along the sides of the test tube. The formation of brown ring at the junction indicated the presence of steroidal glycosides.

Screening for Saponins

Foam Test

The hexane stem fraction was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. formation of 1 cm layer of foam indicated the presence of saponins.

Screening for Triterpenes and Phytosterol

Salkowski Test

The hexane stem fraction was dissolved in chloroform and subsequently treated with a few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicated the presence of triterpenes and steroids.

Libermann Burchard's Test

The hexane stem fraction was dissolved in chloroform. To the chloroform solution few drops of acetic anhydride was added boiled and cooled. Concentrated sulphuric acid was added carefully along the sides of the test tubes. Formation of brown ring at the junction indicated the presence of phytosterols.

Screening for Fixed Oils

Stain Test

The hexane stem fraction in sample quantity was pressed between two filter papers. An oily stain on filter paper

indicated the presence of fixed oils and fats.

Screening for Resins

Acetone- water Test

The hexane stem fraction was dissolved in acetone and filtered. Small amount of water was added to acetone solution and shaken. Appearance of turbidity indicated the presence of resins.

Screening for Phenols

Ferric chloride Test

The hexane stem fraction was treated with few drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

Screening for Flavonoids

Alkaline reagent Test

The hexane stem fraction was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of diluted HCl, indicated the presence of flavonoids.

Lead acetate Test

The hexane stem fraction was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

Shinoda Test

To the alcoholic solution of fractions, a few fragment of magnesium ribbon and concentrated HCl were added. Appearance of magenta color after few minutes indicated the presence of flavonoids.

Screening for Diterpenes

Copper acetate Test

The hexane stem fraction was treated with few drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

Screening for Triterpenoids

Tshugajen Test

The hexane stem fraction was treated with chloroform and filtered. Excess of acetyl chloride and a pinch of zinc chloride were added to the treated fractions, kept aside for some time till the reaction was completed and then warmed on water bath. Appearance of eosin red color indicated the presence of triterpenes.

Screening for tannins

The hexane stem fraction was dissolved in water, after which the solution was clarified by filtration. 10% ferric chloride solution was added to the resultant filtrate. The appearance of a bluish black or brownish green or dark green color will indicated the presence of tannins.

Screening for anthraquinones

The hexane stem fraction was shaken with 10 ml of benzene and filtered. Ammonia solution (10%) was added to the filtrates and the mixture shaken. The formation of a pink, red or violet color on the ammoniacal phase indicated the presence of anthraquinones.

Screening for phlobatannins

A few drops of 1% HCl was added to 1 ml of hexane stem fraction separately and boiled. A red precipitation indicated the presence of phlobatannins.

Gas Chromatography – Mass Spectrometry (GC/MS) Analysis

The hexane fractions of *C. afer* stem was subjected to GC/MS analysis which was carried out at the Department of Chemistry, University of Lagos, Akoka. The GC/MS Specification was: Agilent Technologies model 7890A GC-MS, MSD=5975C (detector) Agilent Technologies, Injector: 7683B series, initial temperature=100 ^{o}C held for 2 min, final temperature=270^{o}C at the rate of 10 ^{o}C /min, 1 µl of 0.2 g/ml fraction was injected. Temperature of heater was 250 ^{o}C, pressure was 3.2652 psi, mode type splitless, column type (HP5MS: 30 M×320 µM×0.25 µM) and carrier gas (helium, 99.9999% purity, flow rate =1.4963 ml/min; average velocity = 45.618 cm/sec). The constituent compounds were determined by comparing the retention times and mass spectra of the authentic samples obtained by GC with the mass spectra from the National Institute of Standards and Technology (NIST) Version 2.0 MS database library.

Results and Discussion

The phytochemical analysis revealed the presence of alkaloids, diterpenes, triterpenes, phytosterols, phenols, phlobatannins and tannins (Table 1). Previous studies have shown that the medicinal value of plant extracts could be attributed to some plant phytochemicals known to inhibit or terminate pro-inflammatory mediators or deleterious chain reactions triggered by free radicals or reactive oxygen species [14,15]. Researches have shown that alkaloids possess anti-inflammatory, antimicrobial, antifungal, antihelminthics, antimalarial and antidepressant activities [13,16,17]. Plant steroids and phlobatannins have been shown to have similar pharmacology attributes compared to animal steroids due to their structural relationship [18]. Cardiac glycosides inhibit the sodium ion/potassium ion pump which is important in the treatment of congestive heart failure and cardiac arrhythmia [19]. It can be deduced that the folkloric use of *C. afer* stem extract in the treatment of arthritis, rheumatism, sore throat, diarrhea, antihelminthics, hemorrhage and wound healing might be due to presence of these phytochemicals.

The GC-MS spectrum of the hexane fraction of *C. afer* stem is shown in Figure 1. The compounds identified in the spectrum together with their relative abundance are shown in Table 2. A coumaran, benzofuran 2,3-dihydro was detected in hexane stem fraction of *C. afer* and previous report had shown that it possesses anti-inflammatory, antidiarrhoeal and anti-helminthic activities [20,21]. Also, hexadecanoic acid, methyl ester and n-hexadecanoic acid detected had been reported to

Table 1: Phytochemical evaluation of hexane fraction of *Costus afer* stem

Chemical constituents	Chemical Test	Hexane stem Fraction
Alkaloids	Meyer's test	+
	Wagner's test	+
Glycosides	Borntrager's test	-
	Lieberman Buchard's test	-
	Legal's test	-
Saponins	Foam's test	-
Triterpenes and Phytosterols	Salkowski's test	+
	Lieberman Buchard's test	-
Fixed oil	Stain test	+
Resins	Acetone-water test	+
Phenols	Ferric chloride tests	+
Flavonoid	Alkaline test	-
	Lead acetate test	-
	Shinoda test	-
Anthraquinone	Anthraquinone test	-
Phlobatannins	Phlobatannins test	+
Tannins	Tannin test	+
Diterpernes	Copper acetate test	+
	Tshugajen's test	+

+ indicates presence; – indicates absence

Figure 1: Photograph showing the arial view of *C. afer* Ker Gawl

possess anti-inflammatory, antioxidant, hypocholesterolemic, 5-alpha reductase inhibitor, nematicide, pesticide and antiandrogenic [22,23]. Similarly, cis-vaccenic acids and oleic acid detected are potent anti-inflammatory and antioxidant compounds [21,22]. Tritetracontane and octacosane detected in hexane stem fraction of *C. afer* had been reported to exhibit insecticidal activity [21]. 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester is known for its plasticizer property [21]. Furthermore, 9,12-octadecadienoic acid, methyl, 11-octadecenoic acid, methyl ester, octadecanoic acid, methyl ester, tricosane, tetracosane, 9-octadecanal (Z), hexacosane and heptadecane were also detected in

Table 2: GC-MS analysis of *C. afer* hexane stem fraction

S/N	PEAK NO.	RETENTION TIME	LIBRARY ID	QUALITY	BIOACTIVITY
1	4	5.198	Benzofuran 2,3-dihydro (Coumaran)	74	Anti-inflammatory, antihelminthics, anti-diarrhoel [20,21]
2	5	12.717	Hexadecanoic acid, methyl ester	99	Antibacterial,antifungal Anti-inflammatory Antioxidant [22,23]
33	6	13.072	n-Hexadecanoic acid (palmitic acid)	99	Anti-inflammatory Antioxidant Hypocholesterolemic Flavour Nematicide Pesticide Antiandrogenic [22,23]
4	8	14.336	9,12-Octadecadienoic acid, methyl	99	No bioactivity reported
5	9	14.388	11-Octadecenoic acid, methyl ester	99	No bioactivity reported
6	10	14.617	Octadecanoic acid, methyl ester (methyl stearate)	99	Raw material , emulsifier or oiling agent for foods No bioactivity (Duke, 2013)
7	11	14.823	Cis-Vaccenic acid	99	Anti-inflammatory Antioxidant [21,22]
8	13	15.252	Oleic acid	87	Anti-inflammatory Antioxidant [21,22]
9	15	16.110	Tricosane	89	No bioactivity reported
10	16	16.934	Tetracosane	98	No bioactivity reported
11	17	17.524	9-Octadecanal (Z)	94	No bioactivity reported
12	19	17.730	Tritetracontane	90	Insecticidal [21]
13	21	18.153	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (phthalate)	91	Plasticizer [21]
14	22	18.491	Hexacosane	95	No bioactivity reported
15	23	19.234	Heptadecane	94	No bioactivity reported
16	27	21.014	Octacosane	96	Insecticidal or mosquitocidal [21]

the hexane stem fraction, however, there has been no reported biological activity.

Conclusion

The hexane fraction of *C. afer* stem contains phytochemicals of biological and medicinal value. These identified bioactive compounds may account for the prophylactic or therapeutic uses of *C. afer* stem extract against chronic inflammatory disease such as rheumatoid arthritis by folklores. Furthermore, *n*-hexane fraction of *C. afer* stem could serve as a potential source of biopharmaceutical agents in the drug discovery process. It is recommended that further studies be carried out on the detected compounds with no known biological activity to unravel their biological activities as they might be contributory to the ethno-medical efficacy of *C. afer* stem extract in treatment of inflammatory disorders.

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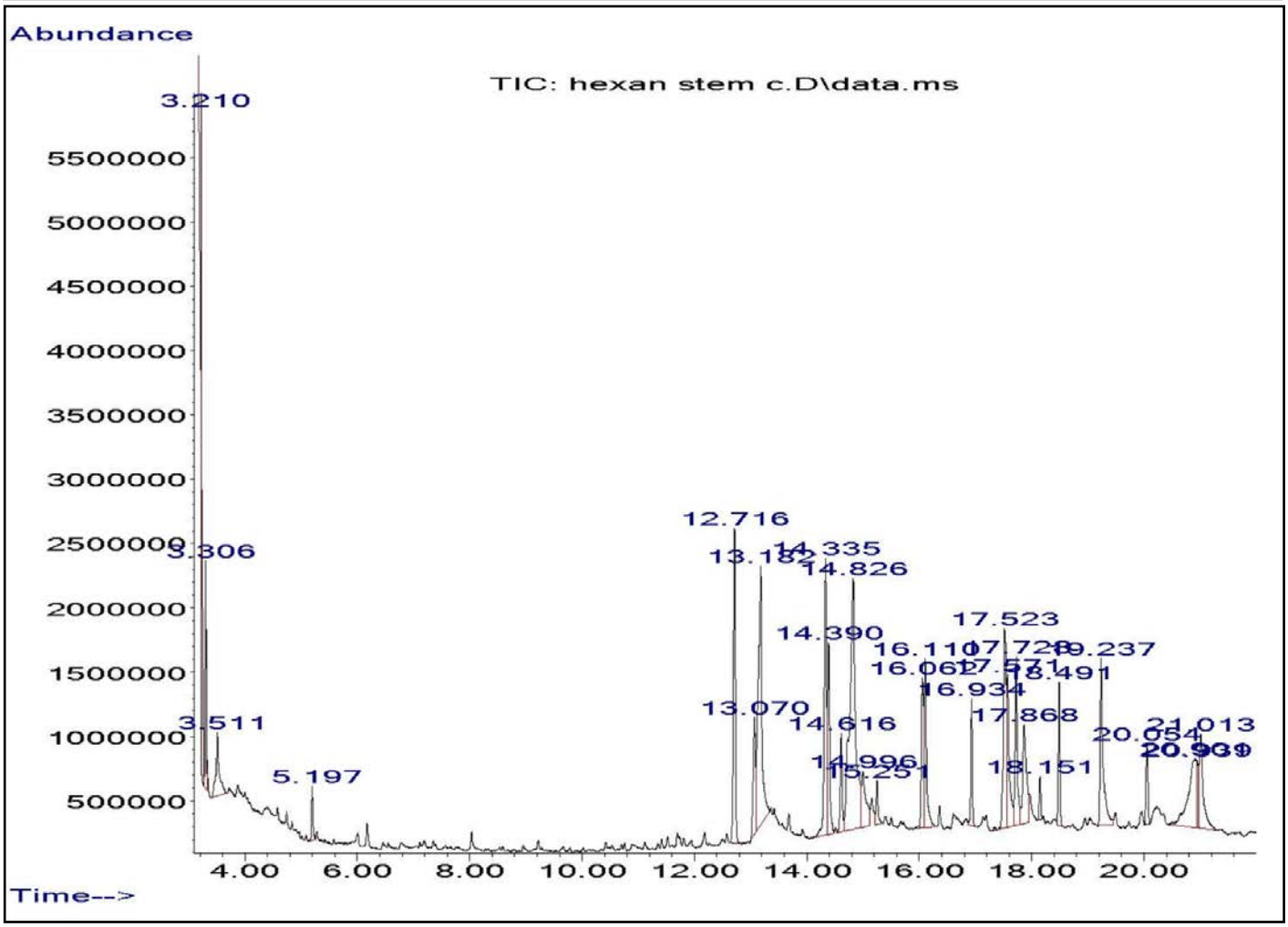

Figure 2: GC/MS chromatogram of hexane fraction of *C. afer* **stem**

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