

Full Length Research Paper

Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae)

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Medicinal plants contain bioactive compounds capable of preventing and fighting oxidative related diseases. These compounds must be screened and assayed before effective drugs are developed. Thus, phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker-Gawl were evaluated. *C. afer* contained flavonoids, phenols, anthraquinones, cardiac glycosides, terpenoids, alkaloids and tannins. Preliminary screening of free radical scavenging activity of extracts with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) using thin layer chromatography tested positive. The aqueous extract had a higher free radical scavenging activity with IC₅₀ (concentration of samples required for 50% inhibition of DPPH radical activity) value of 64.42 µg/ml than methanol extract (92.33 µg/ml). Furthermore, total phenolic content in aqueous extract (0.66 ± 0.02 mg gallic acid equivalent/g) was significantly higher (p < 0.05) than methanol extract (0.52 ± 0.01 mg gallic acid equivalent/g). In addition, inhibition of lipid peroxidation by aqueous extract (80.60 ± 0.28%) was significantly higher (p < 0.05) than methanol extract (77.00 ± 0.84%). Nevertheless, methanol and aqueous extracts of *C. afer* possess anti-oxidative properties as well as bioactive metabolites. Thus, stem extracts of *C. afer* could serve as sources of antioxidants and bioactive compounds for nutrition and therapeutic purposes.

Key words: *Costus afer*, phytochemicals, antioxidant, phenol, nutrition, therapeutic.

INTRODUCTION

The plant *Costus afer* Ker-Gawl. (Costaceae) is among 150 species of stout, perennial and rhizomatous herbs of the genus *Costus* (Edeoga and Okoli, 2000). It can be found in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone and Nigeria (Burkill, 1985; Edeoga and Okoli, 2000). *C. afer* is commonly called bush cane, ireke omode (Yoruba-Western part of Nigerian) and opete (Igbo-Eastern part of Nigeria). It bears white and yellow flowers (Stentoft, 1988). The stem, seeds and rhizomes are harvested from the wild plant and they contain several bioactive metabolites.

Medicinal plants continue to be a major source of drugs and natural products on the basis of their therapeutics (Lown, 1993) in virtually all cultures (Anwannil and Atta, 2006). The plants possess potent bioactive compounds capable of preventing and treating most oxidative related diseases (Dahanuka et al., 2000; Farombi and Fakoya, 2005) and have often been used in folkloric medicine (Wang et al., 2007). In developing countries, the use of medicinal plants in the treatment of infectious disease is rife and reasons include the high cost of effective drugs (Okeke et al., 1999). However, potential indigenous plants exploited for medicinal purposes have to undergo basic phytochemical screening and bioassay as first step towards the ultimate development of drugs (Odebiyi and Sofowora, 1998). Therefore, the present study aims at investigating the potentials of *C. afer* as a source of

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antioxidants for nutritional and therapeutic purposes.

MATERIALS AND METHODS

Plant material

The stem of *C. afer* was procured at Ayepe, Shagamu in Ogun State, Nigeria and identified by Prof. E. B. Esan, a plant scientist in the Department of Chemical and Environmental Sciences, Babcock University, Ilisan-Remo, Ogun State.

Chemicals

DPPH (2,2 diphenyl-1-picrylhydrazyl), gallic acid, ascorbic acid and Folin-Ciocalteu's reagent were purchased from Sigma Aldrich, USA. All other chemicals and reagents used were of analytical grade.

Extraction

Leaves and bark were plucked and peeled off from plant stem while the pitch was cut into tiny pieces and sun-dried for 72 h. 150 g of pulverized dried sample was soaked in 600 ml distilled water and 600 ml methanol for 72 h before extraction. Thereafter, it was filtered through Whatman filter paper No. 1. The filtrate was concentrated under reduced pressure at 80°C using rotary evaporator and preserved in refrigerator at 4°C for further use.

Phytochemical screening

Phytochemical screening was performed on the extracts using standard procedures to identify chemical constituents as described by Trease and Evans (1989), Harborne (1973) and Sofowora (1993).

Screening for alkaloid

0.5 g of the extract was stirred in 5 ml of 1% HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1 ml of the filtrate was treated with a few drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids.

Screening for flavonoids

Two millilitres of dilute sodium hydroxide was added to 2 ml of the extract. The appearance of a yellow colour indicates the presence of flavonoids.

Screening for saponins

One millilitre of distilled water was added to 1 ml of the extract and shaken vigorously. A stable persistent froth indicated the presence of saponins.

Screening for phenols

Equal volumes (1 ml) of extract and Iron (III) chloride were mixed. A deep bluish green solution gave an indication of the presence of phenols.

Screening for tannins

A portion of the extract was dissolved in water, after which the solution was clarified by filtration. 10% ferric chloride solution was then added to the resulting filtrate. The appearance of a bluish black colour indicates the presence of tannins.

Screening for anthraquinones

0.5 g of the extract was shaken with 10 ml of benzene and filtered. 10% of ammonia solution was added to filtrate and the mixture was shaken. The formation of a pink, red or violet colour on the ammoniacal phase indicates the presence of anthraquinones.

Screening for cardiac glycosides

0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 1 drop of ferric chloride solution. This was under layered with 2 ml of concentrated sulphuric acid. A brown ring formation at the inter phase indicates the presence of deoxy sugar characteristics of cardiac glycosides.

Screening for phlobatannins

A few drops of 1% HCl was added to 1 ml of extract and boiled. A red precipitation indicates the presence of phlobatannins.

Screening for terpenoids

0.5 ml of acetic anhydride was mixed with 1 ml of sample extract and a few drops of concentrated H₂SO₄. A bluish green precipitate indicates the presence of terpenes.

Screening for cardenolides

2 ml of benzene was added to 1 ml of the sample extract. The formation of a turbid brown colour is an indication of the presence of cardenolides.

Antioxidant assay

Rapid thin layer chromatography (TLC) screening for antioxidant activity was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. The plates were developed in methanol: ethyl acetate (2:1) and afterwards air-dried and sprayed with 0.2% w/v DPPH spray in methanol. The plates were visualized for the presence of yellow spots. Radical scavenging activity of extracts was performed according to the DPPH spectrophotometric method of Mensor et al. (2001). One millilitre of 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard (100, 200, 300 and 400 µg/ml) and allowed to react at room temperature for 30 min. The absorbance (Abs) of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula:

$$AA\% = [100 - ((Abs_{\text{sample}} - Abs_{\text{blank}}) \times 100)] / Abs_{\text{control}} \quad (1)$$

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solutions of ascorbic acid and gallic acid served as positive controls. This assay was carried out in triplicates for

each concentration. IC_{50} values denotes the concentration of extracts required to scavenge 50% of DPPH free radicals

Determination of total phenolic content

The total phenolic content was estimated as described by Singleton and Rossi (1965) and modified by Gulcin et al. (2003). 1 ml aliquot of extracts or standard solution of gallic acid (10, 20, 30, 40 and 50 mg/l) was added in a volumetric flask containing 9 ml of water. One milliliter of Folin-Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 min at 25°C. The absorbance against reagent blank was determined at 750 nm. A reagent blank was prepared and the amount of phenolic compound in the extract was determined from the standard curve. The total phenolic content of the plant was then calculated as shown in the equation below and expressed as mg gallic acid equivalent (GAE)/g fresh weight. All samples were analyzed in duplicates.

Equation:

$$C = c \cdot m / V \quad (2)$$

Where C = total content of phenolic compound in gallic acid equivalent (GAE)/g, c = the concentration of gallic acid established from the calibration curve ($\mu\text{g/ml}$), V= volume of extract (ml), and m = weight of the crude plant extract (g).

Inhibition of lipid peroxidation

A modified thiobarbituric acid reactive substances (TBARS) assay was used to measure the lipid peroxide formed using egg yolk homogenate as lipid-rich media (Ruberto and Baratta, 2000). Egg homogenate (0.5 ml, 10% v/v) was added to 0.1 ml of extract (1 mg/ml) and the volume made up to 1 ml with distilled water. Thereafter, 0.05 ml of FeSO_4 was added and the mixture incubated for 30 min. 1.5 ml of acetic acid was then added and followed by 1.5 ml of TBA in SDS. The resulting mixture was vortexed and heated at 95°C for 60 min. After cooling, 5 ml of butan-1-ol was added and the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and converted to percentage inhibition using the formula:

$$\text{Inhibition of lipid peroxidation (\%)} = (1 - E/C) \times 100 \quad (3)$$

Where C = absorbance of fully oxidized control and E = absorbance in the presence of extract.

Statistical Analysis

Mann-Whitney U test and Non-linear regression for IC_{50} was performed using GradPad prism version 5.00 for Windows, GraphPad Softwares, San Diego California. USA. P values less than 0.05 were considered significant.

RESULTS

Phytochemical analysis of extracts revealed the presence of flavonoids, phenols, anthraquinones, cardiac glycosides and terpenoids. In addition, aqueous extract of *C. afer* tested positive for alkaloids and tannins. However, all extracts tested negative for saponins, phlobatannins and cardenolides (Table 1).

Table 1. Phytochemical constituents of aqueous and methanol extracts of *C. afer*.

Phytochemical	Methanol extract	Aqueous extract
Alkaloids	-	++
Flavonoids	+	++
Saponins	-	-
Phenols	+	++
Tannins	-	+
Anthraquinones	++	++
Cardiac glycosides	+	++
Phlobatannins	-	-
Terpenoids	++	++
Cardenolides	-	-

++ = Abundant, + = trace and - = absent.

Rapid TLC screening for antioxidant activity was positive for all the extracts. Furthermore, IC_{50} values for methanol, aqueous, ascorbic acid and gallic acid were 92.33, 64.42, 51.40 and 37.92 $\mu\text{g/ml}$, respectively (Table 2).

The total phenolic content of crude extracts was obtained from the regression equation (R) for the calibration curve of standard gallic acid ($y = 0.001x + 0.007$, $R^2 = 1$) and expressed as gallic acid equivalent (GAE). The aqueous extract (0.66 ± 0.02 mg GAE/g) had significantly ($p < 0.05$) higher total phenolic content than methanol extract (0.54 ± 0.01 mg GAE/g) (Table 2). The aqueous extract ($80.6 \pm 0.28\%$) had a significantly ($p < 0.05$) higher percentage inhibition of lipid peroxidation than methanol extract ($77.00 \pm 0.84\%$) (Table 2).

DISCUSSION

The present study investigated the phytochemical constituent of methanol and aqueous extracts of *C. afer*. The results indicated, presence of alkaloids and tannins in aqueous extract. Alkaloids are known to have anti-microbial, antifungal and anti-inflammatory effect (Okwu and Okwu, 2004) and it also acts as an anti-hypertensive agent (Sofowora, 1993). The folkloric use of *C. afer* in the treatment of sore throat, diarrhea, hemorrhage and wound healing might be due to presence of tannins (Okwu and Okwu, 2004). Cardiac glycosides and anthraquinone tested positive in methanol and aqueous extracts. Cardiac glycosides had been reported to be effective in the treatment of congestive heart failure and regulation of heart beat (Leverin and McMatron, 1999). Anthraquinones can induce laxative effect (Muller-Lissner, 1993) and hence, the use of *C. afer* as laxative and nervous system depressant may result from the presence of anthraquinones (Ayoola et al., 2008). Flavonoids and phenols were abundant in aqueous extract than methanol extract. These are potent water soluble antioxidants which prevent oxidative cell damage

Table 2. Quantitative determination of total phenol, antioxidant and lipid peroxidative activity of aqueous and methanol extract of *C. afer*.

Sample extract	Concentration (µg/ml)	Percentage antioxidant activity	Total phenol (mg/g GAE)	Percentage inhibition of lipid peroxidation
Methanol extract of <i>C. afer</i>	100	67.14 ± 1.58	0.54 ± 0.01	77.00 ± 0.84
	200	68.67 ± 0.26		
	300	72.29 ± 0.26		
	400	74.85 ± 0.25		
		92.33*		
Aqueous extract of <i>C. afer</i>	100	68.04 ± 0.09	0.66 ± 0.02	80.6 ± 0.28
	200	71.04 ± 0.18		
	300	79.58 ± 0.08		
	400	83.78 ± 0.08		
		64.42*		
Gallic acid	100	78.36 ± 1.05		
	200	82.24 ± 0.66		
	300	84.73 ± 0.66		
	400	86.67 ± 1.18		
		37.92*		
Ascorbic acid	100	71.87 ± 2.21		
	200	76.43 ± 3.03		
	300	78.77 ± 2.91		
	400	88.97 ± 1.11		
		51.40*		

*IC₅₀ value (concentration of sample required for 50% inhibition of DPPH radical activity).

suggesting antiseptics, anticancer, anti-inflammatory effects and mild anti-hypertensive properties (Del-Rio et al., 1997; Okwu, 2004).

Furthermore, plant phenolics are major group of compounds acting as primary antioxidants or free radical scavengers (Kahkonen et al., 1999). The therapeutic potential of antioxidants in controlling degenerative diseases with marked oxidative damage from reactive oxygen species or free radicals have been reported (Larson, 1988; Tripathi, 1999; Vani et al., 1997). Aqueous extract of *C. afer* with high phenolic content showed higher antioxidant and inhibition of lipid peroxidative activity than methanol extract. These suggest its potential in the treatment and prevention of various oxidative related diseases. Therefore, stem extract of *C. afer* could be exploited as sources of free radical scavengers and bioactive metabolites for nutritional, medicinal and commercial purposes.

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