INTRODUCTION

Chemical compounds of plant origin are increasingly gaining popularity, especially in the development of novel drugs or herbal mixtures used in the treatment of chronic inflammatory and oxidative stress related diseases [1]. In African traditional medicine, several plant parts (root, bark, stem or leaf) are used in the management of inflammatory diseases.

Costus afer Ker Gawl is an indigenous West African medicinal plant of the family of Zingiberaceae now known as costaceae. It is one of the 150 species of stout, perennial, and rhizomatous herbs that grow in moist or shady forests and river banks [2]. It is found in the forest belt region of West Africa from Senegal, East to Ethiopia; and South to Tanzania, Malawi and Angola. C. afer is commonly called Gingerlily or Bush cane and in Nigeria, it is called “ireke omode” in Yoruba, “Okpete” in Igbo, “Kakizawa”
in Hausa, “Mbitem” in Efik and anglophone Cameroon calls it “Monkey sugar cane” [5].

Costus afer stem or leaf are often used as a medicinal herb especially in the treatment of inflammation, rheumatism, arthritis, cough, hepatic disorders, helminthic, miscarriages, epileptic attack, and hemorrhoids. It can also serve as a laxative, diuretic, and an antidote for poison [3-5]. An infusion of C. afer inflorescence or rhizome is taken to treat tachycardia and stomach complaints. A stem decoction or chewed stem or the pounded fruit, sometimes mixed with sugar cane juice, is taken to treat respiratory problems and a sore throat. Leaf sap or a rhizome decoction is taken to treat malaria. In Nigeria, the debarked stem is chewed to treat nausea and to quench thirst. A cold water extract of the stem is taken to treat small epileptic attacks. A rhizome decoction or the raw rhizome is taken to treat leprosy and venereal diseases. In Gabon, the stem sap is rubbed on the body to treat colic [2]. Furthermore, C. afer is used for other socio-cultural purposes such as preparation of ritual ornaments, wrapping of indigenous foods, mat making, and as feed for ruminant animals [6,7].

The aqueous leaves and stem extracts showed significant antibacterial and amoebicidal activity in vitro [8]. The chloroform and methanol extracts from the aerial parts reduced carrageenan-induced rat paw edema [2]. Aqueous and methanol extracts of C. afer stem exhibited antioxidant activity in vitro [9]. The methanol leaf extract showed significant cytotoxicity in the brine shrimp test [5]. The same extract showed moderate local anesthetic activity in guinea pig skin test, and contracted the guinea pig ileum in a concentration-dependent manner [5]. The methanol leaf extract exhibited anti-hyperglycemic activity, and decreased the blood glucose level by 50% in streptozotocin-induced hyperglycemia in male rats [2].

The rhizome of C. afer contains several steroidal sapogenins of which diosgenin is the most important one. It also contains the saponins aferosides A-C, dioscin and paryphyllin C and the flavonoid glycoside kaempferol 3-O-L-rhamnopyranoside [10]. Sesquilavandulyl acetate, β-carophyllene, Z, E-farnesol have been identified in the essential oil of C. afer leaves [11]. To the best of our knowledge no attempts have been made to elucidate the chemical compounds present in n-butanol fractions of C. afer leaf and stem. Therefore, this study was aimed to identify the medicinal compounds in n-butanol fractions of C. afer stem and leaf with the objective of explaining the ethnomedical use of C. afer in the treatment of inflammatory diseases.

MATERIALS AND METHODS

Collection of Plant Materials

Costus afer plants were obtained from a farm land at Irolu in Ikene Local Government Area, Ogun State, Nigeria. The plant was identified and authenticated by Professor Denton, a Crop Scientist in the Department of Crop Sciences, School of Agriculture and Industrial Technology, Babcock University.

A voucher sample was deposited at the Babcock University Horticultural garden.

Plant Processing, Extraction and Solvent Partitioning

The leaves and stem were separated from the root, which was discarded. The leaves and chopped stem pitches were air-dried under room temperature and pulverized using mechanical grinder. Three hundred grams powdered leaf and stem samples were extracted using 1800 mL of 70% methanol at 28°C 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 (BuchiRotavapor RE; Switzerland). The concentrates were reconstituted with distilled water in a ratio of 1:2 (concentrate: distilled water) and defatted using n-hexane. The defatted portion was further partitioned by successive solvent fractionation method starting with ethyl acetate and n-butanol in equal volumes using separating funnel. The n-butanol fraction was subsequently subjected through gas chromatographic-mass spectrometric (GC/MS) analytical method for the chemical compound characterization.

Phytochemical Evaluation

Phytochemical evaluation was performed on the isolated n-butanol fraction of C. afer leaf and stem using standard procedures to identify chemical constituents as described by Tresca and Evans [12], Harbone [13] and Sofowora [14]. The following phytochemical screenings were carried out.

Screening for Alkaloids

Leaf and stem fractions of C. afer were dissolved individually in 1% HCl on the steam bath and filtered while hot. The filtrates were used to test for the presence of alkaloids according to:

Mayer’s Test

Filtrates obtained were treated with Mayer’s reagent (potassium mercuric iodide). The formation of cream colored precipitate indicated the presence of alkaloids.

Wagner’s Test

Filtrates were treated with Wagner’s reagent (iodine in potassium iodide). The formation of brown/reddish brown precipitate indicated the presence of alkaloids.

Screening for Glycosides

Stem and leaf fractions were hydrolyzed with 1% HCl and then subjected to test for glycosides using:

Modified Borntrager’s test

Hydrolyzed fractions were 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**
Hydrolyzed fractions were 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**
Hydrolyzed fractions were treated with chloroform and a few drops of acetic anhydride, boiled and cooled. Concentrated sulfuric 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 fractions were 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 fractions were dissolved in chloroform, chloroform solution was treated with a few drops of concentrated sulfuric acid, shaken and allowed to stand. Appearance of golden yellow color indicated the presence of triterpenes and steroids.

**Liebermann Burchard’s test**
The fractions were dissolved in chloroform. To the chloroform solution, few drops of acetic anhydride were added, boiled, and cooled. Concentrated sulfuric 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**
Small quantities of extract were 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 fractions were 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 fractions were 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 fractions were 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 fractions were 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 fractions were treated with few drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

### Screening for Triterpenoids

**Tshugajen test**
The fractions were 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 until the reaction was completed and then warmed on water bath. Appearance of eosin red color indicates the presence of triterpenoids.

### Screening for Tannins

The fractions were 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 indicate the presence of tannins.

### Screening for Anthraquinones

The fractions were 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 indicates the presence of anthraquinones.

### Screening for Phlobatannins

A few drops of 1% HCl was added to 1 ml of stem and leaf fractions separately and boiled. A red precipitation indicates the presence of phlobatannins.

### GC/MS Analysis

The n-butanol fractions of C. afer leaf and stem were 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°C held for 2 min, final temperature = 270°C at the rate of 10°C/min, 1 μL of 0.2 g/mL fraction was injected. Temperature of heater was 250°C, pressure was 3.2652 psi, mode type split less, column type (HP5MS: 30 M × 0.25 μM × 0.25 μM) and carrier gas (helium, 99.999% purity, flow rate = 1.4963 mL/min; average velocity = 45.618 cm/s). 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 NIST Version 2.0 database library, Washington, DC, USA MS database library.

RESULTS

The phytochemical analysis revealed the presence of alkaloids, saponins, diterpenes, triterpenes, phytosterol, phlobatannins, and tannins in n-butanol fractions of C. afer leaf and stem. Phenols were detected in the n-butanol fraction of the leaves while flavonoids were present in the n-butanol fraction of the stem. Glycosides, fixed oil, resins, and anthraquinones were not detected in the n-butanol fractions of C. afer leaves and stem [Table 1].

The GC/MS spectra of the n-butanol fraction of C. afer leaves and stem are shown in Figures 1 and 2 respectively. Fifteen compounds were identified in the spectrum of n-butanol fraction of C. afer leaves and they are indolizine (0.305%), 2-methoxy-4 vinylphenol (1.202%), 3-butene-2-one 4-(4-hydroxy-2,2,6 trimethyl-7-oxabicyclo[4.1.0] hept-1yl)-(1.448%), hexadecanoic acid, methyl ester (7.176%), dibutyl phthalate (8.196%), n-hexadecanoic acid (7.946%), methyl 10-methyl-hexadecanoate (0.495%), 9,12-octadecanoic acid, methyl ester (1.814%), 11-octadecenoic acid, methyl ester (2.455%), phytol (3.751%), octadecanoic acid, methyl ester (2.459%), oleic acid (7.756%), 9-octadecenal, (Z) (8.226%), eicosane (0.276%) and cis-vaccenic acid (1.127%) [Table 2]. The identified chemical compounds in the spectrum of n-butanol fraction of C. afer leaves and stem [Table 1].

DISCUSSION

Phytochemical evaluation of n-butanol fractions of C. afer leaves and stem revealed the presence of important bioactive compounds. Alkaloids found to be present in the n-butanol fractions of C. afer leaves and stem are known to have antimicrobial, antifungal, antihelminthics, anti-diarrheal and anti-inflammatory effect, and they also act as anti-hypertensive agent, antimalarial, antidepressant, anesthetic and amoebicide [14-16]. Flavonoids and phenols detected are potent antioxidants, anti-inflammatory, anti-allergic, anti-thrombotic, vasoprotective, tumor inhibitory, antiviral, antimicrobial and hypolipidemic agents [17]. Saponins are immune boosters, antidiarrheal, anti-inflammatory, cholesterol lowering and have anticancer property [18]. Tannins, terpenoids and oils have antimicrobials, anti-inflammatory and antidiarrheal properties [19]. Plant steroids and phlobatannins are of interest in pharmacology due to their structural relationship with animal steroid. Plant steroids are known to have cardiotoxic activities, insecticidal and antimicrobial properties [20]. Cardiac glycosides in the n-butanol fractions are known to inhibit the Na+/K+ pump, which is important in the treatment of congestive heart failure and cardiac arrhythmia [21]. It can be deduced that the folkloric use of C. afer in the treatment of arthritis, rheumatism, sore throat, diarrhea, antihelminthics,
hemorrhage and wound healing might be due to the presence of these phytochemicals. Previous studies have shown that the anti-inflammatory and antioxidants properties of plant extracts could be attributed to these identified plant phytochemicals known to inhibit or terminate pro-inflammatory mediators or deleterious chain reactions triggered by free radicals or reactive oxygen species [22].

Further studies using GC/MS analytical method confirmed the presence of chemical compounds detected using phytochemicals screening methods and also quantified them. It identified fatty acids as the major compounds present in both n-butanol leaves and stem fractions. Hexadecanoic acid, methyl ester and n-hexadecanoic acid have been reported to possess anti-inflammatory, antioxidant, hypocholesterolemic, 5-alpha reductase inhibitor, nematicide, pesticide, antiandrogenic [23-25]. 9,12-Octadeconoic acid, methyl ester (linoleic acid methyl ester) also detected has been shown to possess remarkable anti-inflammatory, antihistamine and anti-arthritics properties. It also possesses hepatoprotective and hypcholesterolemic properties [26]. Octadecanoic acid, methyl ester is known to possess antimicrobial and anti-fungal properties [27]. 9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxymethyl) ethyl ester has been reported to inhibit the proliferative effect in keloid fibroblasts [28]. Oleic acid and cis-vaccenic acids are potent anti-inflammatory and antioxidant compounds [24,29]. Eicosane also described as arachidic acid is known for its cytotoxic effects especially as anticancer and antitumor agents [30]. The 2-Methoxy-4-vinylphenol detected is a phenolic derivative known to possess antidiabetic, antiandrogenic [23-25]. 9,12-Octadeconoic acid, methyl ester (linoleic acid methyl ester) also detected has been shown to possess remarkable anti-inflammatory, antihistamine and anti-arthritics properties. It also possesses hepatoprotective and hypcholesterolemic properties [26]. Octadecanoic acid, methyl ester is known to possess antimicrobial and anti-fungal properties [27]. 9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxymethyl) ethyl ester has been reported to inhibit the proliferative effect in keloid fibroblasts [28]. Oleic acid and cis-vaccenic acids are potent anti-inflammatory and antioxidant compounds [24,29]. Eicosane also described as arachidic acid is known for its cytotoxic effects especially as anticancer and antitumor agents [30]. The 2-Methoxy-4-vinylphenol detected is a phenolic derivative known to possess 5-alpha reductase inhibitor, nematicide, pesticide and antiandrogenic [23-25]. 9,12-Octadeconoic acid, methyl ester (linoleic acid methyl ester) also detected has been shown to possess remarkable anti-inflammatory, antihistamine and anti-arthritics properties. It also possesses hepatoprotective and hypcholesterolemic properties [26]. Octadecanoic acid, methyl ester is known to possess antimicrobial and anti-fungal properties [27]. 9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxymethyl) ethyl ester has been reported to inhibit the proliferative effect in keloid fibroblasts [28]. Oleic acid and cis-vaccenic acids are potent anti-inflammatory and antioxidant compounds [24,29]. Eicosane also described as arachidic acid is known for its cytotoxic effects especially as anticancer and antitumor agents [30]. The 2-Methoxy-4-vinylphenol detected is a phenolic derivative known to possess

### Table 2: GC/MS analysis of n-butanol fraction of *Costus afer* leaves

| Peak no. | Retention time (min) | Library ID | Percent of total | Bioactivity |
|----------|----------------------|------------|-----------------|-------------|
| 10       | 6.011                | Indolizine (alkaloids) | 0.305 | Inhibitor of 5-lipoxygenase, anti-inflammatory, analgesic, anti-diabetic, antitumor |
| 11       | 6.182                | 2-methoxy-4-vinylphenol (phenolics) | 1.202 | Antimicrobial, anti-inflammatory, antioxidant, analgesic |
| 20       | 10.354               | 3-buten-2-one, 4- (4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- | 1.448 | Not reported |
| 29       | 12.723               | Hexadecanoic acid, methyl ester (palmitic acid methyl ester) | 7.176 | Anti-inflammatory, antioxidant, hypocholesterolemic, 5-alpha reductase inhibitor, nematicide, pesticide, antiandrogenic |
| 30       | 13.135               | Dibutyl phthalate (plasticizer) | 8.196 | Antimicrobial, anti-fouling |
| 31       | 13.175               | n-Hexadecanoic acid (palmitic acid) | 7.946 | Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematicide, pesticide, antiandrogenic |
| 32       | 13.684               | Methyl 10-methyl-hexadecanolate | 0.495 | Not reported |
| 33       | 14.336               | 9,12-octadecanoic acid, methyl ester (linoleic acid methyl ester) | 1.814 | Anti-inflammatory, hepatoprotective, hypocholesterolemic, anti-arthritis, antihistamine |
| 34       | 14.388               | 11-octadecenoic acid, methyl ester | 2.458 | Not reported |
| 35       | 14.514               | Phytol (diterpene) | 3.781 | Anti-inflammatory, rheumatoid arthritis, antimicrobial, anticancer |
| 36       | 14.617               | Octadecanoic acid, methyl ester | 2.439 | Antifungal, antimicrobial, antibacterial |
| 37       | 14.805               | Oleic acid | 7.756 | Anti-inflammatory, antioxidant |
| 44       | 17.575               | Thiocyanic acid, 2,4-dinitrophenyl ester | 0.258 | Pesticide |
| 49       | 18.485               | Eicosane (arachidic acid) | 0.276 | Antifungal, antibacterial, antitumor and cytotoxic effects |
| 51       | 19.234               | Cis-vaccenic acid | 1.127 | Anti-inflammatory, antioxidant |

### Table 3: GC/MS analysis of n-butanol fraction of *Costus afer* stem

| Peak no. | Retention time (min) | Library ID | Percent of total | Bioactivity |
|----------|----------------------|------------|-----------------|-------------|
| 8        | 5.227                | Benzofuran, 2,3-dihydro (coumaran) | 4.969 | Anti-inflammatory, anti-helminthics, anti-diarrheal |
| 10       | 6.200                | 2-methoxy-4-vinylphenol (phenol) | 1.642 | Antimicrobial, anti-inflammatory, antioxidant, analgesic |
| 27       | 12.717               | Hexadecanoic acid, methyl ester (palmitic acid methyl ester) | 0.482 | Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematicide, pesticide, anti-androgenic |
| 29       | 13.152               | n-Hexadecanoic acid (palmitic acid) | 12.946 | Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematicide, pesticide, antiandrogenic |
| 36       | 14.806               | Cis-Vaccenic acid (omega 7 fatty acid) | 12.285 | Anti-inflammatory, antioxidant |
| 37       | 14.989               | Trans-13-octadecenoic acid | 2.360 | Not reported |
| 38       | 15.149               | Oleic acid (omega 9 fatty acid) | 0.486 | Anti-inflammatory, antioxidant |
| 42       | 17.140               | Thiocyanic acid, 2,4-dinitrophenyl ester | 0.258 | Pesticide |
| 44       | 17.569               | 2-Methyl-Z, Z-3, 13 octadecadienol (terpenoid) | 6.584 | Pesticide, herbicide, insecticide, pheromone |
| 49       | 19.252               | 9-Octadecanoic acid (Z), 2-hydroxy-1-(hydroxymethyl) ethyl ester | 1.904 | Inhibition of proliferative effect in keloid fibroblasts |
| 51       | 19.835               | 17-Pentatriacontene | 2.493 | Not reported |
| 52       | 19.944               | Tricosane | 1.012 | Not reported |
| 53       | 20.190               | Campesterol (ergost-5-En-3-Ol) Steroid | 0.379 | Anticancer, anti-inflammatory, hypocholesterolemic, antioxidant |
| 54       | 20.888               | Stigmasterol (24-Ethylcholesta-5,22-dien-3-ol) steroid | 1.645 | Stimulates proliferation of T lymphocytes, anticancer, antihepatotoxic, antioxidant, estrogenic, sedative |

GC/MS: Gas chromatographic/mass spectrometric
The therapeutic uses of chemical compounds of medicinal value. These identified stress related diseases. Furthermore, management of several chronic inflammatory and/or oxidative further in order to obtain specific drug components.

CONCLUSION

The n-butanol fractions of C. afer leaves and stem contain chemical compounds of medicinal value. These identified bioactive compounds may account for the prophylactic or therapeutic uses of C. afer leaves and stem extracts in the management of several chronic inflammatory and/or oxidative stress related diseases. Furthermore, n-butanol fraction of C. afer could serve as a source for herbal formulation or purified further in order to obtain specific drug components.

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