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Full Length Research Paper

Phytochemical characterization and the antimicrobial property of *Aframomum danielli* extract

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Characterization of preliminary phytochemical components of *Aframomum danielli* seeds was determined. Fractions of the seeds obtained by vacuum liquid chromatographic process were tested for antimicrobial activities. Phytochemical screening revealed the presence of alkaloids, cardenolides, carotenoids and polyphenols. All fractions obtained from the petroleum ether extract exhibited antimicrobial activity on food-borne pathogens with minimum inhibitory concentrations in the range of 100 – 800 microgram per millilitres.

Key words: Phytochemicals, *A. danielli* fractions, antimicrobial properties.

INTRODUCTION

Food preservations in form of synthetic chemicals have been known to be effective as antimicrobial agents and antioxidants (Sherwin, 1990). However, the possible toxicity of synthetic chemicals has been a subject of study for many years (Chang, 1977; Mishra and Dubey, 1994). Commercial antioxidants such as butylated hydroxyanisole (BHA), tetra–butylated hydroquinone (TBHQ) are effective antioxidants but their safety for human consumption is not assured (Ito et al., 1985). Questions concerning the safety of these chemicals in food products have led to increased scrutiny and reappraisal (Inatani et al., 1983). The use of natural occurring materials as presservatives is a promising alternative to the use of chemicals (Howell, 1986).

The potential sources of natural preservative are spices, herbs, fruits, seed, leaves, barks and roots (Pratt and Hudson, 1996). The strong association between increased consumption of these natural products and human diseases prevention has been explained by the content of the phytonutrients (Halliwell and Gutteridge, 1984).

Aframonum danielli is a spice belonging to the genus Aframomum of the family Zingiberaceae (Dalziel, 1948). The seeds are smooth, shining olive – brown with a turpentine – like taste and they are used medicinally. The nutritional profile of *A. danielli* had been reported by Adegoke and Skura (1994). The essential oils of the seed were also reported by Adegoke et al. (2003). Antimicrobial activities of the crude extracts of *A. danielli* against a number of microorganisms have been that the extracts of the spice inhibited the growth of bacteria: *Salmonella enteriditis, Pseudomonas fragi, Pseudomonas fluorescens, Proteus vulgaris, Streptococcus pyogenes, staphyloccus aureus* and molds *Aspergillus flavus, Aspergillus niger* reported (Adegoke and Skura, 1994; Fasoyiro et al., 2001). The antimicrobial properties of many other spices; sage, oregano, allispice, onions, garlic, ginger on food – borne pathogens and molds have been reported (Wu et al., 1982; Zaika et al., 1983; Shelef, 1980). Some components reported in these spices include alcohols, esters, terpenes, phenols and organic acids (Weiser, 1971). This paper reports the preliminary phytochemical compounds in *A. danielli* seeds and the antimicrobial properties of fractionated components of the petroleum ether extract.

MATERIALS AND METHODS

A. danielli pods were obtained from Ogbagi, Ondo state, Nigeria. The seeds were removed from the pods and cleaned of the extraneous material. The seeds were pulverized in a warring blender and sieved (200 μm aperture) and packaged in a polythene bag.

Phytochemical screening

The ground spice was tested for alkaloids, saponins, polyphenols, tannins, cardenolides, anthraquinones, and carotenoids as descrybed by Trease and Evans (2002).

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Table 1. Phytochemical analysis of *A. danielli* ground spice.

Tests	Observations	Indication
Alkaloids		
Meyer Dragendroff	+ ve (cream colour) + ve (red – brown colour)	present
Cardenolides		
Keller-killani Kedde	+ ve (green colour) +ve (brown-purple colour)	present
Anthraquinones		
	+ ve (no pink colour)	absent
Saponins		
Frothing test Emulsion test	+ ve (no frothing) + ve (no emulsion)	absent
Tannins		
$FeCl_3$ test Vanillin – HCL test	+ ve (green colour) + ve (no red colour)	absent
Flavonoids / polyhenols		
$FeCl_3$	+ ve (dusky green colour)	present
Carotenoids		
Conc. H_2So_4 test	+ ve (green colour)	present

Spice extraction and fractionation.

The ground spice was extracted with petroleum ether (40 - 60°C) using the method described by Chang et al. (1977). Extract was identified by thin-layer chromatography (TLC) and retention factor (R_f) was determined using method of Hostettman et al. (1985). The extract was fractionated using vacuum- liquid chromatographic process as described by Odukoya et al. (1999).

Determination of antibacterial activity of *A. danielli* extract and fractions was by the agar diffusion method as described by Hugo and Russell (1983). Overnight both culture of test organism (2 ml) was added to molten and cooled nutrient agar (45°C). This was mixed and poured in a sterile petri-dish. The agar was allowed to set and holes (8 mm cup size) were bored at the periphery and centre of the agar. Extract or fraction (dissolved in l ml 50% ethanol) in the range of 100 to 1000μg/ml were introduced into the holes. Ampicillin (10μg/ml) was used as positive control. Standard deviation of the means was by SAS (1995).

RESULTS AND DISCUSSION

Table 1 shows the results of phytochemical tests for *A. danielli* ground spice. *A. danielli* tested positive to both the Meyer's test and confirmatory Dragendroff's test indicating the presence of alkaloids in the seeds. *A. danielli* also tested positive to the two tests for cardenolides: Keller-killani's test and Kedde's test indicating the presence of sugar as glycosides. These two tests revealed the presence of glycosides in *A. danielli.*

Polyphenols in the forms of tannin, anthraquinones and flavonoids were also tested in *A. danielli*. For both the tannin and flavonoid tests with $FeCl_3$, the spice tested positive, but with further testing with vanillin-HCl, *A. danielli* was confirmed to have no tannin but only flavornoids. Carotenoids were also confirmed present in the seeds. Harbone (1984) reported that occasional phenolic units are elucidated in alkaloids and that the existence of sugar as glycosides usually occurs in the water soluble fractions of phenolic compounds. The glycosides that exist in *A. danielli* are likely to be in form of phenolic glycosides.

Table 2 shows the nature and retention factors of fractions of petroleum ether extract obtained from *A. danielli*. Fraction F1 existed as a yellow - orange oil with R_f in the range of 0.75 - 0.88. Fraction F2 existed as a dark brown viscous oil with a lower R_f value than fraction F1. Fractions F3 and F4 are viscous brown oily solids with lower R_f values than fraction F2. It was observed that polarity of the fractions depends on the nature of the eluting solvents. R_f value indicates the polarity of the fractions. Lower R_f values shows higher polarity. Fraction F4 had the highest polarity and F1, the lowest.

Table 3 shows the zones of inhibition of *A. danielli* crude petroleum ether extract on some food –borne pathogens in comparison with ampicillin. The crude extract had higher antimicrobial activity on *Bacillus subtilis* followed by *Staphylococcus aureus.* The extract had the least activity on *Pseudomonas aeruginosa*. This shows that *A. danielli* extract had higher activity towards gram -positive bacteria.

Table 4 shows the minimum inhibitory concentration (MIC) of *A. danielli* petroleum ether fractions on some

Table 2. Fractions of *A. danielli* petroleum ether extracts obtained by vacuum liquid chromatography (VLC).

Fractions	Nature at room temperature	Eluting solvents	Retention factors (R_f)
F_1	Yellow – orange oil	100% hexane Hexane/EtoAC (90:10)	0.75 0.88
F_2	Dark brown Viscous oil	Hexane/EtoAC (80:20) Hexane/EtoAC (70:30)	0.65
F_3	Dark brown viscous oily solid	Hexane/EtoAC (60:40) Hexane/EtoAC (50:50) Hexane/EtoAC (40:60) Hexane/EtoAC (30:70)	0.63
F_4	Dark brown solid	Hexane/EtoAC (80:20) Hexane/EtoAC (10:90) 100% EtoAC	0.62

Table 3. Zones of inhibition (mm) of *A. danielli* crude petroleum ether extract on some food-borne pathogens in comparison with ampicillin.

Zones of inhibition (mm)		
Food –borne pathogens	**crude petroleum ether extract (100 µg/ml)**	**Ampicillin (10 µg/ml)**
Bacillus subtilis	22.80± 2.20	39.2+ 0.40
Bacillus cereus	8.52± 0.40	10.7 ± 1.30
Staphylococcus aureus	20.5± 1.40	36.2 ± 1.70
Escherichia coli	11.7 ± 1.60	20.3 ± 1.00
Pseudomonas aeruginosa	3.21 ± 0.44	12.3± 0.06

Mean of three readings ± standard deviation

Table 4. Minimum Inhibitory Concentration (µg/ml) of *A. danielli* fractions on food-borne pathogens.

Fractions	*B.subtilis*	*B. cereus*	*S. aureus*	*E. coli*	*Ps. aeruginosa*
F1	400	800	200	>800	>800
F2	400	800	200	800	>800
F3	400	800	100	400	800
F4	400	800	200	400	>800

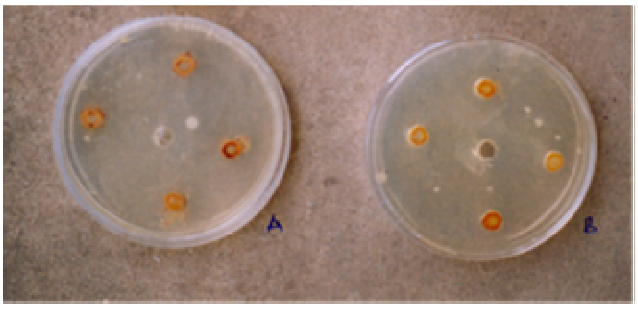

Figure 1. Plates showing zones of inhibition of different concentrations of fraction F3 on *S. aureus.*

food–borne pathogens. All the fractions had similar MIC of 400 µg/ml on *B. subtilis* (400 µg/ml) and *Bacillus cereus* (800 µg/ml). Lower MIC range of 100 - 200 µg/ml of the fractions was needed to inhibit *S. aureus*. Zones of inhibition of different concentrations of fraction F3 is shown in Figure 1. Higher MIC greater than 800 µg/ml was needed for inhibition of *P. aeruginosa,*

Conclusion

This study has been able to highlight some of the phytochemicals present in *A. danielli* spice as alkaloids, carotenoids and polyphenols which could possibly exist as glycosides. Also, the antimicrobial properties of the extracts and fractions show higher activities towards gram -positive bacteria. This shows the possibility of the use of *A. danielli* spice in reducing the incidence of food spoilage and food toxins.

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