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# Chemical constituents in *n*-butanol fractions of *Costus afer* Ker Gawl leaf and stem

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## ABSTRACT

**Aim:** This study was designed to investigate the bioactive compounds in *Costus afer* Ker Gawl, an indigenous African medicinal plant whose leaf and stem extracts are used in the treatment of chronic inflammatory diseases, especially rheumatism and arthritis. **Materials and Methods:** The bioactive compounds present in the *n*-butanol fractions of *C. afer* leaf and stem were identified using qualitative phytochemical evaluation and gas chromatography-mass spectrometry (GC/MS) analytical method, comparing the mass spectra of the identified compounds with those of the National Institute of Standards and Technology database library. **Results:** Qualitative analysis detected alkaloids, saponins, diterpenes, triterpenes, phytosterol, phlobatannins, and tannins in both *n*-butanol fractions of *C. afer* leaf and stem. Phenols were detected in leaves alone while flavonoids were present in stem alone. GC/MS data showed that the bioactive compounds in *n*-butanol fraction of *C. afer* leaf were indolizine, 2-methoxy-4 vinylphenol, phytol, hexadecanoic acid-methyl ester, *n*-hexadecanoic acid, 9,12-octadecanoic acid-methyl ester, eicosane, cis-vaccenic acid and oleic acid while *n*-butanol fraction of *C. afer* stem contain benzofuran, 2,3-dihydro, 2-methoxy-4 vinylphenol, 9-octadecenoic acid (*Z*)-2-hydroxy-1-(hydroxymethyl) ethyl ester, campesterol, stigmasterol, hexadecanoic acid-methyl ester, *n*-hexadecanoic acid, and cis-vaccenic acid. **Conclusion:** The bioactive compounds identified in the *n*-butanol fractions of *C. afer* leaves and stem may explain the folkloric use of *C. afer* plant in the treatment of chronic inflammatory and oxidative stress related diseases.

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## 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, “Mbrirem” in Efik and anglophone Cameroon calls it “Monkey sugar cane” [3].

*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 saponinins 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- $\alpha$ -L-rhamnopyranoside [10]. Sesquilandulyl acetate,  $\beta$ -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 Ikenne 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 Trease 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, was 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 triterpenes.

### 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 leave 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 3.2652psi, mode type split less, 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/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

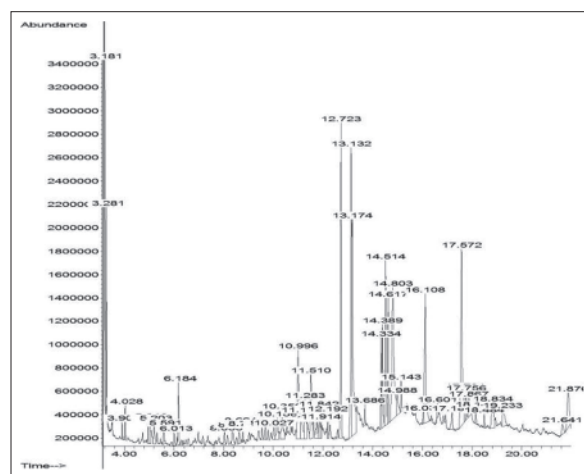
The phytochemical analysis revealed the presence of alkaloids, saponins, diterpenes, triterpenes, phytosterol, phlobatannins, and tannins in *n*-butanol fractions of *C. afer* leaf and stem. Phenols were detected in the *n*-butanol fraction of the leaves while flavonoids were present in the *n*-butanol 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-1-yl)-(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 (2.458%), phytol (3.781%), octadecanoic acid, methyl ester (2.439%), 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* stem were fourteen, and they are benzofuran,2,3-dihydro (4.969%), 2-methoxy-4-vinylphenol (1.642%), hexadecanoic acid, methyl ester (0.482%), *n*-hexadecanoic acid (12.946%), cis-vaccenic acid (12.285%), trans-13-octadecanoic acid (2.360%), thiocyanic acid 2,4-dinitrophenyl ester (0.258%), 2-methyl-Z,Z-3,13 octadecadienol (6.584%), 9-octadecenoic acid (Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester (1.904%), 17-pentatriacontene (2.493%), 17-pentatriacontene (2.493%), tricosane (1.012%), campesterol (0.379%) and stigmasterol (1.645%) [Table 3].

## 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, antidiarrheal 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 for their cardiotoxic activities, insecticidal and antimicrobial properties [20]. Cardiac glycosides in the *n*-butanol fractions are known to inhibit the Na<sup>+</sup>/K<sup>+</sup> 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,



**Figure 1:** Gas chromatography-mass spectrometric spectrum of *n*-butanol fraction of *Costus afer* leaves

**Table 1: Qualitative phytochemical evaluation of fractions of *Costus afer* leaf and stem**

Chemical constituents	Chemical test	Butanol leaf fraction	Butanol 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	Salkowski's test	-	+
Phytosterols	Lieberman Buchard's test	+	+
Fixed oil	Stain test	-	-
Resins	Acetone-water test	-	-
Phenols	Ferric chloride tests	+	-
	Alkaline test	-	+
Flavonoid	Lead acetate test	-	+
	Shinoda test	-	+
	Anthraquinone test	-	-
Anthraquinone	Anthraquinone test	-	-
Phlobatannins	Phlobatannins test	+	+
Tannins	Tannin test	+	+
Diterpernes	Copper acetate test	+	+
	Tshugajen's test	+	+

+: Indicates presence, -: Indicates absence

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

Peak no.	Retention time	Library ID	Percent of total	Bioactivity
10	6.011	Indolizine (alkaloids)	0.305	Inhibitor of 5-lipoxygenase, anti-inflammatory, analgesic, antidiabetic, antitumor
11	6.182	2-methoxy-4vinylphenol (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-1yl)-	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, nematocide, pesticide, antiandrogenic
30	13.135	Dibutyl phthalate (plasticizer)	8.196	Antimicrobial, antifouling
31	13.175	<i>n</i> -Hexadecanoic acid (palmitic acid)	7.946	Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematocide, pesticide, antiandrogenic
32	13.684	Methyl 10-methyl-hexadecanoate	0.495	Not reported
33	14.336	9,12-octadecanoic acid, methyl ester (linoleic acid methyl ester)	1.814	Anti-inflammatory, hepatoprotective, hypocholesterolemic, anti-arthritic, 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	9-octadecenal, (Z)	8.226	Not reported
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

GC/MS: Gas chromatographic/mass spectrometric

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

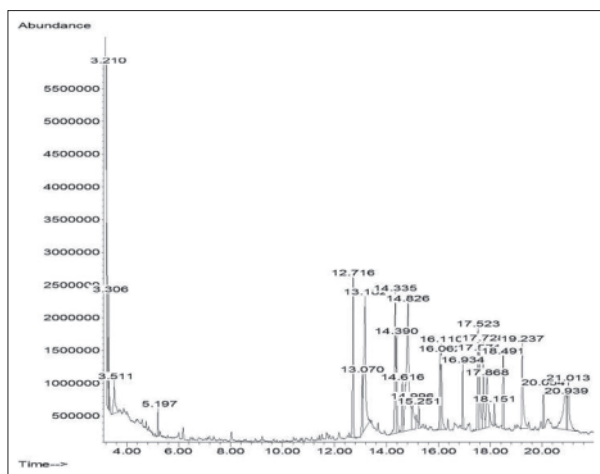
Peak no.	Retention time	Library ID	Percent of total	Bioactivity
8	5.227	Benzofuran, 2,3-dihydro (coumaran)	4.969	Anti-inflammatory, anti-helminthics, antidiarrheal
10	6.200	2-methoxy-4vinylphenol (phenol)	1.642	Antimicrobial, anti-inflammatory, antioxidant, analgesic
27	12.717	Hexadecanoic acid, methyl ester (palmitic acid methyl ester)	0.482	Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematocide, pesticide, anti-androgenic
29	13.152	<i>n</i> -Hexadecanoic acid (palmitic acid)	12.946	Anti-inflammatory, antioxidant, hypocholesterolemic, flavor, nematocide, 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-Octadecenoic 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

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, nematocide, pesticide and antiandrogenic [23-25]. 9,12-Octadecanoic 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 hypocholesterolemic properties [26]. Octadecanoic acid, methyl ester is known to possess antimicrobial and antifungal 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 antimicrobial and antitumor agents [30]. The 2-Methoxy-4 vinylphenol detected is a phenolic derivative known to possess



**Figure 2:** Gas chromatography-mass spectrometric spectrum of *n*-butanol fraction of *Costus afer* stem

antioxidant, analgesic, anti-inflammatory and antimicrobial properties [24]. Thiocyanic acid, 2,4-dinitrophenyl ester has active pesticidal activity [24]. Phytol detected only in *n*-butanol fraction of *C. afer* leaves is one of the most important diterpenes and it has been shown to possess remarkable anti-inflammatory, anti-arthritic, anticancer and antimicrobial activities [24,31]. 2-Methyl-Z,Z-3, 13 octadecadienol also a terpenoids compound detected only in *n*-butanol fraction of *C. afer* stem is known to possess pesticidal, herbicidal and insecticidal activities [24]. Indolizine found only in *n*-butanol fraction of *C. afer* leaves is an organic compound containing two condensed rings (5- and 6-membered) and a nitrogen atom junction [32]. Indolizine possesses biological activities such as anti-inflammatory [33], hypoglycemic activities [34], 5HT<sub>3</sub> receptor antagonist [35], anti-acetylcholine [36], central nervous system depressant activity [37], estrogen receptor binding [38] antioxidant property [39], antimicrobial and analgesic activity [40], many amino acid derivatives with an active indolizine nucleus have been utilized in cancer therapy [41]. Benzofuran, 2,3-dihydro is a coumaran and research has shown that it possesses anti-inflammatory, antidiarrheal and anti-helminthic activities [25]. Campesterol (ergost-5-en-3-ol) and stigmaterol (24-ethylcholesta-5,22-dien-3-ol) were steroids found only in the *n*-butanol fraction of *C. afer* stem and several studies have shown that they possess anti-inflammatory, antioxidant, anticancer, hypocholesterolemic, hepatoprotective, stimulation of T lymphocytes proliferation, estrogenic, and sedative properties [23,742-44].

## 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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