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# Gas chromatography-mass spectrometry and high performance liquid chromatography analyses of *Costus afer* stem ethyl acetate fraction

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## Graphical Abstract

**EXTRACTION AND FRACTIONATION**

**GCMS**

**1,6 Octadien-3-ol, 3,7-dimethyl-cyclohexene; Estragole; Caryophyllene; 1,3,6,10-Dodecatetraene; Humulene**

**HPLC**

**Chlorogenic acid; Catechin; Rutin hydrate; Caffeic acid; Quercetin; Ellagitannin; Tannic acid; Gallotannin. p-coumaric acid; Ferulic acid; Gallic acid; Apigenin**

# Abstract

*Costus afer* is a medicinal plant commonly found in Africa that is known for its many medicinal properties. In this study, the bioactive compounds present in the ethylacetate fraction of the stem extract were identified using Gas Chromatography Mass Spectroscopy (GCMS) and High-Performance Liquid Chromatography (HPLC). The GCMS revealed that the ethylacetate fraction contains 1,6 Octadien-3-ol, 3,7-dimethyl-cyclohexene, estragole, caryophyllene, 1,3,6,10-Dodecatetraene and humulene while the HPLC identified chlorogenic acid, catechin, rutin hydrate, caffeic acid and quercetin as well as ellagitannin, tannic acid and gallotannin. Also identified were p-coumaric acid, rutin, ferulic acid, gallic acid, apigenin and ascorbic acid. We conclude that *Costus afer* stem contains a number of bioactive compounds that may have important medicinal properties.



**Keywords:** *Costus afer;* Flavonoids; GCMS screening; HPLC analysis; Tannins; Phytochemicals; Polyphenols

# Introduction

- Gas chromatography-mass spectroscopy (GC-MS) is an important technique employed in the determination of volatile and semi-volatile organic compounds in a wide variety of samples (Razack et al. 2015).

- Alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters, and amino acids can all be detected using GC–MS, which is one of the best, rapid, and accurate procedures available.

- The GC-MS has applications in plant, pharmacological, and medical metabolomic studies, and is regarded as one of the most suitable techniques for the accurate determination of primary metabolites, however, it is sometimes, compromised in determining some secondary metabolites (Salem et al. 202).

- High-performance liquid chromatography is an important type of column chromatography commonly employed to separate, identify, and quantify bioactive compounds (Martin and Guiochon, 2005).

- Basically, HPLC has a column that contains the packing material (stationary phase); a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules.

- Normally, the retention time of a compound depends on the interactions between the stationary phase, the molecules being analysed, and the solvent(s) used (Liu and Lee, 2006).

- *Costus afer* is a medicinal plant commonly found in Africa. Some of its reported medicinal properties include cardioprotective, antioxidant, antimalaria, anti-inflammatory and analgesic effects (Boligon et al. 2014).

- In addition, an infusion of the rhizome is used in the treatment of stomach ache while the stem decoction is used in the treatment of cough, sore throat as well as other respiratory diseases (Burkill, 2000).

- Also, the leaf sap is used as eye while the stem sap is used to treat urethral discharges, venereal diseases, jaundice and to prevent miscarriage (Burkill, 2000).

- In addition, the stem decoction has been reported to be effective in the treatment of rheumatoid arthritis.

# Results and discussion

## Table 1. Phytochemical Constituents of *Costus afer* Stem

| S/N | Phytoconstituents | Relative Abundance |
|---|---|---|
| 1 | Phenolics | +++ |
| 2 | Flavonoids | ++ |
| 3 | Tannins | + |
| 4 | Terpenoids | + |
| 5 | Alkaloids | + |
| 5 | Glycosides | + |
| 7 | saponins | + |

- The preliminary phytochemical screening of ethylacetate fraction of *Costus afer* stem extract showed the presence of phytochemicals such as flavonoids, tannins, phenolics, saponins, glycosides, alkaloids and terpenoids.

- Some of these identified phychemicals have been reported to possess antioxidant, cardioprotective effects and other pharmacological properties.

- Flavonoid-rich vegetables are widely used functional foods since they can be used to treat cardiovascular diseases (Njoku et al. 2017).

- Saponins exert anti-inflammatory, antiallergic, cytotoxic, antidiabetic as well as cardioprotective effects [17]. In addition, terpenoids and alkaloid compounds have also been reported to have potent activity against gastric ulcers (Abdelhak and Soraya, 2018).

- Terpenes have been reported to relax cardiovascular smooth muscle by inhibition of $Ca^{2+}$ influx in vascular smooth muscle or via quenching of reactive oxygen species (ROS) and stimulation of nitric oxide (NO) synthesis (Alves-Silva et al. 2016).

# Chromatogram for the GC-MS analysis of *Costus afer*

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File        :C:\msdchem\1\TOTAL SCAN\ Pharm. Marthins\UNN.D
Operator    : DR EMEKA A
Acquired    : 13 Jun 2019  18:12              using AcqMethod Total _SCAN.M
Instrument :   CRL ILORIN
Sample Marthin
Misc Info   :
Vial Number: 9
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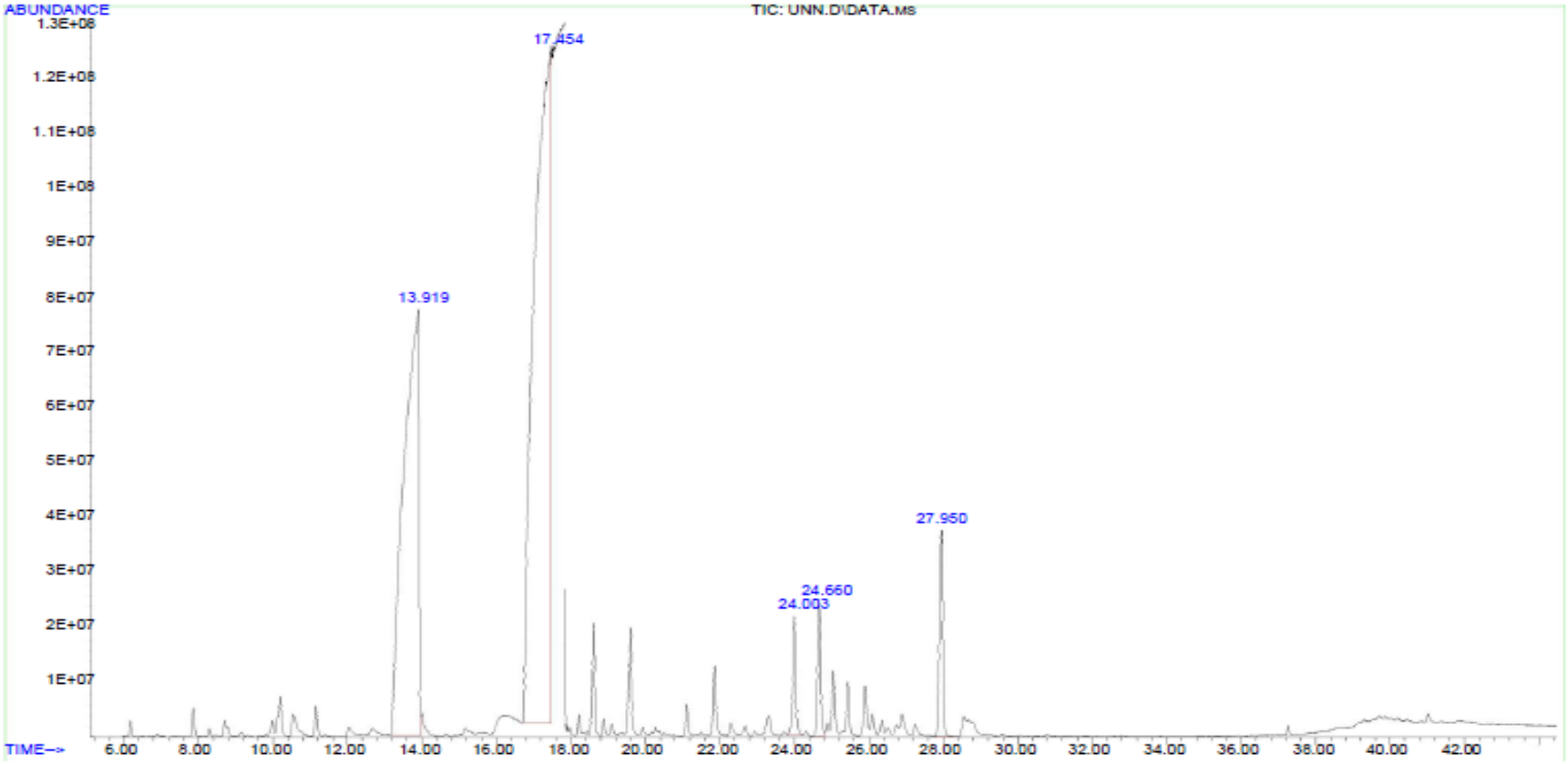

# Bioactive Compounds Identified in Ethylacetate Fraction of *Costus afer* using GC-MS

| S/N | RT (mins) | NAME OF COMPOUND | MOLECULAR FORMULAR | M.WT g/mol | PEAK AREA (%) |
|---|---|---|---|---|---|
| 1 | 13.92 | 1,6 Octadien-3-ol, 3,7-dimethyl-cyclohexane | $C_{10}H_{18}O$ | 154.25 | 44.84 |
| 2 | 17.45 | Estragole | $C_{10}H_{12}O$ | 148.20 | 44.84 |
| 3 | 24.00 | Caryophyllene Bicyclo [7.2.0] undec-4-ene, 4,11,11, -trimethyl-8-methelene, [1R*,4Z,9S*)] | $C_{15}H_{24}$ | 204.35 | 2.62 |
| 4 | 24.66 | 1,3,6,10-Dodecatetraene 3,7,11-trimethyl-, (Z, E)- | $C_{15}H_{26}$ | 206.37 | 2.91 |
| 5 | 27.95 | Humulene | $C_{15}H_{24}$ | 204.35 | 4.78 |

- 1,6 Octadien-3-ol. 3,7-dimethyl-cyclohexane possesses several pharmacological properties such as antiinflammation, antioxidant and anticarcinogenic effects (Al-Marzoqi et al. 2015).
- Estragole has many biological effects including antioxidant and antimicrobial activities as well as induce contraction of skeletal muscle and relaxation of ileal and other vascular smooth muscle (Silva-Alves et al. 2013).
- Caryophyllene has been shown to exert several biological activities including anti-inflammatory, antioxidant, and as well as ability to modulate the activity of natural killer cells (Kumar et al. 2021).
- Alpha humulene exhibits marked anti-inflammatory properties in a murine model of airways allergic inflammation via reduction of inflammatory mediators, adhesion molecule and transcription factors activation (Rogerio et al. 2009).

# Flavonoids present in the ethylacetate fraction of *Costus afer* stem extract

| Peak No. | Peak ID | Ret Time | Height | Area | Conc. µg/100mg |
|---|---|---|---|---|---|
| 1 | Chlorogenic acid | 7.540 | 87519.945 | 822502.813 | 60.5252 |
| 2 | Catechin | 8.632 | 95571.477 | 2173638.500 | 114.2422 |
| 3 | Rutin hydrate | 10.498 | 8064.095 | 101499.523 | 12.5329 |
| 4 | Caffeic acid | 14.998 | 8422.607 | 290748.656 | 17.2555 |
| 5 | Quercetin | 18.815 | 17574.744 | 596835.938 | 36.8938 |
| 6 | Unidentified | 0.065 | 1108.444 | 22058.301 | 0.5505 |

- Chlorogenic acid is an essential bioactive dietary polyphenol that has pharmacological properties such as antioxidant, hepatoprotective, cardioprotective, anti-inflammatory, neuroprotective, antiviral,anti-hypertension, and central nervous system stimulating effect (Naveed et al. 2018).

- Catechin has been reported to exert antidiabetic, antihyperlipidemic, anticoagulant and antiplatelet as well as cardioprotective effects (Babu et al. 2008).

- Rutin possesses antibacterial, anti-inflammatory, cytoprotective, vasoactive, hypolipidemic, antiplatelet, antispasmodic as well as cardioprotective effects (Yanga et al. 2008).

- Caffeic acid possesses antioxidant and anticarcinogenic effects while quercetin is an important polyphenolic flavonoid found in various food products that has been reported to possess numerous pharmacological activities, such as anticancer, antiviral, antiprotozoal, and antimicrobial effects.

# Tannins present in the ethylacetate fraction of *Costus afer* stem extract

| Peak No. | Peak ID | Ret Time | Height | Area | Conc. µg/100mg |
|---|---|---|---|---|---|
| 1 | Ellagitannin | 1.182 | 856.684 | 6116.454 | 0.7662 |
| 2 | Tannic acid | 1.865 | 21753.490 | 456793.094 | 57.2252 |
| 3 | Gallotannin | 2.465 | 8510.612 | 278634.625 | 34.9062 |
| 4 | Unidentified | 0.140 | 270.804 | 1467.920 | 0.1839 |
| 5 | Unidentified | 0.273 | 237.062 | 1884.030 | 0.2360 |
| 6 | Unidentified | 1.707 | 4138.291 | 53341.492 | 6.6824 |

- Ellagitannin contains one or more hexahydroxydiphenoyl (HHDP) unit(s) on a glucopyranose core. It has pharmacological properties such as anti-inflammatory, anticancer, antioxidant and antimicrobial activities (Yoshida et al. 2010).

- Tannic acid possesses anti-lipogenic, anticarcinogenic, antiinflammatory, antioxidant, and free radicals scavenging activities (Donglai et al. 2020).

- Gallotannins have been reported to exert antioxidant, anti-inflammatory, immunomodulatory and anticarcinogenic activities (Kiss and Piwoarski, 2016).

- The anti-inflammatory effects of gallotannin involves the partial or complete inhibition of expression of inflammatory cytokines, chemokines, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Al-Halabi et al. 2011).

- Gallotannin known to inhibit the proliferation of different cancer cells such as colorectal carcinoma, prostate cancer as well as acute myeloid leukaemia, without showing any adverse effects to the normal cells (Al-Halabi et al. 2011).

# Phenolics present in the ethylacetate fraction of *Costus afer* stem extract

| Peak No. | Peak ID | Ret Time | Height | Area | Conc. µg/100mg |
|---|---|---|---|---|---|
| 1 | p-coumaric acid | 7.548 | 4252.901 | 21508.475 | 9.3104 |
| 2 | Rutin | 8.632 | 2639.610 | 16884.359 | 7.3088 |
| 3 | Ferrulic acid | 9.840 | 1250.384 | 11539.640 | 4.9952 |
| 4 | Gallic acid | 10.182 | 2025.692 | 25039.217 | 10.8388 |
| 5 | Apigenin | 13.657 | 172.649 | 1325.500 | 0.5738 |
| 6 | Ascorbic acid | 5.323 | 26847.186 | 136650.109 | 59.1522 |
| 7 | Unidentified | 15.123 | 755.820 | 18067.199 | 7.8208 |

- p-coumaric acid is an antioxidant and a scavenger of reactive oxygen species (ROS) and other free radicals.

- Rutin has some pharmacological properties such as antioxidant, cytoprotective, vasoprotective, anticarcinogenic, analgesic, anti-inflammatory, antidiabetic, neuroprotective and cardioprotective activities (Ganeshpurkar and Saluja, 2017).

- Ferulic acid is characterized by its low toxicity as well as other pharmacological properties such anti-inflammatory, antimicrobial, anti-arrhythmic, and antithrombotic activity (Chen et al. 2002).

- Gallic acid is known to exert antioxidant, anti-inflammatory, and antineoplastic actvities. It is also effective against gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders (Kahkeshani et al. 2019).

- Apigenin activates several anti-inflammatory pathways such as p38/MAPK and PI3K/Akt, and inhibits the IKB degradation and nuclear translocation of the NF-κB, and thereby inhibiting cyclooxygenase-2 activity (Salehi et al. 2019).

## Conclusions

- This present study has demonstrated that *Costus afer* stem contains some very important secondary metabolites.

- The presence of these bioactive compounds may be responsible for the several pharmacological properties such as antioxidant, anti-inflammatory, cardioprotective, antimicrobial, anticarcinogenic, as earlier reported.

- The purification of the bioactive compounds and the continued use of the plant in folk medicine is highly recommended

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