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Original Research

Proximate composition, mineral content and *in vitro* antioxidant activity of leaf and stem of *Costus afer* (Ginger lily)

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ABSTRACT

Aim: This study was designed to determine the proximate composition and mineral content of *Costus afer* leaf and stem, as well as to identify the most active antioxidant fraction. **Materials and Methods:** The proximate composition and mineral analysis of *C. afer* leaf and stem were performed using the standard methods described by Pearson and Association of Official Analytical Chemist while the 1,1 diphenyl 2 picryl hydrazyl (DPPH), thiobarbituric acid reactive species (TBARS), lipid peroxidation (LPO), and total antioxidant capacity (TAC) assays were used to determine the *in vitro* antioxidant activity of aqueous, *n*-butanol, ethyl acetate and hexane fractions of *C. afer* leaf and stem. **Results:** Proximate analysis revealed that the carbohydrate content was highest in the leaf (55.83 ± 3.71%) and stem (50.38 ± 1.27%) while crude fat content was lowest in the leaf (1.83 ± 0.43%) and stem (1.75 ± 0.48%). The minerals detected in appreciable quantity in both the leaf and stem samples were calcium, magnesium, potassium, sodium, chromium, lead, manganese, nickel, and copper. Further study showed that the aqueous leaf fraction exhibited a significantly ($P < 0.05$) high DPPH scavenging activity ($IC_{50} = 259.07\ \mu g/ml$) and TAC (7.95 ± 0.37 mg ascorbic acid equivalent/g) compared with the other test fractions while the aqueous stem fraction had the highest TBARS scavenging activity ($IC_{50} = 0.37\ \mu g/ml$) and inhibition of LPO ($IC_{50} = 41.15\ \mu g/ml$) compared with the other test fractions. **Conclusion:** The findings from this study indicate that *C. afer* could serve as a source of nutrient and minerals for animal nutrition and human metabolism. It also showed that the aqueous fractions of *C. afer* leaf and stem possess high antioxidant activity than the other fractions. In addition, this study may also explain the folkloric use of crude *C. afer* leaf or stem extracts in the treatment of oxidative stress associated diseases, including rheumatoid arthritis and hepatic disorder.

KEY WORDS: Antioxidant, *Costus afer,* mineral, plant, proximate

INTRODUCTION

Costus afer Ker Gawl of the family Zingiberaceae now known as Costaceae is a tall perennial herbaceous, unbranched medicinal plant with creeping rhizome [1]. It can be found in shady forest and riverbanks of Senegal, South Africa, Guinea, Nigeria, Ghana and Cameroon [2]. It is commonly called bush cane, ginger lily or spiral ginger, and in Nigeria it can be identified by several names such as Ireke omode-Western Nigeria, Okpete-Eastern Nigeria, Kakizawa-Northern Nigeria and Mbritem-Southern Nigeria, Akan asante in Ghana and Monkey sugar cane in Cameroun [2].

C. afer is a medicinal plant used traditionally for the treatment of rheumatoid arthritis, hepatic diseases, stomach ache, cough, measles, malaria, eye defects, and could also serve as an antidote for snake poisoning [2,3]. It is also important for other sociocultural purposes, including wrapping of indigenous food items, mat making, and feed for ruminants as well as an ornament for ritual purposes [4].

The major constituents of *C. afer* are steroidal sapogenins, saponins aferosides A-C, and dioscin, which are important biopharmaceuticals [3]. Phytochemical analysis of *C. afer* leaf and stem crude extracts has revealed the presence of flavonoids, saponins, alkaloids, tannins, phenols, and glycosides [5,6]. Antioxidant activity of the crude stem extracts has been reported [6]. *C. afer* leaf and stem crude extracts possess hepatoprotective activity [7-9].

Phytochemical compounds possess a wide range of nutritional, biological, and pharmacological effects [10,11]. Antioxidant property, anti-inflammatory activity, hormonal action, stimulation of enzymes, interference with deoxyribonucleic acid replication, anti-inflammatory effect, and physical action are some of the known possible effects of phytochemicals [2,10,11]. This study was designed to evaluate the proximate composition and mineral content of *C. afer* leaf and stem, as well as to identify the fraction of *C. afer* with the most active *in vitro* antioxidant activity.

MATERIALS AND METHODS

Collection of Plant Materials

C. 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 O.A. Denton, a crop scientist in the Department of Agronomy and Landscape Design, School of Agriculture and Industrial Technology, Babcock University, Ilisan-Remo, Ogun State, Nigeria. A voucher sample with number of FHI-108001 has been deposited at Forestry Herbarium Ibadan (FHI).

Plant Processing, Extraction, and Solvent Partitioning

The leaves and stem were separated from the roots. The roots were discarded while 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 separately using 1800 ml of 70% methanol at 28°C with intermittent shaking for 48 h. The extracts were filtered using Whatman No.1 filter paper and the filtrates were subsequently concentrated using rotary evaporator at 30°C (Buchi Rotavapor RE; Switzerland). The concentrates were reconstituted with distilled water in a ratio of 1:2 (concentrate: distilled water) and partitioned by successive solvent fractionation method using separating funnel containing equal volume of reconstituted suspension and solvents in the following order: hexane, ethyl acetate, *n*-butanol and distilled water. The fractions obtained were concentrated again using rotary evaporator at 30°C and kept in the refrigerator at 4°C until further use a stock.

Proximate and Mineral Analysis

The proximate composition and mineral analysis were carried out on the plant stem and leaf for the quantitative determination of physicochemical constituents using standard procedures as described by Pearson [12] and Association of Official Analytical Chemist [13].

Determination of Antioxidant Activity using *In vitro* Methods

1,1-diphenyl 2-picryl hydrazyl (DPPH) assay

Free radical scavenging potentials of test fractions (aqueous, butanol, ethyl acetate, and hexane fractions), which is based on the capacity of the test fractions to reduced 1,1-diphenyl-2-picryl hydrazyl was adopted according to the procedure described by Mensor *et al*. [14]. 1 mm of 0.3 mM DPPH in methanol was added to 2.5 ml solution of varying concentrations of test fractions or standard Gallic acid at different concentrations of 50, 100, 250, and 500 μg/ml prepared using 10% dimethyl sulfuroxide (DMSO) and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm and converted to percentage antioxidant activity (AA %), using the formula:

$$AA\% = 100 - \frac{([\text{Abs sample} - \text{Abs blank}] \times 100)}{\text{Abs control}}$$

AA% indicates antioxidant activity of fractions; Abs sample indicates absorbance of sample; Abs blank indicates absorbance of blank (methanol and DPPH) and Abs control indicates absorbance of control. One milliliter of 0.3 mm DPPH plus methanol (2.5 ml) served as a control. This assay was carried out in triplicates for each concentration. The IC_{50} values were calculated for the samples. IC_{50} denotes the concentration of fractions required to scavenge 50% of DPPH radicals.

Thiobarbituric acid (TBA) assay

The thiobarbituric acid method of Ottolenghi [15] as modified by Kikuzaki and Nakatani [16] was used for the determination of antioxidant activity of the various leaf and stem fractions of *C. afer*. Gallic acid and ascorbic acid standards at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 μg/ml were prepared and made up to 1.0 ml in test tubes using 10% DMSO. Similarly, test leaf and stem fractions (aqueous, butanol, ethyl acetate and hexane fractions) concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 μg/ml were also prepared and made up to 1.0 ml using DMSO. To these test solutions, 2 ml of 20% TCA and 2 ml of 0.67% of thiobarbituric acid solutions were added. Another set of test tubes containing the above reagents without any sample were used as control. The test tubes were placed in a boiling water bath (Uniscope, SM801A England) for 10 min. They were then cooled and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 552 nm. This assay was carried out in triplicate.

Calculation

$$\text{Inhibition of TBA } (\%) = 1 - \frac{E}{C} \times 100$$

Where C = absorbance of fully oxidized control and E = absorbance in the presence of fraction/standard. The IC_{50} values were also computed for the standards and test fractions.

Inhibition of lipid peroxidation (LPO) assay

The inhibition of LPO by test samples and two standards (Gallic acid and ascorbic acid) was carried out by using egg

yolk homogenate as lipid-rich media according to the method described by Ruberto and Barrata [17]. A stock solution of 1 mg/ml was prepared for standards Gallic acid, ascorbic acid, and test fractions (aqueous, butanol, ethyl acetate and hexane leaf and stem). Egg yolk homogenate (0.5 ml, 10% v/v) was added to varying volumes of 10, 20, 50, and 100 μl of standards and test sample fractions and the volume made up to 1 ml with distilled water. Thereafter, 0.05 ml of $FeSO_4$ was added. The reaction mixture was subsequently incubated for 30 min at 37°C. After incubation, 1.5 ml of acetic acid was added to the reaction mixture, followed by 1.5 ml of 0.67% TBA in 20% sodium dodecyl sulfate. The resultant solutions were mixed in a vortex mixer and heated at 95°C for 60 min. After cooling, 5 ml of butan-1-ol was added, and the mixture centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and converted to the percentage inhibition using the formula:

$$\text{Inhibition of lipid peroxidation } (\%) = \frac{1-E}{C} \times 100$$

Where C = absorbance of fully oxidized control and E = absorbance in the presence of test fraction/standard.

Total antioxidant capacity (TAC)

The TAC of the test fractions of *C. afer* leaf and stem was carried out using phosphomolybdenum method as described by Priesto *et al.* [18]. An aliquot of 0.1 ml of the various 1 mg/ml test fractions was combined with 1 ml of working reagent solution containing 0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate. The working reagent was prepared by dissolving 0.1092 g of 28 mm sodium phosphate and 0.1236 g of 4 mm ammonium molybdate in 25 ml of 0.6 M H_2SO_4. The standard consisted of 0.1 ml of varying concentrations of 1 mg/ml ascorbic acid (water soluble antioxidant) in methanol with 1 ml of the working reagent. The blank consisted of 0.1 ml methanol and 1 ml of the working reagent. The solutions were incubated in a water bath at 95°C for 90 min. After the samples had cooled, the absorbance values were determined using a spectrophotometer at a wavelength of 695 nm against blank. The antioxidant activity of the extracts was measured and expressed as ascorbic acid equivalents.

Statistical Analysis

Statistical analysis was carried out with the aid of SPSS for windows; SPSS Inc., Chicago, Standard version 17.0 to determine differences between the mean of the test fractions using Mann–Whitney U-test analysis. Linear regression analysis was performed to determine IC_{50} for the test fractions. *T*-test analysis was also performed. $P < 0.05$ was considered significant. All analyses were performed in triplicate. Data were reported as mean ± standard error of the mean.

RESULTS

Table 1 shows that the proximate composition of carbohydrate, crude protein, crude fat, and crude ash content in *C. afer* leaf sample were not significantly ($P > 0.05$) different from that of the stem. However, the moisture content of the dried leaf (18.63 ± 2.11%) was significantly ($P < 0.01$) higher than that of stem (6.76 ± 0.67%) while the crude fiber content of stem (27.28 ± 1.54%) was significantly ($P < 0.05$) higher than that of the leaf (21.16 ± 0.86%).

Table 2 shows that there were no significant differences ($P > 0.05$) between the amount of minerals detected in the leaf and stem. However, the amount of calcium, magnesium, and sodium were appreciably elevated in both stem and leaf samples compared with the other minerals detected. Cadmium was not detected in both samples.

The data in Figure 1 and Table 3 shows that all test fractions scavenged DPPH radical in a concentration dependent manner. However, the aqueous leaf fraction (IC_{50} = 259.07 μg/ml) exhibited a significantly ($P < 0.05$) high DPPH scavenging

Table 1: Proximate composition of *C. afer* leaf and stem

Parameters	Leaf (%)	Stem (%)
Moisture content	18.63±2.11	6.76±0.67
Crude fiber content	21.16±0.86	27.28±1.54
Crude ash content	11.47±1.47	10.91±0.50
Crude fat content	1.83±0.43	1.75±0.48
Crude protein content	2.75±0.56	2.93±1.16
Carbohydrate content	55.83±3.71	50.38±1.27

C. afer: Costus afer

Table 2: Mineral composition of *C. afer* leaf and stem

Mineral element	Leaf (mg/kg)	Stem (mg/kg)
Calcium	7.69±1.12	7.92±0.25
Magnesium	4.01±1.25	3.64±1.15
Potassium	1.02±0.34	0.95±0.03
Sodium	1.97±0.12	2.25±1.07
Chromium	0.07±0.01	0.10±0.05
Lead	0.01±0.00	0.02±0.00
Manganese	0.82±0.02	0.75±0.12
Nickel	0.17±0.01	0.12±0.05
Copper	0.44±0.02	0.52±0.11
Cadmium	ND	ND

ND: Not detected, *C. afer: Costus afer*

Table 3: Fifty percent inhibitory concentrations of different fractions of *C. afer* leaf and stem in different antioxidant system *in vitro*

Fractions of *C. afer*	IC_{50}		
	DPPH (50-500 μg/ml)	TBARS (0.1-1.0 μg/ml)	LPO (10-100 μg/ml)
Butanol stem	367.65	0.68	57.94
Butanol leaf	555.56	0.49	47.21
Hexane stem	537.63	0.49	51.02
Hexane leaf	333.33	0.48	52.19
Ethyl acetate stem	2500.0	0.40	60.39
Ethyl acetate leaf	1923.08	0.41	48.40
Aqueous stem	271.74	0.37	41.15
Aqueous leaf	259.07	0.38	43.90
Gallic acid	206.61	0.36	44.40
Ascorbic acid		0.40	44.09

DPPH: 1,1 diphenyl 2 picrylhydrazyl, TBARS: Thiobarbituric acid reactive species, LPO: Lipid peroxidation

activity compared with the other test fractions while ethyl acetate stem fraction (IC_{50} = 2500.00 μg/ml) had the least DPPH scavenging activity.

The data in Figures 2 and 3 and Table 3 shows that the test different leaf and stem fractions, Gallic acid and ascorbic acid scavenged thiobarbituric acid reactive species (TBARS) in a concentration dependent manner. The aqueous stem (IC_{50} = 0.37 μg/ml) had the highest TBARS scavenging activity while butanol stem had the lowest scavenging activity of TBARS. The LPO inhibitory activity of *C. afer* leaf and stem fractions showed that aqueous stem fraction (IC_{50} = 41.15 μg/ml) had the highest inhibition of LPO while ethyl acetate stem fraction (IC_{50} = 60.39 μg/ml) had the lowest inhibition of LPO activity [Figure 4 and Table 3].

TAC of the different fractions of *C. afer* leaf and stem showed that the aqueous leaf fraction had a significantly ($P < 0.05$) high TAC (7.95 ± 0.37 mg ascorbic acid equivalent/g); followed by hexane leaf fraction (7.07 ± 0.06 mg ascorbic acid equivalent/g)

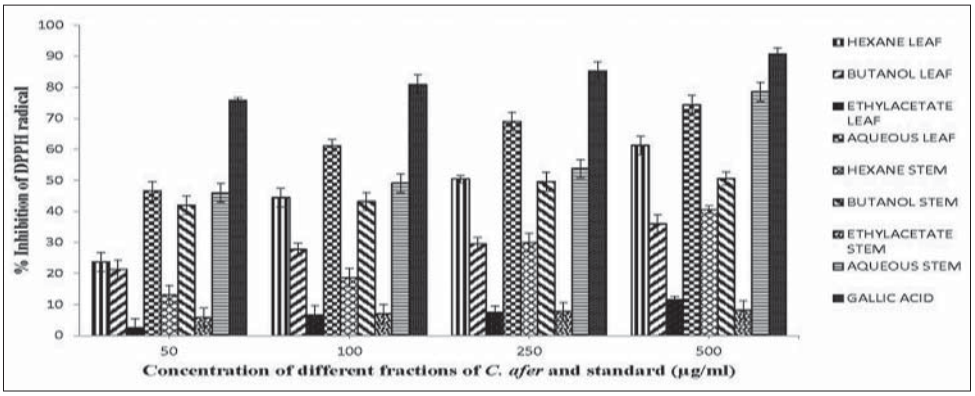

Figure 1: Percentage inhibition of 1,1 diphenyl 2 picrylhydrazyl radical by different concentration of *Costus afer* fractions

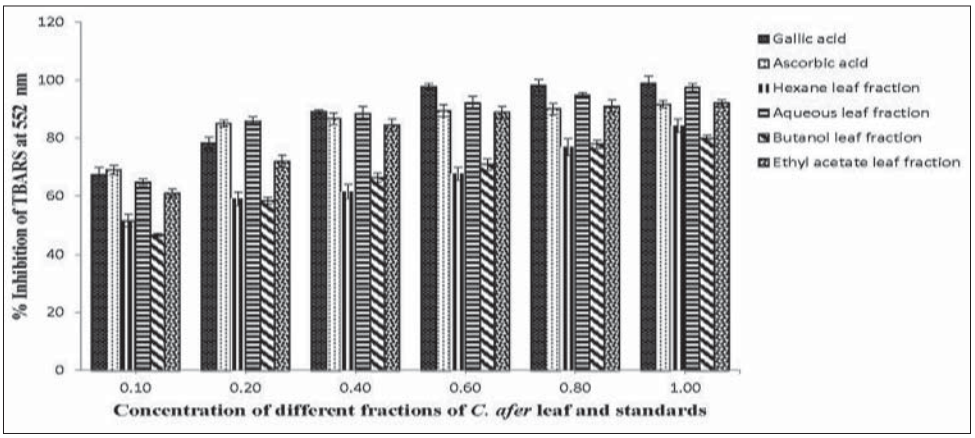

Figure 2: Percentage inhibition of thiobarbituric acid reactive species by different concentration of *Costus afer* leaf fractions

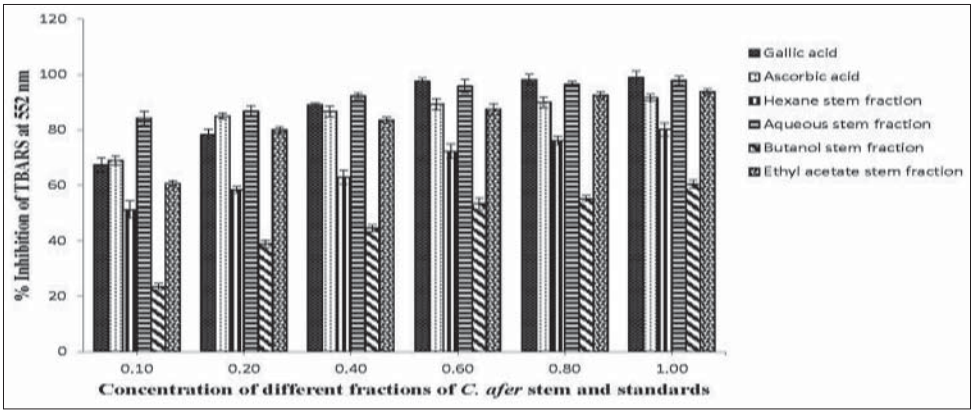

Figure 3: Percentage inhibition of thiobarbituric acid reactive species by different concentration of *Costus afer* stem fractions

and aqueous stem fraction (6.85 ± 0.36 mg ascorbic acid equivalent/g) when compared with other test fractions [Figure 5 and Table 3].

DISCUSSION

The proximate analysis data indicated that *C. afer* leaf and stem contained an appreciable amount of carbohydrate and crude fiber. These suggest that *C. afer* could serve as a source of energy and dietary fiber. Previous studies have shown that consumption of carbohydrates provides the body with the necessary energy required to drive cellular metabolism while dietary fiber could prevent the incidences of cardiovascular diseases, arteriosclerosis and increase intestinal transit time [19]. This may account for its use as folder in livestock production. The ash content also indicates that it could be a good source of minerals. The moisture content of the stem might be due to the succulent nature of the plant stem and could serve as a readily available source of fluid for quenching of thirst when dehydrated, especially in hot farm areas where water is not easily assessable to farmers, while the moisture content of the leaf could serve as a source of water to farm animals. However, the elevated moisture content of dried *C. afer* leaf compared to the dried stem indicates that the leaf may have reduced shelf life. The crude fat and protein contents of the leaf and stem suggested that they could be an important source for dietary fat and protein feed supplementation.

The mineral analysis revealed that the levels of calcium, magnesium, sodium, potassium, and manganese in *C. afer* leaf and stem were appreciably high compared to the other minerals detected. These minerals are essential in the body system for disease prevention and control [20]. The presence of these minerals may account for the ethnomedical use of *C. afer* in the treatment and management of inflammatory diseases. Calcium and potassium are important for growth and maintenance of strong bones, muscular function, synthesis of enzymes, and normal physiological function of the body [20]. Potassium and sodium also helps in the maintenance of acid-base balance in the body and osmotic pressure [21]. Magnesium serves as a cofactor for enzymes activation and biological structure promoter [22]. Manganese is an important modulator of cells functions and play vital role in the control of diabetes mellitus [23]. Chromium has been shown to participate in sugar metabolism and possible in the prevention of diabetes [24]. Nickel serves as a cofactor of important antioxidant enzymes such as superoxide dismutase [25]. Copper is a very powerful pro-oxidant and catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid [26]. The lead levels of 0.02 mg/kg stem and 0.01 mg/kg leaf may not lead to any health hazard in consumers since it is lower than the maximum permissible limit of 30 mg/kg lead for vegetables (FAO/WHO/2001)[20] and thus are within safe limits for the use of *C. afer* stem and leaf as herbal medicine.

Evaluation of the antioxidant activity of the different fractions of *C. afer* leaf and stem *in vitro* showed that they reduced DPPH to 1,1 diphenyl 2 picrylphenylhydrazine in a concentration dependent manner as evidenced by the decolorization of DPPH radical from purple to yellow color. Previous study had shown that when DPPH radicals react with suitable reducing agent

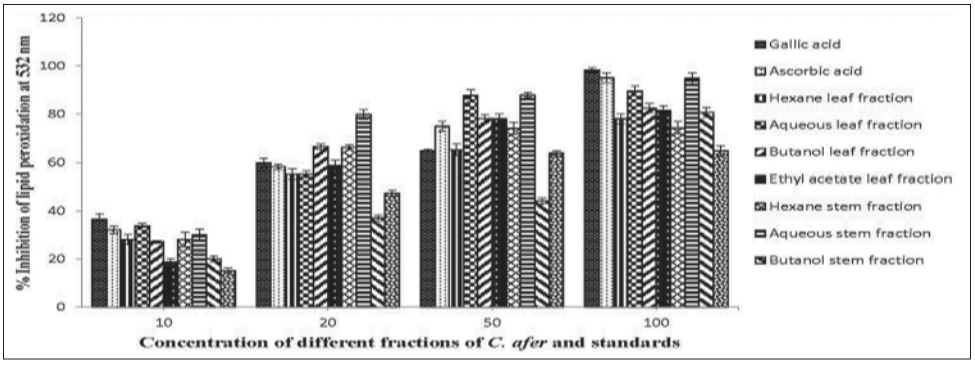

Figure 4: Percentage inhibition of lipid peroxidation by different concentration of *Costus afer* fractions

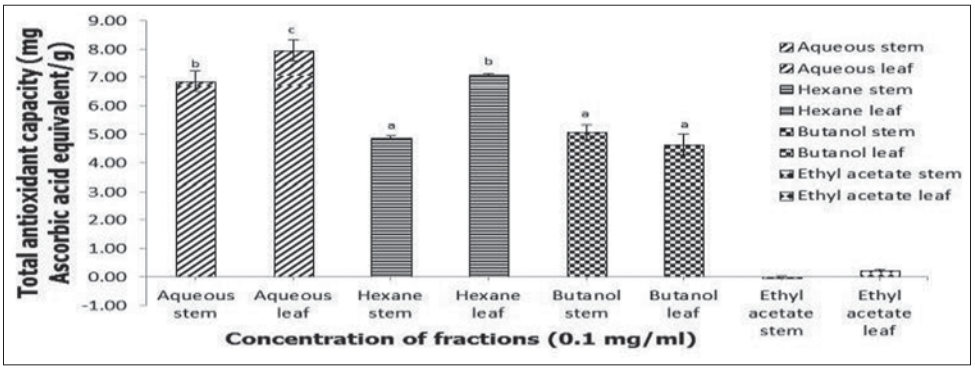

Figure 5: Total antioxidant capacity of *Costus afer* leaf and stem fractions

it loses color stoichiometrically depending on the number of electrons taken up [27]. The aqueous leaf fraction exhibited the highest DPPH scavenging activity when compared with the other test fractions. This indicates that the polar fraction of *C. afer* may contain higher antioxidant compounds than the non-polar fractions. Previous studies have shown that the majority of the antioxidant compounds are usually polar in nature. This is due to the presence of the hydroxyl groups present in the polyphenol and flavonoid ring structures of the antioxidant compounds [28].

In TBARS scavenging assay, the different test fractions inhibited the formation TBARS in a concentration dependent manner. The aqueous fractions of *C. afer* exhibited the highest TBARS scavenging activity when compared to the other test fractions. This also indicates that the polar fractions contained higher antioxidant activity than the non-polar fractions. Furthermore, the capacity of the various test fractions to break the chain reactions generated by propagating LPO was also in a concentration dependent manner. The aqueous fraction exhibited the highest inhibition of LPO when compared with the other test fractions. This further suggests that the polar fractions possess higher antioxidant activity than the non-polar fractions. LPO of membranes is part of the early events that takes place during the inflammatory response by stimulated polymorphonuclear cells and thus agents capable of preventing LPO of membranes could serve as a suitable candidate in anti-inflammatory drug discovery process [29].

Further study also demonstrated that the aqueous leaf fraction had a significantly ($P < 0.05$) high TAC when compared with the other test fractions using phosphate molybdenum assay, which is based on the reduction of molybdenum (VI) to molybdenum (V) with the subsequent formation of a green phosphate-molybdenum (V) complex in acidic condition. This further suggests that the aqueous fraction of *C. afer* leaf may possess a higher antioxidant activity *in vitro* when compared with the other test fractions. The reduction of molybdenum (VI) to molybdenum (V) may be attributed to the hydrogen and electron donating potentials of polyphenolic compounds present in *C. afer* [18]. This may also account for the higher antioxidant activity exhibited by the polar fractions in the various studied models as they tend to contain a lot of polyphenolic compounds [28].

CONCLUSION

The data from this study showed that the *C. afer* leaf and stem could serve as an important source nutrients and minerals. The leaf fractions of *C. afer* had more antioxidant activity than the stem fractions *in vitro*. More so, among the test fractions, the most active antioxidant fraction was the aqueous fraction followed by hexane, *n*-butanol and ethyl acetate fraction. These findings may explain the use of *C. afer* stem and leaf extracts in the ethnomedical practice as a therapeutic agent against most of the inflammatory and oxidative stress related diseases including rheumatoid arthritis and hepatic disorders.

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