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Research Article

Assessment of Fatty Acid, Proximate and Quantitative Phytochemical Compositions of Matured Stem of *Costus afer* (Bush Cane).

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ABSTRACT

This study estimated the fatty acid, proximate and quantitative phytochemical compositions of *Costus afer* matured stem collected from Choba campus of University of Port Harcourt, in Choba community, Rivers State. This was carried out using standard procedures of analysis (fatty acid and proximate) as well as gas chromatographic method (quantitative phytochemical analysis). The fatty acid analysis of the plant stem revealed high contents of linolenic acid (32.26%), linoleic acid (25.89%) and palmitic acid (25.48%) and moderate levels of oleic acid (7.11%) and stearic acid (6.36%) while myristic acid, palmitoleic acid, arachidonic acid, behenic acid and lignoceric acid was low and caprylic acid, capric acid, lauric acid, margaric acid, arachidonic acid, erucic acid were absent. The results of the proximate composition of the stem of *Costus afer* indicated that total carbohydrate composition of *Costus afer* stem was the highest (54.98%) while crude fat had the lowest value of 1.15%. The moisture content had a moderate value of 22.15% while total ash (4.60%), crude protein (7.72%) and crude fibre (9.40%) values were low. The gas chromatographic analysis of the stem indicated that the total alkaloid composition was 70.59mg/100g with high compositions of papaverine (44.72%), methyl morphine (23.24%), morphine (17.92%) and narcotine (14.11%). The total flavonoids concentration was 28.29mg/100g. Myricetin (69.79%) had the highest value with moderate levels of quercetin (14.88%) and kaempferol (9.78%). The total composition of saponin was 2.87mg/100g. The saponin content of the stem (39.20%) was the highest with moderate levels of diosgenin (26.13%), saponine (22.12%) and tigonine (9.76%) while gitogenin value (2.28%) was observed to be low. The analysis of the plant stem showed the presence of glycosides (22.35mg/100mg) made up mainly of costugenin (65.60%), digitoxin (18.73%), digoxin (6.28%), salicin (4.76%) and low levels of ouabain (1.95%) and kaemferol-3-rhamnoside (1.08%). The study has shown that matured stems of *Costus afer* can contribute greatly towards meeting human nutritional requirements and suggest the possibility of its use in folklore medicine.

Keywords: *Costus afer*, phytochemicals, proximate composition, fatty acids.

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INTRODUCTION

Plants are important sources of foods and natural drugs. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, mainly based on their use in traditional medicines or phytomedicines. In Nigeria many indigenous plants are used as spices, food or medicine. These plants often exhibit a wide range of biological and pharmacological activities. Extracts from the roots, barks, seeds and fruits of these plants are used in the preparation of syrups in traditional medicine as cough suppressant and in the treatment of oxidative related diseases.^[1] It is generally assumed that the active

constituents contributing to these protective effects are the phytochemical constituents, vitamins and minerals.^[1] Phytochemical constituents exhibit a wide range of biological effects resulting in their protective or disease preventive properties. These are some possible actions: antioxidant, hormonal action, stimulation of enzymes and interference with DNA replication, anti-microbial effect, and physical action.^[2] ^[3] ^[4] Medicinal plants play a great role in human life and have substances that are used for traditional therapeutic and modern drug production purposes in primary health care delivery. These medicinal plants might contain one or more compounds having medicinal activity. These compounds could be used or

mixed together to make effective medicines.^[5] Medicinal plants have long been a subject of curiosity and need. Herbal medicines are usually in the form of vegetable drugs or their extracts that are utilized by man for the treatment of disease or to maintain a state of improved health. With the increasing interest in medicinal plants, arises the need to investigate the biochemical basis of the action of plants and their extracts. This further emphasizes the need to link phytochemistry and pharmacology. In addition, some of these medicinal plants have been found to be rich in nutrients, thus necessitating the investigation of their nutrient composition and potential as sources of nutrients or as food. Therefore the knowledge of the chemical composition of foods is the first essential in dietary treatment of disease or in any quantitative study of human nutrition.^[6] Indigenous people of Africa employ at least 20,000 plant species for medicines and related purposes.^[7] In addition to medicinal properties many of the plants are high in protein, vitamins, mineral and carbohydrate for both human and livestock.

The plant *Costus afer* is among 150 species of stout, perennial and rhizomatous herbs of the genus *Costus*^[8].

Costus afer is found in the forest belt from Senegal east to Ethiopia and south to Tanzania, Malawi and Angola. It is often planted in home gardens for medicinal purposes. *Costus afer* belongs to the family Costaceae a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady and river banks forest of West and Tropical African countries including Senegal, South Africa, Guinea, Nigeria, Ghana and Cameroon.^[8] ^[9] *Costus afer* is pantropical and comprises of about 70 species, which about 40 species are found in tropical America, about 25 in tropical Africa and about 5 in South-East Asia.^[10] *Costus afer* is commonly called bush cane.^[11] ^[12] It is known as "Okpete" or "Okpoo" in Igboland, "Kakizuwa" in Hausa, "tete-egun" in Yoruba and "Mbritem" in Efik, all in Nigeria. Anglophone Cameroon calls it "Monkey sugar cane."^[12] ^[13]

Costus afer is perennial, rhizomatous herb that can attain a height up to 4m. Leaves are arranged spirally, simple and entire. Sheath is tubular, closed, and green with purple blotches; ligule 4-8mm long; blade is elliptical to obovate, 15 - 35cm x 3.5 - 9.5cm, base is rounded to subcordate, apex is acuminate, and margin is sparsely hairy, usually glabrous above and sometimes shortly hairy beneath. Inflorescence is a very compact, terminal, conical spike 2.5cm - 7.5cm long, sessile; bracts is oblong, convex, 3.5cm long, densely inbricate, upper ones often smaller, apex is truncated to rounded, green with purple markings, each subtending 2 flowers; bracteoles is boat shaped, 2.5cm x 1cm, keel is thick and ridged, pale green with pink markings and thin papery margin. Flowers are bisexual and zygomorphic.^[10]

MATERIALS AND METHODS

Collection and Identification of Plant Samples

Fresh stems of *Costus afer* were obtained from Choba campus of University of Port Harcourt, in Choba community. They were authenticated by a Plant taxonomist at the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria where a voucher specimen was also deposited. The stems were washed, cut into pieces and sun dried. They were later ground into fine powder with the aid of a clean dry electric grinder ((Sorex SHB-520, Korea)) and stored in an air tight container.

Determination of Proximate Composition

Proximate analysis to determine the moisture, crude protein, fat, ash, fiber and total carbohydrate contents of the samples were carried out according to the standard methods.^[14]

Determination of Moisture Content

Procedure

An empty silica dish was allowed to cool in the desiccator, after which its weight was taken. Then 1.0g of the sample was weighed into the silica dish and placed in the oven at about 105°C for 24 hours. It was cooled in desiccator to the room temperature. The silica dish and contents was weighed and later placed back in the oven for another 24 hours to ensure complete drying. The cooling process in the desiccator was repeated until a constant weight was obtained. The calculation is shown in appendix C.

Determination of Ash Content

Procedure

To a pre-weighed, clean, empty petri-dish, 1.0g of the sample was added and placed in the muffle furnace at 550°C for 4 hours. The sample was allowed to cool in the desiccator. This was repeated until a constant weight was obtained. The weight of the petri-dish and residue was taken. The calculation is shown in appendix C.

Determination of the Protein Content

Procedure

Digestion

To 1.0g of the sample in a 100ml Kjeldahl digestion flask, was added 3g of Kjeldahl digestion catalyst, 20ml of the 1.25% concentrated sulphuric acid and a few anti-bumping agents. The flask was fitted to a reflux condenser and gently heated until foaming had ceased, and the contents became completely liquefied. Then the content of the flask was heated intensely, with occasional rotation of the flask, until the colour of the digest changed from ash to blue-green or pale green colour. The flask was allowed to cool and its contents were quantitatively transferred into a 100ml volumetric flask and made up to the 100ml mark with distilled water.

Distillation

Twenty ml of this diluted digest was transferred into a 150ml distillation flask. The flask into which some anti-bumping chips have been added was connected to a condenser whose receiver was attached to a Buchner funnel immersed in a 400ml beaker containing 10ml of 2% boric acid solution masked with 2 drops of double (methyl red-methylene blue) indicator. Then, 20ml of 40% NaOH solution was added to the flask using a syringe. Distillation was stopped when the volume in the beaker was about the same in the original volume, and the colour of the boric acid in the receiver flask changed from purple to pale green. The ammonia was liberated into the boric acid solution. The distillation unit was dismantled and rinsed with distilled water.

Titration

The distillate (boric acid-ammonia solution) was titrated with 0.1M hydrochloric acid, until the colour changed to pink, which marked the end of titration. The titre value was recorded and this was used to determine the nitrogen content from which the protein value was calculated by

multiplying with the Nitrogen factor, 6.25. The calculation is shown in appendix C.

Determination of the Crude fat content

Procedure

Extracting flasks (250ml) capacity was dried in the oven at 105°C, transferred to the desiccator to cool to the laboratory temperature and their weights were measured. Petroleum ether (250ml) was measured into the dried flasks while 0.25g of the sample was weighed into labelled porous thimbles and placed in the condenser of the soxhlet extractor, and the sample was extracted for 4 hours. The thimbles were removed with care and the petroleum ether in the top container (tube) was collected for reuse. The extraction flask was removed from the heating mantle arrangement when it was almost free of petroleum ether. The extraction flask with the oil was oven dried at 105°C for the period of 1 hour. The flask containing the dried oil was cooled in the desiccator and the weight of the cooled flask with the dried oil was measured.

Determination of Fiber Content

Procedure

Two grams of the pulverized sample was weighed into 1 litre conical flask and 200ml of 1.25% sulphuric acid was added and allowed to boil gently for 30minutes and contents were filtered through the Buckner funnel and rinsed well with hot deionised water. To the filtrate 200ml of the boiling 1.25% of sodium hydroxide was added, allowed to boil gently for 30minutes and later filtered through the Buckner funnel. The residue was washed with hot deionized water, with 10% hydrochloric acid, and then followed by dimethyl ether and then dried in oven overnight at 110°C. The residue was then transferred into the desiccator to cool before weighing. After weighing, it was ashed in the muffle furnace at 550°C for 90 minutes. After ashing, it was transferred to the desiccator to cool and then weighed. The calculation is shown in appendix C.

Determination of Total Carbohydrate

The total carbohydrate was determined by getting the percentage difference between the summations of values of crude protein, crude fat, crude fibre, ash and moisture content of the sample.

Energy value: The energy value was calculated using the Atwater factors 4, 9, and 4 for protein, fat and carbohydrate respectively.

Phytochemical Analysis

Calibration, Identification and Quantification

The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards.

Chromatographic Analysis

Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev A 09.01 (1206) software. The capillary column was an AC-5 Column (30m × 0.32mm × 0.25μm film thickness).

Determination of Alkaloid Composition [15]

Procedure

Five grams of the pulverized sample was macerated in hexane of 25ml for about 72 hours. The extract was filtered and the residue was air dried, later treated with 10% aqueous ammonia and macerated in chloroform for 24 hours. After filtration and evaporation at reduced pressure, the resultant filtrate was treated with 7.5ml, 5% aq. HCl. The aqueous phase was made alkaline with aqueous ammonia and extracted thrice with chloroform. The chloroform was washed with water. The extract was poured into the round bottom flask of the rotary evaporator arrangement. It was separated by driving the solvent off the extract. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis.

Determination of Flavonoid Composition [16]

Procedure

One gram of the sample was weighed into the 250ml conical flask capacity with addition of distilled water and boiled for 10minutes. The flavonoid extract was obtained by pouring 100ml of the boiling methanol: water (70:30) v:v into the samples in the test tubes. The mixture was allowed to macerate for about 4 hours and then filtered with Whatman filter paper No.1. The filtrate was concentrated to 5ml for gas chromatography analysis.

Determination of Saponin Composition [17]

Procedure

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

Determination of Glycoside Composition [18]

Procedure

One gram of the pulverized sample was weighed into a pre-cleaned borosilicate beaker, and extracted by pouring 10ml of ethanol/water (7:3v/v) mixture on it, and allowing to stand for 2hrs. The mixture was filtered with Whatman No. 1 filter paper. The extract was purified by washing with lead acetate. The purified extract was further purified by adding sodium hydrogen phosphate. The extract was concentrated to 1ml, for gas chromatographic analysis.

Determination of Fatty acid Composition [19]

Procedure

Fifty milligrams of the extracted fat content of the sample was saponified (esterified) for five minutes at 95°C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. Three millilitres (3ml) of the 14% boron trifluoride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90°C to achieve complete methylation process. The Fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1ml for gas chromatography analysis.

RESULTS AND DISCUSSION

Proximate Composition of *Costus afer* Stem.

The results of the proximate composition of the stem of *Costus afer* are presented in Table 4.1. The total carbohydrate composition of *Costus afer* stem was the highest (54.98%) while crude fat had the lowest value of 1.15%. The moisture content had a moderate value of 22.15% while total ash (4.60%), crude protein (7.72%) and crude fibre (9.40%) were low.

Table 4.1: Proximate Compositions of *Costus afer* stem

Parameter	Composition
Moisture (%)	22.15
Total Ash (%)	4.60
Crude Protein (%)	7.72
Crude Fat (%)	1.15
Crude Fibre (%)	9.40
Total Carbohydrate (%)	54.98
Caloric value (kcal)	261.15

Fatty acid Composition

Table 4.2: Fatty acid composition of *Costus afer* stem

Compounds	<i>Costus afer</i>	Retention time (min)	Composition (%)
Caprylic acid (C8:0)		8.908	0.00
Capric acid (C10:0)		10.363	0.00
Lauric acid (C12:0)		12.100	0.00
Myristic acid (C14:0)		13.743	0.02
Palmitic acid (C16:0)		15.160	25.48
Palmitoleic acid (C16:1)		16.248	2.06
Margaric acid (C17:0)		17.230	0.00
Stearic acid (C18:0)		18.057	6.37
Oleic acid (C18:1)		18.844	7.11
Linoleic acid (C18:2)		19.523	25.90
Linolenic acid (C18:3)		21.824	32.27
Arachidonic acid (C20:0)		22.359	0.52
Arachidonic acid (C20:4)		23.234	0.00
Behenic acid		23.970	0.20
Erucic acid		24.793	0.00
Lignoceric acid		25.619	0.07
Total fatty acids			100.00

The content of fatty acids present in the stem of *Costus afer* investigated is shown in Table 4.2. The plant showed high contents of palmitic acid (25.48%), linoleic acid (25.89%), and linolenic acid (32.26%) and moderate levels of oleic acid (7.11%) and stearic acid (6.36%) while myristic acid, palmitoleic acid, arachidonic acid, behenic acid and lignoceric acid was low and caprylic acid, capric acid, lauric acid, margaric acid, arachidonic acid, erucic acid were absent.

Phytochemical Profile

The results of the phytochemical analysis of the stem of *Costus afer* are shown below. Table 4.3a shows the alkaloid composition of the stem of *Costus afer*. The total alkaloid composition was 70.59mg/100. The plant showed high composition of papaverine (44.72%), methyl morphine (23.24%), morphine (17.92%) and narcotine (14.11%) while biflorin, daphnoline, aromoline, homoaromoline, ambelline, 6-hydroxyphanidine, monocrotalline, 6-hydroxypowelline and nitidine concentrations were negligible.

Table 4.3a: Alkaloid composition of *Costus afer* stem

Compounds	<i>Costus afer</i>	Retention time (min)	Composition(mg/100g)
Morphine		12.412	12.65
Methyl morphine		13.794	16.41
Papaverine		15.111	31.56
Biflorin		15.722	0.01
Narcotine		16.368	9.96
Daphnoline		16.664	0.00
Aromoline		18.042	0.00
Homoaromoline		18.988	0.00
Ambelline		19.665	0.00
6-Hydroxybuphanidine		20.599	0.00
Monocrotalline		21.251	0.00
6-Hydroxypowelline		21.794	0.00
Nitidine		22.559	0.00
Total alkaloids			70.59

The flavonoid composition of the stem of *Costus afer* is presented in Table 4.3b. The total flavonoids concentration was 28.29mg/100g. Myricetin (69.79%) had the highest value with moderate levels of quercetin (14.88%) and kaempferol (9.78%) while catechin, resveratrol, apigenin,

daidzein, butein, naringenin, biochanin, luteolin, epicatechin, salvogenin, epicatechin-3-gallate, gallicatechin, sinensetin, kaempferol-3-arabinoside, quercitrin, isorquercetin, orientin, isoorientin and rutin levels were negligible.

Table 4.3b: Flavonoid Composition of *Costus afer* stem

Compounds	<i>Costus afer</i>	
	Retention time (min)	Composition(mg/100g)
Catechin	13.549	0.00
Resveratrol	14.904	0.00
Apigenin	16.036	0.92
Daidzein	16.246	0.06
Butein	16.458	0.00
Naringenin	16.671	0.00
Biochanin	17.357	0.00
Luteolin	17.769	0.07
Kaempferol	18.050	2.77
Epicatechin	19.395	0.00
Salvogenin	20.467	0.00
Epicatechin-3-gallate	21.501	0.00
Gallocatechin	22.065	0.00
Quercetin	22.597	4.21
Isorhamnetin	23.471	0.00
Myricetin	23.965	19.75
Sinensetin	24.997	0.00
Kaempferol-3-arabinoside	25.360	0.00
Naringenin	26.041	0.00
Quercitrin	27.294	0.04
Isoquercetin	27.480	0.07
Orientin	27.910	0.00
Rutin	28.195	0.39
Isoorientin	28.529	0.02
Total flavonoids		28.29

Table 4.3c: Saponin Composition of *Costus afer* stem

Compounds	<i>Costus afer</i>	
	Retention time (min)	Composition(mg/100g)
Gitogenin	17.545	0.08
Solagenin	18.588	0.01
Diosgenin	19.516	0.75
Tigogenin	20.115	0.28
Neohecogenin	20.979	0.00
Hecogenin	21.819	0.00
Sapogenin	22.600	1.12
Euphol	24.185	0.00
Saponine	25.480	0.63
Total saponins		2.87

The concentrations of saponin compounds in the stem of *Costus afer* investigated are presented in Table 4.3c. The total composition of saponin was 2.87mg/100mg. The sapogenin content of the stem (39.20%) was the highest

with moderate levels of diosgenin (26.13%), saponine (22.12%) and tigonine (9.76%) while gitogenin (2.28%) was low and solagenin, neohecogenin, hecogenin and euphol were absent.

Table 4.3d: Glycoside composition of *Costus afer* stem

Compounds	<i>Costus afer</i>	Composition(mg/100g)
	Retention time (min)	
Kampferol-3-O-Rhamnoside	15.395	0.24
Arbutin	17.464	0.21
Salicin	18.758	1.06
Amygdalin	19.513	0.00
Ouabain	20.469	0.44
Digitoxin	21.436	1.03
Vitexicarpin	22.688	0.15
Digoxin	23.110	1.40
Costugenin	23.963	14.66
Total glycosides		22.35

The concentrations of glycoside compounds in the stem of *Costus afer* investigated are presented in Table 4.3d. Costugenin (65.60%) had the highest value with moderate levels of digitoxin (18.73%), salicin (4.76%), digoxin (6.28%) and low levels of ouabain (1.95%) and kaemferol-3-rhamnoside (1.08%) while arbutin, amygdalin and vitexicarpin were absent.

DISCUSSION

Proximate composition of the stems of *Costus afer* in Table 4.1 showed that its moisture content was 22.15% and is high compared to 11.23% reported for the stems of *Balanites aegyptiaca* [20] but lower than those of *Tridax procumbens* (88.30%) and *Ocimum gratissimum* (82.60%).[21][22] The moisture content of any food is used as a measure of stability and the susceptibility to microbial contamination.[23] This implies that *Costus afer* may have a short shelf life due to its high moisture content.

The study indicated that the ash content of the stem was 4.60% and is lower than 13.67% reported for the stem of *Ocimum gratissimum*[22] which implies that the stems of *Costus afer* is a poor source of mineral element since the ash content of a plant material is an index of mineral contents in biota.

The protein content of the stem of *Costus afer* was 7.72% which is lower than 37.44% reported for *Tridax procumbens*[21] but higher than those of *Ocimum gratissimum* (1.65%).[22] Proteins act as enzymes, hormones and antibodies. They are responsible for the formation of bones, teeth, hair and the outer layer of skin and they help maintain the structure of blood vessels and other tissues.[24] The result revealed that the stems of the plant are poor sources of protein.

The crude fat content of the plant was 1.15% and is lower than that of *Aspilia africana* which was 3.86%[25]. The finding showed that the plant is a poor source of plant lipid thus advantageous healthwise in avoiding overweight.[26] The crude fibre content was 9.40% and is high compared to that of *Eugenia uniflora* (0.67%).[27] Adequate intake of dietary fibre can lower the serum cholesterol level, and thus risk of coronary heart disease, hypertension, diabetes, breast cancer and constipation. [28][29] Dietary fibers alter the colonic environment in such a way as to protect against colorectal diseases. They provide protection by increasing fecal bulk, which dilutes the increased colonic bile acid concentrations that occur with a high fat diet. [30]

The study revealed the carbohydrate content of the plant to be 54.98% which is higher than that of *Tridax procumbens* (41.03%)[21] but is lower than 58.35% reported for the stems of *Balanites aegyptiaca*.[20] Carbohydrates are the human body's key source of energy,

providing 4 calories of energy per gram. Carbohydrates provide the body with a source of fuel and energy that is required to carry out daily activities and exercise.

The caloric value of the plant (261.15 kcal/100g) is lower than those of *Ocimum gratissimum* (278.42 kcal) and *Tridax procumbens* (321.54 kcal).[21][22]

The result of the fatty acid composition of the plant (Table 4.2) indicates the presence of linolenic acid which had the highest value of 32.27%. Linolenic acid has been beneficial in lowering body fat. α -linolenic acid is a polyunsaturated (Omega-3) fatty acid. Preliminary research has found evidence that α -linolenic acid is related to a lower risk of cardiovascular disease. Dietary α -linolenic acid has been assessed for its role in cardiovascular health.[31] Linoleic acid was also found to be present at the concentration of 25.90%. It is a polyunsaturated fatty acid used in the biosynthesis of arachidonic acid and thus some prostaglandins. [32] Linoleic acid is an essential fatty acid that must be consumed for proper health. These polyunsaturated fatty acids are able to decrease the plasmatic levels of VLDL, LDL cholesterol and increases in serum High density lipoprotein cholesterol expression of LDL receptors in liver. These LDL receptors increases uptake and subsequent removal of LDL, VLDL and thus restore cholesterol homeostasis.

Palmitic acid was found to be 25.48%. Palmitic acids are needed for energy, hormone production, organ padding and cellular membranes. It is also needed for important signaling and stabilization processes in the body.

Oleic acid composition was 7.11%. It is a monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low density lipoprotein (LDL) cholesterol and possible increased high density lipoprotein (HDL) cholesterol. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil. [33]

The result of the phytochemical analysis indicated the presence of alkaloids, saponins, flavonoids, glycosides. Alkaloids are known to have anti-microbial, antifungal and anti-inflammatory effect[3] and it also acts as an anti-hypertensive agent.[34] Table 4.3a shows the result of the quantification of alkaloid compounds present in the stems of *Costus afer*. The plant contained papaverine, an alkaloid which had the highest value of 31.56mg/100g than other alkaloid compounds. It is used to treat spasms of the gastrointestinal tract, bile ducts and ureter and for use as cerebral and coronary vasodilators in subarachnoid hemorrhage[35] and coronary artery bypass surgery.[36] It relaxes veins and arteries, which makes them wider and allows blood to pass through them more easily, thereby increasing the amount of oxygen rich blood in the brain,

heart and muscles. It is also used as an erectile dysfunction drug, alone or sometimes in combination.^{[37][38]}

Morphine, an alkaloid with the value 12.65mg was present in the plant and they are narcotic analgesics used to relieve severe pain. It is primarily used to treat both acute and chronic pain. Also used for pain due to myocardial infarction and for labour pains.^[39] Also methyl morphine an alkaloid referred to as codeine with the value 16.41mg/100mg was present in the plant. It is used as a cough suppressant, analgesic and hypnotic. It is also used to treat diarrhea.^[40] Narcotine (9.96mg/100g) was also present. It has an antitussive effect. It is currently under investigation for use in the treatment of several cancers and hypoxic ischemia in stroke patients.

Table 4.3b shows the result of flavonoid compounds present in the stems. Collectively, flavonoids are of particular importance in the human diet as there is evidence that they act as antioxidants, antiviral and anti-inflammatory agents^[41] and are associated with reduced risk of cancer and cardiovascular diseases.^{[42][43]} Myricetin, a flavonoid was found to have the highest concentration of 19.74mg/100g in the plant. Myricetin in high concentrations can modify LDL cholesterol such that uptake by white blood cells is increased and also lowers rates of prostate cancer.^[44] Invitro studies show that flavonoids have anti-diarrheal activities.^[45] Flavonoids such as Quercetin (4.21mg/100g) was also present and it has efficacy against the Group 1 carcinogen *helicobacter pylori*^[46] and kaempferol (2.77mg/kg) was also detected. Kaempferol, quercetin and myricetin reduced the risk of pancreatic cancer by 23 percent in an 8 year study.^[47]

The presence of flavonoids suggests that the plant might have anti-oxidant, anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities.

Table 4.3c shows saponin compounds present in the stem of *Costus afer*. Saponins are reported to have broad range of pharmacological properties.^[41] The presence of saponin

(2.86mg/100g) in *Costus afer* stems suggests that the plant may act as anti-inflammatory, anti-fungal, expectorant, vasoprotective, hypocholesterolemic, anti-parasitic, hypoglycaemic and many others.^{[48][49]} Nwauche et al., 2014 reported that the stem of *Costus afer* has anti-hyperglycemic potentials. Sapogenin had the highest value of 1.12mg/100g. Diosgenin, a saponin had a concentration of 0.75mg/100g. Diosgenin is the precursor for the semi-synthesis of progesterone^[50] which in turn was used in early combined oral contraceptive pills.^[51] It has an estrogenic activity and can reduce the level of serum cholesterol.^{[52][53]} Saponins are used as adjuvants in vaccines, they form complexes with cholesterol to form pores in cell membrane bilayers.^[54] They are anti-inflammatory compounds that lower blood cholesterol and prevent heart disease as well as some cancers.^[55]

Table 4.3d shows the compositions of glycoside compound present in the plant. Costugenin had the highest value of 14.66mg/100g. Cardiac glycosides are used in the treatment of heart diseases like congestive heart failure and arrhythmia. Digoxin and Digitoxin were present in the plant with values 1.40mg/100g and 1.03mg/100g. They are widely used in the treatment of various heart conditions, namely atrial fibrillation and sometimes heart failure that cannot be controlled by other medication. Digoxin and Digitoxin increases the strength of heart contraction. Salicin content was 1.06mg/100g. Salicin has been shown to have anti-inflammatory, anti-pyretic effect. Salicin aids in lowering production of two enzymes, prostaglandins and thromboxanes, which reduces inflammation and the potential of platelets to stick to one another, by acting as a natural oil cleanser to wipe up the sticky and greasy layer on platelet surfaces, lessening risk of blood clots, heart attacks and strokes.^[56] The presence of cardiac glycosides in *Costus afer* stems shows that the plant is good for the treatment of diseases associated with heart. The alkaloid composition (70.58mg/100g) of the plant was higher than other phytochemicals present.

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