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Full Length Research Paper

Phytochemical characterization of the extracts of *Aframomum danielli* flower, leaf, stem and root

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Phytochemical screening of methanol extracts of the leaves, flowers, stems and roots of Aframomum danielli revealed the presence of alkaloids, cardenolides, flavonoids and polyphenols. Fractions from the extracts were tested for antioxidant activities and lipoxygenase enzyme inhibition. Flower fraction FF5 and leaf fraction LF4, which were the most potent fractions, gave antioxidant effectiveness values of 85.32 ± 0.27 and 53.92 ± 0.03%, respectively on 1,1, diphenyl 1,2 picrylhydrazyl (DPPH) free radicals at a level of 750 µg/ml. The lipoxygenase enzyme inhibition at 750 µg/ml was 87.11 ± 5.13% (FF5) and 82.55 ± 2.62% (LF4). All fractions inhibited Bacillus subtilis, staphylococcus aureaus, Escherichia coli, Salmonella enteriditis, Candida albicans and Aspergillus flavus.

Key words: Phytochemical, *Aframomum danielli,* leaf, flower, stem, root, characterization.

INTRODUCTION

It is well known that food which is rich in natural antioxidants leads to a limited incidence of cardio and cerebro-vascular diseases (Hertog et al., 1993). Natural anti-oxidants are present in compounds belonging to several classes of phytochemical components such as phenols, flavonoids carotenoids and tannins (Vincenzo et al., 1999). These compounds are able to scavenge free radicals such as oxygen, hydroxyl or liquid peroxyl radical in plasma (Hertog et al., 1993; Miller et al., 2000[a,b,]; Frei et al., 1988). Besides, free radical oxidation of the lipid components in foods is a major problem for food manufacturers and thus the early attempts to measure antioxidative activity were mainly focused on lipid protection (Frankel, 1980). So far synthetic materials such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are being applied to reduce problems associated with lipid oxidation in foods. However reports on the harmful effects of these compounds call for research into the use of plant products as alternatives to synthetic additives (Adegoke and Odesola, 1996; Mclellan et al., 1995; Adegoke and GopalaKrishna, 1998). The potent sources of natural antioxidants are spices and herbs (Caraleuro et al., 2006; Helle et al., 2004; Rita et al., 2009; Fasoyiro and Adegoke, 2007*). Aframomum danielli* is a spice belonging to the genus Aframomum and the family zingiberaceae. The seed of *A. danielli* plant has been found to contain phytochemicals (Fasoyiro and Adegoke, 2007).

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MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade, obtained from fluka chemical.

Plant materials

The leaf, flower, stem and roots of freshly harvested plant of *A. danielli* were collected from a farmer in Ibadan, Oyo State, Nigeria, sun dried for a week and trimmed to remove extraneous materials. The samples were pulverized using a warring blender. The ground spice was sieved (200µm aperture sieve) and packaged in a polythene bag till used.

Phytochemical screening of *A. denielli* samples

This was carried out on ground spice using the method described

Table 1. Phytochemical screening of plant parts.

	Test			
Alkanoids	**Leaf**	**Flower**	**Stem**	**Root**
Dragendraff	+ve	+ve	+ve	+ve
Mayer's test	+ve	+ve	-ve	+ve
Wagner's test Cardenolides	+ve	+ve	+ve	+ve
Keller-killani	+ve	+ve	+ve	+ve
Kedde	-ve	+ve	-ve	+ve
Antihraquinones saponins	-ve	-ve	-ve	-ve
Frothing Tannins	+ve	+ve	+ve	+ve
Ferric chloride	+ve	-ve	+ve	+ve
Phenolic group	+ve	+ve	-ve	+ve
Flavones	±	+ve	-ve	+ve
Carotenoids	±	+ve	-ve	-ve

+ve = presence of the phytochemical, -ve = absence of the phytochemical, ± = colour undefined.

by Trease and Evans (2002). Preparation of extracts from powder of *A. danielli* leaf, flower, stem and root: 200 g of each of the powdered, *A. danielli* samples was percolated with 1000 mi of 100% methanol at room temperature for 24 h and filtered. The extracts obtained were concentrated in vacuo at 50°C to give the crude extracts of the plant parts.

Fractionation of *A. danielli* leaf, flower, stem and root crude extracts

This was carried out using the method described by Hostettman et al. (1985). Five fractions were collected for each of the leaf and flower, while two were collected for each of the stem and root.

Anti-oxidative activities of *A. danielli* leaf, flower, stem and root of purified fractions

These were determined by 1,1,-diphenyl-1, 2-picrylhydrazyl (DPPH) assay as described by Aderogba et al. (2004). Ascobic acid was used as positive control.

Lipoxygenase (15-LO) enzyme inhibition 15

15-Lipoxygenase enzyme was used for peroxidation of linoleic acid as described by Lyckander and malternel (1992).

Anti-microbial effect of *A. danielli* leaf, flower, stem and root fractions

This was carried out by Agar diffusion method (Hugo and Russel, 1983). The test organisms were: *Bacillus subtilis, staphylococcus aureus, Escherichia coli ,Salmonella enteriditis, Candida albicans* and *Aspergillus flavus.* Ampicillin (100 ppm) and Tioconazole (100 ppm) were used as controls.

RESULTS AND DISCUSSION

The phytochemical screening of *A. danielli* leaf, flower, stem and root revealed the presence of phytochemicals which are of anti-oxidative and anti-microbial interest. The result of phytochemical screening is presented in Table 1. *A. danielli* parts tested positive to Meyer's test and confirmatory Dragendroff's test indicated the presence of alkaloids. The flower and roots were tested positive for cardenolides; Keller-killanis and Kede's test indicated the presence of sugar as glycosides. Flavones (flavonoids) were present in flower and root but not as intense in leaf.

Anti-oxidant activities (%) on DPPH free radical

The result of the anti-oxidant activities of the fractions is given in Table 2. Flower fraction FF5 had average anti-oxidant activities of 93.38 ± 3.15, 91.54 ± 1.36 and 85.94 ± 0.27% at concentrations of 250, 500 and 750 µg/ml respectively. The leaf, stem and root fractions are not so potent in scavenging DPPH radicals; the leaf, stem and root showed maximum anti-oxidant activities of 53.92 ± 0.11, 53.84 ± 0.39 and 52.69 ± 0.71% respectively at concentrations of 750 µg/ml on DPPH free radicals. The activities in leaf, stem and root fractions in this assay may be caused by lack of hydrogen donating capacity of the leaf, stem and root.

Lypoxygenase enzyme (15-LO) inhibition by leaf, stem and root fractions (Table 3)

The leaf fractions showed higher anti-oxidant activities towards 15-LO enzyme in comparism to DPPH radicals. The flower fraction FF5 followed closely by leaf fraction LF4 had high antioxidant activities values of 87.11 ± 5.13 and 82.51 ± 2.62%, respectively. This could be due to the fact that involvement of proton donation from the active compounds may be less important for 15-LO inhibition (Lyckander and Malternal, 1992).

Table 2. Anti-oxidant activities of purified fractions of *A. danielli* flower, leaf, stem and root on 1,1-diphemyl 1,2 picrylhydrazy (DPPH) free radical at various concentrations.

Sample fractions	Average anti-oxidant activity (%) of sample fractions				
	25 µg/ml	50 µg/ml	250 µg/ml	500 µg/ml	750 µg/ml
FF1	-82.23 ± 3.04	-1.0 ± 0.11	1.39 ± 0.30	46.63 ± 0.70	55.56 ± 0.96
FF2	-66.67 ± 4.32	5.87 ± 1.21	25.36 ± 1.00	69.19 ± 0.52	64.21 ± 0.51
FF3	-73.90 ± 5.12	3.12 ± 0.92	48.82 ± 0.21	64.29 ± 2.27	73.46 ± 1.02
FF4	-82.25 ± 2.56	4.23 ± 0.18	70.15 ± 0.23	80.72 ± 0.09	80.08 ± 1.39
FF5	11.93 ± 1.05	86.53 ± 3.21	93.38 ± 3.15	91.54 ± 1.36	85.94 ± 0.27
LF1	-37.78 ± 3.51	-28.32 ± 1.51	-53.83 ± 0.83	18.35 ± 4.04	16.90 ± 0.12
LF2	-33.66 ± 1.91	-24.76 ± 3.20	-40.80 ± 6.78	22.71 ± 0.36	33.17 ± 0.23
LF3	-35.71 ± 3.31	-26.84 ± 3.90	-13.15 ± 1.46	20.99 ± 0.41	34.95 ± 0.03
LF4	-38.23 ± 1.59	-17.36 ± 3.96	-10.94 ± 0.25	36.60 ± 2.38	53.92 ± 0.11
LF5	-66.31 ± 5.59	-55.68 ± 4.17	-8.75 ± 5.90	41.10 ± 0.10	51.33 ± 0.21
RF1	-70.34 ± 3.79	-51.31 ± 6.30	-37.57 ± 3.10	38.42 ± 1.28	32.12 ± 0.21
RF2	-36.42 ± 4.11	-15.52 ± 2.30	-13.71 ± 0.10	45.72 ± 2.41	52.69 ± 0.71
SF2	-47.99 ± 3.92	-24.08 ± 4.10	-11.67 ± 0.70	18.18 ± 1.16	52.17 ± 1.20
Ascobic acid	84.01 ± 1.34	92.01 ± 1.22	92.56 ± 1.02	93.06 ± 1.52	85.21 ± 1.07

* Mean of three readings, ± Standard deviation, FF – Flower fraction, LF – Leaf fraction, RF – Root fraction, SF – Stem fraction.

Table 3. Inhibition of 15-lipoxygenase (15-LO) enzyme by *A. danielli* flower leaf stem and root fractions.

Sample	Enzyme inhibition activity (%) of sample fractions		
	250 µg/ml	500 µg/ml	750 µg/ml
FF1	25.11 ± 1.11	33.11 ± 0.16	40.55 ± 1.12
FF2	21.79 ± 1.09	29.12 ± 1.10	47.36 ± 1.26
FF3	17.11 ± 1.18	48.11 ± 2.16	52.15 ± 1.19
FF4	63.76 ± 2.17	76.12 ± 2.51	82.33 ± 2.26
FF5	75.11 ± 3.42	79.11 ± 3.11	87.11 ± 1.13
LF1	44.10 ± 1.61	45.12 ± 1.17	47.88 ± 2.29
LF2	37.35 ± 1.51	51.11 ± 1.01	55.34 ± 2.21
LF3	53.13 ± 1.49	53.22 ± 2.00	60.11 ± 1.78
LF4	61.35 ± 2.11	77.11 ± 3.52	82.51 ± 2.62
FL 5	62.55 ± 3.12	70.05 ± 2.99	77.30 ± 2.51
RF1	45.19 ± 2.10	44.17 ± 1.76	46.55 ± 1.79
RF2	42.11 ± 3.42	46.12 ± 3.11	51.16 ± 2.11
SF1	37.35 ± 101	45.13 ± 2.81	43.26 ± 0.70
SF2	38.11 ± 1.82	39.23 ± 1.76	40.11 ± 0.30

*Mean of three reading, ± Standard deviation, Ff – Flower fractions, LF – leaf fractions, RF – Stem fractions, BF – stem fraction.

Zones of inhibition of *A. denielli* leaf, flower, stem and root fractions on food born pathogens

Table 4 shows the zones of inhibition of the partially purified fractions *A. danielli* plant on some food borne pathogens in comparison with ampicillin. Flower fraction FF5 had the highest antimicrobial activity on *B. subtilis, S. aureus* and *Salmonella enteridits* among all the fractions. The flower fraction FF_5 and root fraction RF_2 had higher antimicrobial activity on *S. enteridits* in comparison with ampicillin. Root fraction RF_2 had higher activity on *E. coli* compared to ampicillin, leaf fraction F1 had the least activity on the pathogenic bacteria. Table 5 shows the zones of inhibition of *A. danielli* plant parts on *Candida albicaus* and *A. flavus*. Flower fraction FF_5 had higher antimicrobial activities on *C. albicans* and *A. flavus* compared to Tioconazole. Stem fraction SF_1 and root fraction RF_1 had the least activities on *C. abbicaus* and

Table 4. Zones of inhibitions (mm) of flower, leaf, root and stem fractions on some food borne pathogens at 750 µg/ml.

Fraction	Test organisms			
	B. subtilis	***S. aureus***	***E. coli***	***S. enteriditis***
FF_1	15.50 ± 1.50	15.80 ± 2.00	12.00 ± 2.00	11.00 ± 1.00
FF_2	18.50 ± 0.50	16.10 ± 0.30	14.00 ± 1.00	13.50 ± 1.00
FF_3	15.00 ± 3.00	12.00 ± 1.00	11.00 ± 0.50	12.00 ± 0.50
FF_4	17.00 ± 1.00	15.00 ± 2.0	14.00 ± 2.0	11.00 ± 1.00
FF_5	29.33 ± 1.00	30.00 ± 4.50	16.50 ± 0.75	24.50 ± 0.50
LF_1	9.50 ± 0.50	10.00 ± 0.50	-	-
LF_2	10.50 ± 0.50	10.70 ± 1.00	-	-
LF_3	-	-	10.00 ± 1.00	-
LF_4	28.33 ± 2.50	20.50 ± 1.00	20.00 ± 1.00	18.00 ± 2.00
LF_5	10.60 ± 00	11.00 ± 0.50	12.00 ± 0.50	-
RF_1	13.00 ± 1.00	14.00 ± 1.00	-	11.50 ± 1.00
RF_2	15.33 ± 1.25	12.33 ± 0.50	21.67 ± 0.32	19.70 ± 1.50
SF_1	12.00 ± 1.00	13.00 ± 2.00	14.00 ± 0.50	10.00 ± 0.50
SF_2	11.50 ± 0.50	18.00 ± 2.00	15.00 ± 15.00	0
Methanol (50%)	0	0	0	19.00 ± 0.50
Ampicillin (10 µg/ml)	28.66 ± 0.20	32.00 ± 0.50	20.50 ± 0.50	

*Mean of three readings, ± Standard deviation, FF – flower fractions, LF – Leaf fractions, RF – Root fractions, SF – Stem fractions.

Table 5. Zones of inhibition (mm) of *A. danielli* flower, leaf root and stem fractions on A. *flavus* and *C. albicans* at 750 µg/ml.

Fractions	***Candida albicans***	***Asperigillus flavus***
FF1	17.20 ± 0.40	14.20 ± 0.80
FF2	16.80 ± 1.20	16.50 ± 1.50
FF3	13.90 ± 0.60	17.20 ± 1.00
FF4	22.20 ± 0.70	20.50 ± 1.50
FF5	26.50 ± 1.50	28.00 ± 1.00
LF1	11.10 ± 0.40	10.00 ± 0.50
LF2	10.20 ± 0.60	9.50 ± 2.00
LF3	17.10 ± 0.40	12.50 ± 0.50
LF4	26.00 ± 1.00	22.00 ± 2.00
LF5	11.20 ± 0.30	10.50 ± 0.50
RF1	16.40 ± 0.70	9.00 ± 0.50
RF2	18.40 ± 1.50	20.00 ± 2.00
SF1	9.00 ± 1.00	11.00 ± 1.00
SF2	9.50 ± 1.00	10.00 ± 0.50
Methanol (50%)	0	0
Tioconazole (100 µg/ml)	25.00 ± 1.00	26.00 ± 1.00

* Mean of three readings, ± standard deviation.

flavus, respectively.

Conclusion

This study had been able to highlight some of the phytochemical components present in the leaf, flower, stem and root of *A. danielli* plants. This showed that this plant parts could serve as antioxidant and anti-microbial agent in food when purified and concentrated.

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