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# Quantitative phytochemicals and essential oil constituents of *costus lucanusianus* stem

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## Abstract

The use of medical plants as raw materials in the production of new drugs is ever increasing because of their potentials in combating the problem of drug resistance in microorganisms. The study was aimed at determining quantitative phytochemicals and essential oil constituents of *costus lucanusianus* stem (spiral ginger). Quantitative phytochemicals and essential oil constituents were determined using high performance liquid chromatography (HPLC) and Gas chromatography mass spectrometry (GC- MS) respectively. Result revealed in decreasing order total saponins (837.38mg/100g) with predominant diosgenin (533.83mg/100g), total tannins (485mg/100g), total flavonoids (261.31mg/100g) with myricetin (138.98mg/100g) as highest, glycosides (51.57mg/100g) with costusoside (25.99mg/100g) as highest, total carotenoids (44.20mg/100g) with carotene (15mg/100g) as highest and lycopene the lowest (3.39×10-6mg/100g) total phytosterol (26.97mg/100g) with sitosterol (18.12mg/100g) as highest phenolics (22.46mg/100g) ferulic (5.00mg/100g) was highest, total isoflavone was (16.96mg/100g), with daidzein (5.39mg/100g) being highest others includes anthocyanins (7.73mg/100g), anthroquinone (1.90mg/100g), total terpinoids (0.91mg/100g), total lignin (0.36mg/100g) and total alkaloids was (0.19mg/100g). Essential oil constituents included cis-carvone oxide (3.17g/100mg), Trans geranyl acetate (2.17g/100mg), linanyl acetate (1.26g/100mg), β-mycrene (1.83g/100mg), ɒ-pinene (1.03g/100mg) and carvone and p-acetyl toluene were the least (0.01g/100mg). *costus lucanusianus* stem is rich in phytochemicals hence could serve as herbal agents in pharmaceutical and cosmetic industries.

**Keywords:** Phytochemicals; *Costus lucanusianus* stem; Essential oil

## 1. Introduction

Herbal medicine is the use of plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems or in a more conventional medical sense to treat, cure, or prevent disease in animals or humans [1]. In recent years, herbal prescriptions have received considerable attention as an alternative way to compensate for perceived deficiencies in orthodox pharmacotherapy worldwide [2]. Despite a lack of medical evidence to support their therapeutic efficacy and toxicological effects, the use of herbal medicine has increased considerably [2]. According to World Health Organization (WHO), up to 80% of the world's population in underdeveloped and developing countries relies on traditional medicine practices for their primary health care needs [3]. This is why, traditional medicines have been accorded greater acceptance in Africa because of the unavailability, unwanted side effects and high costs associated with orthodox medicines, inadequate health facilities and healthcare professionals, coupled with inadequate training of health workers [4]. According to [5], medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The therapeutic effects of these medicinal plants can justifiably be attributed to among others, the phytochemicals in them especially the flavonoids,

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alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins and saponins. Phytochemicals (from the Greek word phyto ,meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [6]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds.

Recently, the demand for the development of natural products from medicinal and aromatic plants as substitutes for artificial additives and as pharmacologically active agents has increased significantly [7]. Essentials oils are oily substances produced by different parts of the plants, including flowers, buds, leaves, twigs, stems, seeds, and fruits [8]. Generally, these oils are comprised of complex mixtures of volatile substances that are biosynthesized by plants. These substances can be broadly classified into several groups, such as aromatic and aliphatic compounds, terpenes, and terpenoids [9]. Among the different natural products, essential oils have gained immense popularity in various industries, including the food, cosmetics, and pharmaceutical industries, because of their remarkable characteristics such as, strong odor, unique colors, and high volatility [10, 11].

The plant *Costus lucanusianus* (Costaceae) is among 150 species of stout, perennial and rhizomatous herbs of the genus *Costus* [12]. It can be found in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone, Cameroun, Gabon, Fernando Po and the southern part of Nigeria [12, 13]. *Costus lucanusianus is* commonly called monkey sugarcane, in the Niger Delta region of Nigeria but known as spiral ginger in other parts of the world. *Costus lucanusianus* is medicinal plant commonly used treat tachycardia and stomach complaints, warmed stem sap or the pounded fruit are taken to treat cough, bronchitis and a sore throat; the stem is also mashed or chewed to treat cough. Leaf sap is acidic and is used as eye drops to treat eye troubles and headache with vertigo, and in frictions to treat oedema and fever. Leaf sap is used as nose drops and leaf pulp is rubbed on the head to calm insanity. Stem sap is applied to treat urethral discharges, venereal diseases and jaundice, and to prevent miscarriage. Stem sap is rubefacient and burns on open wounds, but it is also anodyne and healing, and is applied to mumps and measles. Rhizome pulp is applied to abscesses and ulcers to mature them, and mixed with water it is taken to treat diarrhoea. A stem decoction is widely taken to treat rheumatism. The pulped stems taken in water are strongly diuretic. In Gabon stem sap is used as eye drops to control filariasis. In Gabon young shoots are cooked and eaten as a substitute for those of *Hibiscus sabdariffa L.*; they have a slightly acid taste. Stem sap is used to coagulate latex. *Costus lucanusianus* is commonly used for ceremonial and religious purposes. It is sold in Western countries as an ornamental container plant. In Nigeria, most spiritual homes will always request for the juice from the stem which is usually very expensive to purchase and used for the prevention of certain diseases and possesses such properties as antioxidants, hormonal actions, stimulation of enzymes, interference with DNA replication and physical action [14]. It is commonly used for diarrhea, pyrexia, pain, inflammatory conditions dysmenorrhea [15].

## 2. Material and methods

### 2.1. Sample collection and identification

Matured stem of *Costus lucanusianus* was harvested from a pit at the back of Anatomy and physiology Department, University of Port Harcourt, and was identified and authenticated by Dr. Ekeke chimezie of the Department of Plant science and Biotechnology (PSB), University of Port Harcourt, with herbarium number UPH/V/1292 and specimen deposited at herbarium.

### 2.2. Sample preparation



**Figure 1** *Costus lucanusianus* plant stem

*Costus lucanusianus* stem was detached from the plant, cut into pieces and oven dried in an oven at 50°C for 24 hours. The dried samples were crushed into powder with an electric grinder to obtain the coarse powder and kept in an air tight container till further use.

### 2.3. Phytochemicals screening.

Quantitative phytochemicals constituent of *costus lucanusianus* stem were determined by high performance liquid chromatography. The phytochemicals determined were alkaloids, saponins, tanins, phenolics, glycosides, flavonoids, terpenoids, lignin, carotenoids, isoflavones, anthocyanins phytosterols and anthraquinones.

#### *2.3.1. Determination of Flavonoids*

Exactly 1.0g of the plant sample was weighed into a 250ml conical flask capacity with the addition of 100ml of de-ionized water; the mixture was boiled for 10 minutes. 100ml of mixture of the boiling methanol and water were added to the sample in the conical flask in a ratio of 70:30. The mixture was allowed to macerate for 4 hours on the laboratory bench. The whole solution was filtered through Whatman filter paper. The standards of different concentrations were prepared for injection into the HPLC system for calibration and correlation coefficient establishment. The samples were injected into the HPLC system following the same procedure as standard mixtures.

#### *2.3.2. Determination of Alkaloids*

A weight of 5.0g of the sample was put into 250ml beaker and 25ml of hexane was added and allowed to stand for 72 hours. The extract was filtered and the residue was air dried and treated with 10% aqueous $NH_3$ and macerated in $CHCl_3$ for 24 hours at reduced pressure. The whole solution was filtered and evaporated. The resultant crude extract was treated with 5% aqueous HCl of 7.5ml. The aqueous phase was made alkaline with aqueous $NH_3$ and extracted thrice with $CHCl_3$ that was washed with water. The extract was poured into a round bottom flask of the rotatory evaporator arrangement. It was separated by driving the solvent off the extract. The concentrated extract was dried of water by using an anhydrous sodium sulphate before gas chromatography analysis.

#### *2.3.3. Determination of Saponins*

The sample was pulverized and saponin was extracted three times with redistilled methanol. The saponin was removed with 20ml of the solvent for 20 minutes with the aid of the sonication. The combined extracts were concentrated to syrup under reduced pressure and were then suspended in water. The suspension was extracted with petroleum ether, chloroform and 1-butanol saturated with water to give the respective extract after removal of the solvent. The combined extract was filtered and concentrated to 1ml in a vial for a gas chromatography analysis and 1µl was injected into the injection pot of gas chromatography.

#### *2.3.4. Determination of Glycoside*

Sample of 1.0g weight was extracted by soaking for 2hours with 10ml of 70% alcohol and filtered. Redistilled hexane was used to replace the initial solvent and the hexane was concentrated to 1ml in the vial for gas chromatography analysis. 1µl was injected into the injection port of gas chromatography.

#### *2.3.5. Determination of Lignan*

A sample of 1.0g weight was pulverized and the organic constituent was extracted with methanol over night by stirring. The lignan was removed by suction filtration and the filtrate shaken overnight with hexane/dichloromethane in the ratio of (60:40). The aqueous layer was removed in separator funnel; the organic solvent was washed with saturated sodium chloride, dried over sodium sulphate and the solvent was evaporated using rotary evaporator to obtain viscous dark oil. The sample of the oil was dissolved in acetone and concentrated to 1ml in the vial for the gas chromatography analysis and 1µl was injected into the injection pot of gas chromatography analysis.

#### *2.3.6. Determination of Carotenoids*

Sample of 5.0g weight was homogenized, 75ml of acetone was kept in the dark at a room temperature for 1 hour and the homogenate was filtered through a filter paper by suction. Extraction was repeated thrice with the same volume of acetone, the extracts were combined and evaporated under reduced pressure, and the residue was extracted by a mixture of diethyl ether and petroleum ether in an equal ratio. The extract was poured into a round bottom flask of the rotator evaporator arrangement, it was concentrated by evaporation, and the concentrated extract was dried of water by using an anhydrous sodium sulphate before gas chromatography.

*2.3.7. Determination Phenolic Compound*

Two stage extraction procedures were followed for the effective removal of polyphenols.

- Stage 1: Sample of 50.0mg was extracted with 5ml of 1M NaOH for 16 hours on a shaker at an ambient temperature. After extraction, the sample was centrifuged, rinsed with water, and was centrifuged again. The supernatants were combined and placed in a disposable glass tube and was heated at 90°C for 2 hours to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4ml HCl to pH a less than 2.0 and was diluted to 10ml with a deionized water, it was centrifuged to remove the precipitate. The supernatant obtained was saved for subsequent purification and the residue was extracted further in stage 2.
- Stage 2: The residue from stage 1 was extracted with 5ml of 4M NaOH, heated to 160° C in Teflon. After cooling, the mixture was filtered, supernatant was collected and the residue was washed with water. Supernatant was combined and adjusted to pH less than 2.0 with 4MHCl. The filtrate was combined for further purification.

Purification of extracted phenolic acids: An aliquot (5-15ml) of the various supernatants was passed through a conditioned Varian Bond Elut PPL (3-mL size with 200-mg packing) solid-phase extraction tube at 5mL miñ$^{1}$ attached to a Visiprep. The tubes were placed under a vacuum (-60kPa) until the resins was thoroughly dried after which the Pas were eluted with 1 ML of ethyl acetate into gas chromatography auto sampler vials. The PPL tubes were conditioned by first passing 2mL water (pH<2.0).

*2.3.8. Determination of Phytosterols*

The sample of 5.00g was weighed and transferred to a Stoppard flask and treated with petroleum ether until the powder was fully soaked. The flask was shaken every hour for the first 6 hours and was kept aside and shook again after 24 hours. This process was repeated for three days and then the extract was filtered. The extract was collected and evaporated to dryness by using nitrogen stream.

Exactly 0.5g of the extract from the sample was added to the screw-capped test tube. The sample was saponified at 95° C for 30 minutes by using 3ml of 10% KOH in ethanol to which 0.20ml of benzene had been added to ensure miscibility. 3ml of de-ionized water was added and 2ml of hexane was used in extracting the non-saponifiable materials. Three extractions each with 2ml of hexane were carried out for 1 hour, 30 minutes and 39 minutes respectively to achieve complete extraction of the sterols

The hexane was concentrated to 2ml in an Agilent vial for gas chromatography analysis.

*2.3.9. Determination of Tannins*

A pulverised sample of 0.2g weight was measured into 5ml borosilicate beaker, 20ml of a 50% methanol was added and covered with paraffin and placed in a water bath at 80° C for 1 hour. The content was stirred with a glass rod to prevent lumping, and the extract was quantitatively filtered using a double layered Whatman filter paper into a 100ml volumetric flask using 50% methanol to rinse. This was concentrated to 2ml in the borosilicate vial for the gas chromatography analysis. 1.0 micro-litre was injected into the injection port of the gas chromatography

*2.3.10. Determination of Terpenoids*

The sample was pulverized and terpene constituents extracted with re-distilled chloroform. The terpene was removed with 10ml of the solvent for 15 minutes. The mixture was filtered and concentrated to 1ml in the vial for gas chromatography analysis and 1µl was injected into the injection port of gas chromatography.

**2.4. Essential Oil Analysis**

The bark and flower of the plant were peeled off leaving the stem which was then washed and air dried at room temperature and ground to a fine particle using an electric blender. The ground plant sample was introduced into the Soxhlet extractor to obtain a methanol extract of the sampled plant stem. For pure oil extraction, the reagents used were n-hexane. Essential oil constituent of *costus lucanusianus* was determined by Gas chromatography mass spectrophotometer.

*2.4.1. Procedure for GC Analysis*

The prepared samples (methanol extract and pure oil extract) were calibrated with various compositions, with the amount of Isopropyl alcohol fixed to equal 2ml in each sample. The hydrogen supply was switched on and all parameters

set as follows: oven, trap column detector (TCD) and Injector temperature = 170 $^0$C, 150 $^0$C and 200 $^0$C respectively. Carrier Gas Pressure was 4kg/cm$^2$. The isotherm was started, and the baseline of the recorder was perfectly horizontal before the sample was injected. This indicates that the GC is stabilized or conditioned properly. The sample was injected with a micro syringe at the injector port, the RUN was started as all the peaks were attained the run was stopped and the results were integrated.

### 2.5. Statistical Analysis

Results were analyzed using statistical package for social sciences (SPSS) version 15. All data were represented as mean ±standard deviation (M±SD) using descriptive statistics and analysis of variance (Anova) confidence level was fixed at $P \leq 0.05$.

## 3. Results and discussion

**Table 1** Quantitative characterization of alkaloids constituent in *costus lucanusianus* stem

| Alkaloids | Concentration mg/100g |
|---|---|
| Betaine | $1.51\times10^{-7}$ |
| Saussurine | $1.42\times10^{-1}$ |
| Vasicine | $1.37\times10^{-7}$ |
| Ergometrine | $8.60\times10^{-8}$ |
| Tylophorine | $4.51\times10^{-2}$ |
| Ergovaline | $4.98\times10^{-6}$ |
| Ergotamine | $1.40\times10^{-6}$ |
| Dihydroergotamine | $1.60\times10^{-6}$ |
| Vasicinone | $3.31\times10^{-7}$ |
| Total | $1.88\times10^{-1}$ |

**Table 2** Quantitative characterization of flavonoids constituent in *costus lucanusianus* stem

| Flavonoids | Concentration mg/100g |
|---|---|
| (+)- Catechin | $5.67\text{x}10^{-3}$ |
| (+)- Gallocatechin | $1.27\text{x}10^{-4}$ |
| Coumarin | $2.96\text{x}10^{-6}$ |
| Dihydroxycoumarin | $4.51\times10^{-5}$ |
| Apigenin | $2.46\times10^{-3}$ |
| Butein | $4.99\times10^{-5}$ |
| Naringenin | $6.15\times10^{-3}$ |
| Luteolin | $1.39\times10^{-4}$ |
| Kaemferol | 7.58 |
| (-)- Epicatechin | $4.16\times10^{-2}$ |
| Quercritrin | $4.28\times10^{-5}$ |
| (-)- Epigallocatechin | $1.51\times10^{-5}$ |
| Myricitrin | $3.14\times10^{-6}$ |

| | |
|---|---|
| Quercetin | 114.68 |
| (-)- Epicatechin-3-gallate | $4.64\times10^{-4}$ |
| Hyperin | $7.23\times10^{-5}$ |
| (-)- Epigallocatechin-3-gallate | $4.30\times10^{-6}$ |
| Isorhammetin | $1.00\times10^{-5}$ |
| Robinetin | $2.92\times10^{-4}$ |
| Abzelin | $5.16\times10^{-4}$ |
| Baicalein | $3.87\times10^{-5}$ |
| Isoquereitrin | $2.33\times10^{-5}$ |
| Myricetin | 138.98 |
| Baicalin | $3.67\times10^{-5}$ |
| Silymarin | $2.79\times10^{-7}$ |
| Total | 261.31 |

**Table 3** Quantitative characterizations of phenolics constituent of *costus lucanusianus* stem

| **Phenolics** | **Concentration mg/100g** |
|---|---|
| Salicyclic Acid | $6.85\times10^{-5}$ |
| Cinnamic Acid | 3.04 |
| Protocatechuic Acid | $3.12\times10^{-4}$ |
| p-Coumaric Acid | $1.63\times10^{-4}$ |
| Vanillic Acid | 4.67 |
| O-Coumaric acid | $6.79\times10^{-5}$ |
| p-hydro×ybenzoic Acid | $2.34\times10^{-5}$ |
| Gallic Acid | 2.51 |
| Ferulic Acid | 5.00 |
| Syringic Acid | 2.71 |
| Caffeic Acid | 4.50 |
| Piperic Acid | $8.92\times10^{-6}$ |
| Sinapinic Acid | $5.71\times10^{-4}$ |
| Ellagic acid | $1.030\times10^{-1}$ |
| Chlorogenic Acid | $2.31\times10^{-4}$ |
| Rosmarinic Acid | $2.26\times^{-4}$ |
| Total | 22.46 |

**Table 4** Quantitative determination of tannins constituent of *costus lucanusianus* stem

| Tannins | Concentration mg/100g |
|---|---|
| Tannic acid | 485.26 |
| Total | 485.26 |

**Table 5** Quantitative characterization of saponins constituent of *costus lucanusianus* stem

| Saponins | Concentration mg/100g |
|---|---|
| Hispogenin | $4.90x10^{-4}$ |
| Saponin B | $83x10^{-4}$ |
| Saponin C | $3.03x10^{-4}$ |
| Solagenin | $8.32x10^{-4}$ |
| Dioscin | 144.31 |
| Diosgenin | 533.83 |
| Justicisaponin-1 | $5.77x10^{-4}$ |
| Tigogenin | 46.31 |
| Neochlorogenin | $1.62x10^{-3}$ |
| Hecogenin | $6.97x10^{-4}$ |
| Sapogenin | 109.49 |
| Tribuloin | $4.09×10^{-4}$ |
| Yanogenin | $3.32×10^{-4}$ |
| Conyzorgin | 1.18×10-4 |
| Gracillin | 3.13 |
| Saponine | $2.89×10^{-1}$ |
| Total | 837.38 |

**Table 6** Quantitative characterizations of Terpenoids constituent of *costus lucanusianus* stem

| Terpenoids | Concentration mg/100g |
|---|---|
| Euphorbioside A | $1.24x10^{-7}$ |
| Euphorbioside B | $1.31x10^{-7}$ |
| Taraxerol | $1.34x10^{-7}$ |
| Taraxerone | $2.72x10^{-7}$ |
| Alpha-amyrin | $1.28x10^{-3}$ |
| Deglucosyleuphorbioside A | $6.073x10^{-8}$ |
| Clovandiol | $1.58x10^{-7}$ |
| Beta-amyrin | $9.11x10^{-1}$ |
| Lupeol | $4.73x10^{-6}$ |

| | |
|---|---|
| Euphaginol | $5.89x10^{-7}$ |
| Bauerenol acetate | $1.68x10^{-4}$ |
| Total | $9.13x10^{-1}$ |

**Table 7** Quantitative characterization of glycosides constituent of *costus lucanusianus* stem

| **Glycosides** | **Concentration mg/100g** |
|---|---|
| Afzelin | $4.14\times10^{-7}$ |
| Arbutin | $1.57x10^{-7}$ |
| Salicin | $2.04x10^{-6}$ |
| Allantoin | $4.17x10^{-6}$ |
| Amygdalin | $2.22x10^{-6}$ |
| Kaempferitrin | $4.16x10^{-6}$ |
| O-sitosterol-3-B-glycoside | $4.98x10^{-6}$ |
| Rutin | 1.64 |
| B-sitosterol glucoside | 13.92 |
| Costusoside I | 25.99 |
| Costusoside J | 10.00 |
| Kampferol 3-o- glucopyranoside | 4.35 |
| Total | 51.57 |

**Table 8** Quantitative characterization of Phytosterols constituent of *costus lucanusianus* stem

| **Phytosterols** | **Concentration mg/100g** |
|---|---|
| Cholesterol | $2.65\times10^{-9}$ |
| Cholestanol | $2.26\times10^{-3}$ |
| Tinyaloxin | $5.71x10^{-9}$ |
| Daucosterol | $5.26x10^{-9}$ |
| Ergosterol | $1.82x10^{-3}$ |
| Campesterol | 3.78 |
| Stig-Massterol | 2.59 |
| Savenasterol | 2.46 |
| Sitosterol | 18.12 |
| Total | 26.97 |

**Table 9** Quantitative characterization of Anthraquinone constituents of *costus lucanusianus* stem

| **Anthraquinone** | **Concentration mg/100g** |
|---|---|
| 2,6- Dimethoxbenzoquingne | $3.76x10^{-1}$ |
| 6-Methoxyquinoline-1-oxide | $1.03x10^{-1}$ |
| Soranjidiol | $2.97x10^{-1}$ |
| Damnacanthal | $1.70x10^{-2}$ |
| Damnacanthol | $2.37x10^{-3}$ |
| Heterophylline | 3.00 |
| Total | 1.09 |

**Table 10** Quantitative characterization of Carotenoids constituent of *costus lucanusianus* stem

| **Carotenoids** | **Concentrations mg/100g** |
|---|---|
| Malvidin | $8.04x10^{-5}$ |
| Beta- cryptoxanthin | $6.23x10^{-3}$ |
| Lycopene | $3.93x10^{-6}$ |
| Carotene | 15.18 |
| Lutein | 7.33 |
| Xanthophyll | 4.16 |
| Anthera-xanthin | 5.5 |
| Asta-xanthin | 7.83 |
| Viola-xanthin | 5.34 |
| Neo-xanthin | 5.82 |
| Total | 44.20 |

**Table 11** Quantitative characterization of Anthocyanins constituent of *costus lucanusianus* stem

| **Anthocyanins** | **Concentration mg/100g** |
|---|---|
| Cyanidin 3-sophoroside-5-glucoside | $4.92\times10^{-1}$ |
| Peonidin 3-sophoroside-5-glucoside | $1.95\times10^{-1}$ |
| p-hydroxybenzolated (Cyanidin3-sophoroside-5-glucoside | $3.64\times10^{-1}$ |
| Caffeoylated (Cyanidin 3-sophoroside-5-glucoside) | $6.62\times10^{-1}$ |
| p-hydroxybenzolated(Peonidin 3-sophoroside-5-glucoside) | $5.46\times10^{-1}$ |
| Caffeoylated( Peonidin 3-sophoroside-5-glucoside) | $1.78\times10^{-1}$ |
| Feruloylated (Cyanidin3-sophoroside-5- glucoside) | $1.90\times10^{-1}$ |
| Cyanidin 3-(6,6'-caffeoyl-p-hydroxybenzoylso) | 2.26 |
| Cyanidin 3-(6,6'-dicaffeoylsophoroside)-5-glucoside | $8.79\times10^{-1}$ |
| Cyanidin 3-(6, -caffeoylsophoroside)-5-glucoside | $2.56\times10^{-1}$ |

| | |
|---|---|
| Cyanidin 3-(6,6'-caffeoylferuloylsophoroside) | $4.01\times10^{-1}$ |
| Peonidin 3-(6,6'-dicaffeoylsophorous)-5-glucosde | $9.01\times10^{-1}$ |
| Peonidin 3-(6,6'-caffeoyl-p-hydroxybenzoylso) | $1.18\times10^{-1}$ |
| Peonidin 3-(6, -caffeoylsophoroside)-5-glucoside | $1.17\times10^{-1}$ |
| Peonidin 3-(6,6'-caffeoylferuloylsophoroside) | $1.58\times10^{-1}$ |
| Total | 7.73 |

**Table 12** Quantitative characterization of Lignan constituents *of costus lucanusianus* stem

| **Lignan** | **Concentration mg/100g** |
|---|---|
| 2-Allyic-5- ethoxy-4-methoxyphenol | $3.45x10^{-5}$ |
| Lariciresinol | $1.40x10^{-2}$ |
| (9E,12E,15E)-9,12,15-Octadecatrien-1-ol | $4.47x10^{-6}$ |
| Matairesinol | $9.56x10^{-3}$ |
| Apigenin-4',7-dimethyl ether | $6.73x10^{-5}$ |
| Epipinoresinol | $7.16x10^{-2}$ |
| Pinoresinol | $9.42x10^{-2}$ |
| Secoisolariciresinol | $3.22x10^{-2}$ |
| Dehydroabietic acid | $5.22x10^{-2}$ |
| Ratusin | $5.26x10^{-2}$ |
| Galgravin | $1.41x10^{-3}$ |
| Epieudesmin | $1.48x10^{-5}$ |
| Sakuranin | $2.86x10^{-5}$ |
| Total | $3.62x10^{-1}$ |

**Table 13** Quantitative characterization of Isoflavones constituent of *costus lucanusianus* stem

| **Isoflavones** | **Concentrations mg/100g** |
|---|---|
| Daidzein | 5.39 |
| Genistein | 4.20 |
| Glycitein | 3.99 |
| Daidzin | 1.59 |
| Prunetin | 7.66×10-3 |
| Gentstin | 1.75 |
| Formonetin | 7.28×10-3 |
| Puerarin | 1.56×10-3 |
| Total | 16.92 |

**Table 14** Essential oil constituents of *costus lucanusianus* stem

| Compounds | Concentration (g/100g |
|---|---|
| ɒ-pinene | 1.03 ± 0.01 |
| β-mycrene | 1.83 ± 0.02 |
| Limonene | 0.06 ± 0.01 |
| Cis-carvone oxide | 3.17 ± 0.01 |
| β-Linalool | 0.98 ± 0.01 |
| ɒ-terpineol | 0.29 ± 0.07 |
| Perilla alcohol | 0.02 ± 0.00 |
| Cis-Geraniol | 0.04 ± 0.01 |
| Trans-Geranyl acetate | 2.17 ± 0.01 |
| Linalyl acetate | 1.26 ± 0.01 |
| Thymol | 0.04 ± 0.00 |
| Germacrene D | 0.17 ± 0.01 |
| p-Acetyl toluene | 0.01 ± 0.00 |
| Limona ketone | 0.21 ± 0.00 |
| Carvone | 0.01 ± 0.01 |

Data were represented in mean ± standard deviation (M ± SD) in triplicate determination

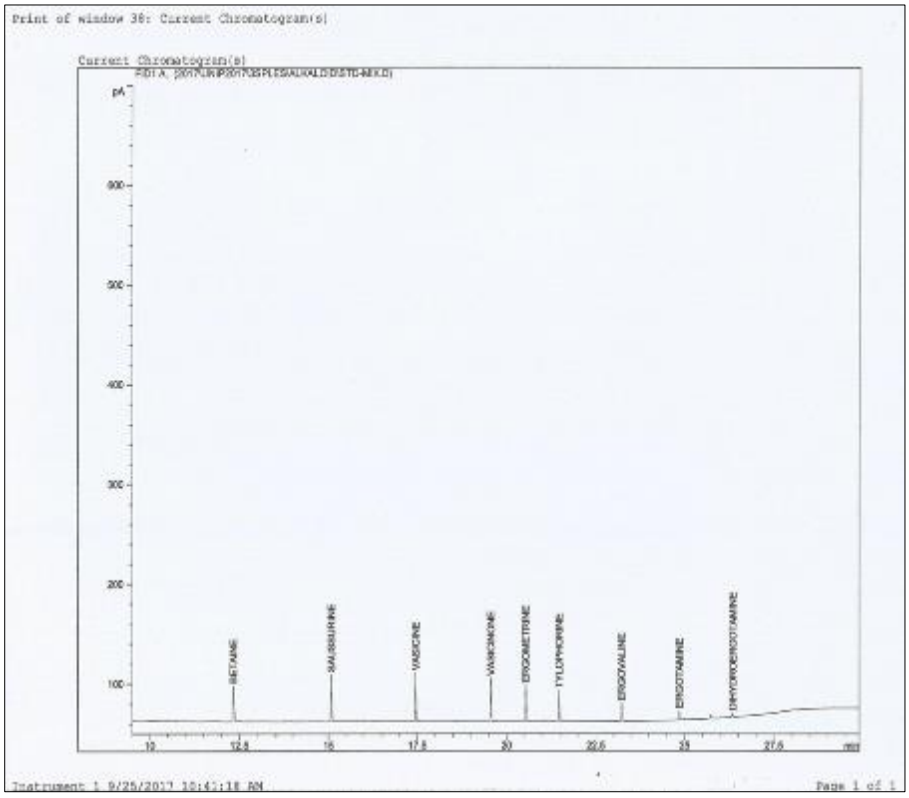



**Figure 2** Chromatogram of phytochemicals constituents of *costus lucanusianus* stem

## 4. Discussion

Results from Table 5: Shows saponins composition of the stem of *costus lucanusianus*. The total saponins composition was 837.38mg/100g.With Diosgenin having the highest concentration (533.83mg/100g) and Conyzorgin the lowest concentration ($1.18\times10^{-4}$ mg/100g). This is higher than the amount reported by [16] in the stem, leaf, and rhizhome of *costus afer* and that reported by [17,18]. Saponins are another type of bioactive chemical constituent which are involved in plant diseases resistance because of their antimicrobial activity [19]. They help to reduce congestive heart failure by inhibiting sodium ions into the cell [20].

Table 4 shows the composition of tannins compounds present in the plant stem. With a total (485.26mg/100g), this value is higher than what was obtained in researches by [16, 17, 21] on tannin contents of *costus afer* stem and relatively higher than tannin contents found in other plants stem as reported by [22, 23, 24, 25]. Tannin containing plant extracts are used as astringents, against diarrhea, as diurectics, against stomach and duodenal tumors [26]. Tannins are also used as anti-inflammatory, antiseptic, and hemeostatic pharmaceuticals [27].

Table 2 shows the result of flavonoids present in the plant stem, with total of (261.31mg/100g). Myricetin was found to have been the highest with concentration of (138.98mg/100g) and Silymarin ($2.79\times10^{-7}$mg/100g) the lowest. This result is higher than what was reported on flavonoid content of *costus afer* stem by [17] and that of six other medicinal plant stem from Malaysia [28] and other researches on stems of other plants [29,30,22]. Flavonoids are antioxidant and free radical scavengers which prevent oxidation; they have strong antiulcer activity and also protect the cell against all stages of carcinogenesis [31, 32].

Table 7 shows the presence of glycoside (51.57·mg/100g) in the stem of *costus lucanusianus* plant with costusoside and Arbutin having the highest and lowest concentrations respectively. This value is higher than the value recorded for *costus afer* stem by [16, 18] and for other plant stems [33, 23, 34, 35].

Table 10 shows Carotenoids constituents of the stem of *costus lucanusianus*, with a total of 44.20mg/100g. Carotene and Lycopene were the highest and lowest constituents respectively of ($3.39\times10^{-6}$mg/100g). This value is higher than that reported by [36] for *jatropha curcas* stem. Fruit and vegetables constitute the major sources of carotenoids in human diet [37]. Based on epidemiological studies a positive link is suggested between higher dietary intake and tissue concentrations of carotenoids and lower risk of chronic diseases [38]. The antioxidant properties of carotenoids have been suggested as being the main mechanism by which they exert their beneficial effects [39].

Total of 26.96mg/100g phytosterols was present in the stem of *costus lucansianus* Table 8 with Sitosterol having the highest concentration and Cholesterol the lowest. Phytosterols are secondary metabolites occurring in small quantities, with the highest concentrations in vegetable oil. They reduce total and low density lipoprotein cholesterol lever in plasma by inhibiting it absorption from small intestine. Hence, they lower the atherosclerotic risk and offer protection against cardiovascular diseases [40].

Total phenolics constituent of *costus lucanusianus* stem was found to be (22.46mg/100g) Table 3. With ferulic acid having the highest concentration and piperic acid having the lowest concentration. This is higher than the values reported by [17] and [19] for *costus afer* stem. Phenolics compounds are famous group of secondary metabolites with wide pharmacological activities. They increase bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as *staphylococcus aureus* are some of biological activities. Phenolics compound posseses diverse biological activities for instance, anti-ulcer, anti- inflammatory and antioxidant [41]. Cytotoxic and anti-tumor, antispasmodic and antidepressant activities [42].

From Table 13, the total concentration of isoflavones was revealed to be 16.96mg/100g. Daidzein had the highest concentration of (5.39mg/100g) and Puerarin ($1.56\times10^{-3}$mg/100g) the lowest. This value is higher than what was obtained by [43] (0.79mg/100g) on *trifolium pratense l*. stem. Isoflavones protect the body against age related diseases including cardiovascular diseases, osteoporosis, and hormone dependent. They also act as detoxifying agent [44].

Table 11 shows the presence of anthocyanins in the stem of *costus lucanusianus* plant with a total of 7.72mg/100g. With Cyanidin 3-(6,6'-caffeoyl-p-hydroxybenzoylso) having the highest concentration of (2.26mg/100g) and Peonidin3-(6, -caffeoylsophoroside)-5-glucoside ($1.17\times10^{-1}$mg/100g) the lowest concentration. Anthocyanins from plants have been used for treatment of hepato biliary issues such as hyperbilirubinemia, obstructed bile ducts and treatment of lack of appetite. These compounds are also reported to lower the level of nitric oxide by inhibiting the activity of nitric oxide synthase [45].

Result from Table 9 shows a total of 1.09mg/100g anthroquinone present in the stem of *costus lucanusianus* with 2,6-Dimethoxybenzoquinone having the highest concentration of ($3.76x10^{-1}$mg/100g) and Damnacanthol ($2.37x10^{-3}$mg/100g) the lowest. Anthroquinone are used in constipation, they are reported to possess antiviral, antibacterial, antifungal properties and treatment of fungal skin diseases [46, 47].

Table 6 reveals total terpinoids concentration as 0.91mg/100g. Beta-amyrin had the highest concentration of ($9.11x10^{-1}$mg/100g) and Deglucosyleuphorbioside A ($6.073x10^{-8}$mg/100g) the lowest. Terpeniods is class of natural product which has been derived from the five carbon isoprene units. Many of the terpenoids are commercially interesting because of their use as flavours and fragrances in foods and costmetics example menthol and sclareol or because they are important for quality of agricultural products, such as the flavor of fruits and fragrance of flowers like linalool [48].

Lignan total concentration of *costus lucanusianus* plant stem from Table 12 was 0.36mg/100g. Pinoresinol and (9E, 12E, 15E)-9, 12, 15-Octadecatrien-1-ol ($4.47x10^{-6}$mg/100g) were highest and lowest in concentration respectively. Lignans are also reported to suppress the receptor binding of platelet activating factor and inhibit the replication of human immuno deficiency virus at the integration stage. These compounds are also known to inhibit tumor necrosis factor-alpha from lipopolysaccharide-triggered murine microphage [49].

Table 1 revealed the total alkaloids constituent of *costus lucanusianus* stem as (0.19mg/100g). With Saussurine and Ergometrine as highest and lowest in concentration respectively of ($1.42\times10^{-1}$mg/100g) and ($8.60\times10^{-8}$mg/100g) the lowest which is in variance with result *costus afer* plant stem reported by [17] and [18]. Alkaloids are known to have blood glucose lowering activity. Alkaloids are also believed to elicit antimicrobial and trypanocidal activity by inhibition of protein biosynthesis and by interaction with neuro receptors [50].

Result from Table 14. Shows the essential oil constituents of *costus lucanusianus* stem as cis-carvone oxide (3.17g/100mg), Trans-Geranyl acetate (2.17g/100mg), β-mycrene (1.83g/100mg) and Linalyl acetate (1.26g/100mg) were the four predominant essential oils.

Cis-carvone oxide concentration of *costus lucanusianus* stem (3.17g/100mg) was higher than the value reported by [51] (0.1g/100mg) in leaf and stem of *origanum vulgare l.* Cis-Carvone has pharmacologic property of anti-infective agent. Trans-Geranyl acetate is used as component of perfumes for creams and soaps and as flavouring agent and β-mycrene an important intermediate used in the perfumery industry. It has a pleasant odor, but is rarely used directly [52]. The result for β-mycrene was found to be (1.83g/100mg). β-mycrene concentration in *costus lucanusianus* stem agrees with the result reported by [53] for *Cinnamomumkusteeri* and *cinnamomum canbodianum* stem bark and [54] for *calophyllum inophylum* stem. Linalyl acetate has a cytocytic effect on several types of human tumor cells, small cell lung carcinoma and colorectal cancer cell lines were the most sensitive [55].

## 5. Conclusion

The quantitative phytochemicals and essential oil constituents of *costus lucanusianus* stem in this study revealed the presence of saponins, flavonoids, glycosides, caratenoids, phytosterols, phenolic acids, alkaloids, lignan, terpenenoids, isoflavone anthocyanins, anthraquinone at varying concentrations. The predominant essential oil constituents were cis-carvone and trans-geranyl acetate. linanyl acetate, β-mycrene and α-pinene. Hence *costus lucanusianus* stem possess bioactive constituents that could serve as raw material in pharmaceuticals and cosmetics industries.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

All the authors contributed in the design, interpretation of data, discussion and proof reading of the manuscript hence do not have any conflict of interest.

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