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Characterisation and Partial Purification of Antioxidant Component of Ethereal Fractions of *Aframomum danielli*

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Abstract: Petroleum ether fractions of Aframomum *danielli* obtained by vacuum liquid chromatography were characterized using ultraviolet, infrared and proton nuclear magnetic spectroscopy. Antioxidant activities of the fractions were obtained by their reducing power. Among the fractions, fraction F3 had the strongest antioxidant property on the basis of their reducing power. Infrared spectrum of fraction F3 showed the presence of a quinone group which was also confirmed by the nuclear magnetic spectroscopy.

Key words: *Aframomum danielli* · Petroleum ether fractions · Antioxidant activities · Quinone compound

INTRODUCTION

Free radicals can be generated by metabolic pathways in the body tissues or in food systems. Many synthetic chemicals such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), though very effective as antioxidants, have been known to have toxic and carcinogenic effects on humans [1]. Synthetic antioxidant may result in liver swelling and influence liver enzyme activities [2]. Numerous studies have shown the antioxidant potentials of aromatic, spicy, medicinal plants [3-5]. The use of these plant materials as natural antioxidants for food, cosmetics and other application becomes necessary because of food safety issues. Natural antioxidants as food additives for inactivation of free radicals receive a lot of attention nowadays, not only for their scavenging properties, but also because they are natural, non-synthetic products and are more readily acceptable to the consumers [6, 7]. Different degrees of antioxidant activities have been reported from extracts of spices and herbs.

Aframomum danielli is a local spice found in tropical Africa. The spice has been reported to exhibit antioxidant properties in different oil systems [8-11]. With no report of the nature of the antioxidant constituents of the spice, we report here findings on the antioxidant components of ethereal fractions of *A. danielli.*

MATERIALS AND METHODS

Extraction of antioxidants: The fresh pods of *A. danielli* (obtained from Ogbagi, Ondo State) were sun-dried for five days before removing the seeds. The seeds sorted to remove extraneous materials were winnowed to remove adhering particles and dirts and were later air-dried at 27±2°C for 3 days and pulverized using warring blender. The ground spice was sieved (200 µm aperture sieve) and was extracted using a modified method of Chang *et al*., [3] with petroleum ether (40-60°C) in a soxhlet apparatus for 10 h. The extracts were concentrated by distillation and evaporated to dryness in a vacuum oven at 40°C to remove the solvent.

Fractionation of *A. danielli* crude antioxidant extracts: Using vacuum liquid chromatography (VLC) according to the modified method of Odukoya *et al*., [12], sample was prepared by dissolving 10 g of antioxidant extract in 150 mL of petroleum ether (used in extraction). Sixty gram of absorbent powder (Kielselgel 60G Merck, TLC grade) was added and the mixture was dried in a vacuum oven (Gallenkamp) at 60°C to remove the solvent. Whatman filter paper (No. 4) was placed in chromatographic column and 10 g of the adsorbent powder was dry-packed under vacuum. Another filter paper was placed on the adsorbent powder and was then conditioned with the initial mobile phase (hexane for petroleum ether extract and toluene for

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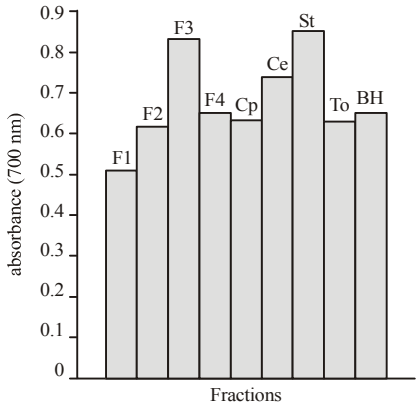

Fig. 1: Reducing power of ethereal fractions of *A. danielli* Legend: F1 = fraction 1; F2 = fraction 2; F3 = fraction 3; F4 = fraction 4; Cp = crude *A. danielli* petroleum ether extract; Ce = crude *A. danielli* ethanolic extract; St = stabex; To = tocopherol; BHT = butylated hydroxytoluene

ethanol extract) and the absorbent powder was sucked dry under vacuum. The free-flowing pre-adsorbed sample was packed under vacuum in a buchner funnel (used as chromatographic column) eluted with hexane-ethyl acetate (9:1) to ethyl acetate alone of increasing polarity under vacuum, sucked dry to collect 50 mL of each fraction.

Identification of components: Four coloured fractions obtained were identified by TLC, ultraviolet (UV) and infrared (IR) spectra [13]. The antioxidant activity of each fractions was determined as described by Amarowicz *et al*., [14].

Partial purification of *A. danielli* active fraction: Flash chromatography described by Hostettmann *et al.*, [15] was modified as follows: 2 g of each fraction was dissolved in 10 mL of acetone, mixed with 12 g of absorbent (Kielselgel 60G, Merck) and dried in the oven at 60°C. Glass column (20 mm size) was filled with mobile phase (hexane) after which ten grams of absorbent powder was added followed by the pre-adsorbed fraction. The column was connected to a low-pressure pump (Dyna Model 1000). The mobile phase was run off the column until it almost reached the stationary phase and hexane: ethyl acetate (30:70) was used for elution. Eluates were spotted on silica gel TLC aluminium sheet using petroleum ether: ethyl acetate (1: 1) as mobile phase. Eluates with similar R_f were collected and dried at 27±2°C.

RESULTS AND DISCUSSION

The crude extract obtained from *A. danielli* seeds using petroleum ether was a dark brown viscous oil with a minty odour and the yield was 2.71%. Thin layer chromatography of petroleum ether extract using a developing solvent of ethyl acetate/ hexane (70:30 v/v), gave two distinct spots. Eleven eluates were collected from the petroleum ether extract by vacuum liquid chromatography using solvent systems hexane: ethyl acetate for elution in increasing order of polarity. Using thin layer chromatography with different solvent systems, ethyl acetate/ hexane and ethyl acetate/ methanol in different ratios, the fractions obtained were bulked into four major fractions based on the spots observed and their retention factors. The reducing power of *A. danielli* fractions is shown in Fig. 1. Fraction F3 fractions exhibited the highest activity amongst the fractions and its activity is higher than that of BHT. Reducing power of stabex was not significantly different ($p<0.05$) from that of F3, while fraction F2 had similar reducing power with dl-α-tocopherol ($p<0.05$). Among the *A. danielli* fractions, F1 had the lowest reducing power. Thus, fractions F3 will serve as good electron and hydrogen donors. The reducing power of antioxidants is important in the delay or inhibition of initiation or propagation steps of lipid oxidation. Antioxidants terminate free radical chain reactions by donating hydrogen or electron to free radicals generated in such chain reactions thereby converting them to stable compounds [16].

Ultraviolet spectra of the fractions of *A. danielli* are shown in Table 1. All the fractions had identical ultraviolet absorption peak at 215 nm with optical density at 3.00 except fraction 4. Fractions with similar peaks indicate the presence of similar conjugation system (arrangement of double bonds). Scott [17] showed that peak at 215 nm in UV spectrum indicate the presence of unsaturated six ring ketone. When alkali (NaOH) was added to alcoholic solutions of the crude extracts and the fractions, the spectra were characteristically shifted towards the longer wavelengths indicating that the compounds present showed bathochromic shift characteristic of phenolic compounds [13]. Figure F2 shows the UV spectrum of fraction F3. All fractions showed 3521-3350 cm^{-1} absorption in the IR spectra (Table 1). This indicated that the compounds have hydroxyl (-O-H) and hydrogen bonds characteristic of phenols or alcohols. This is similar to the findings of Hershenson [18], Harborne [13] and Osawa *et al*., [4].

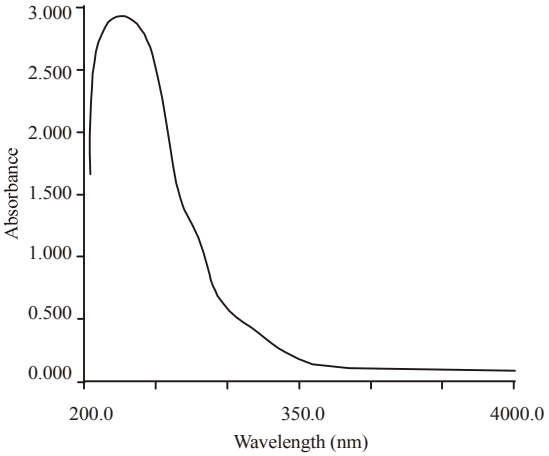

Fig. 2: UV spectrum of fraction F3 of *A. danielli*

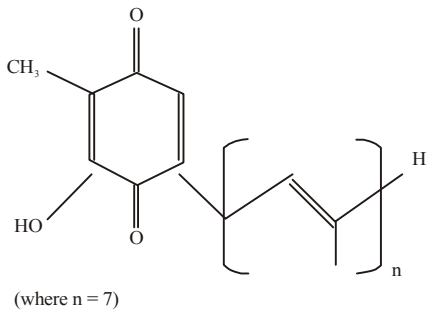

Fig. 3: Proton Nuclear Magnetic Resonance of fraction F3 of *A. danielli*

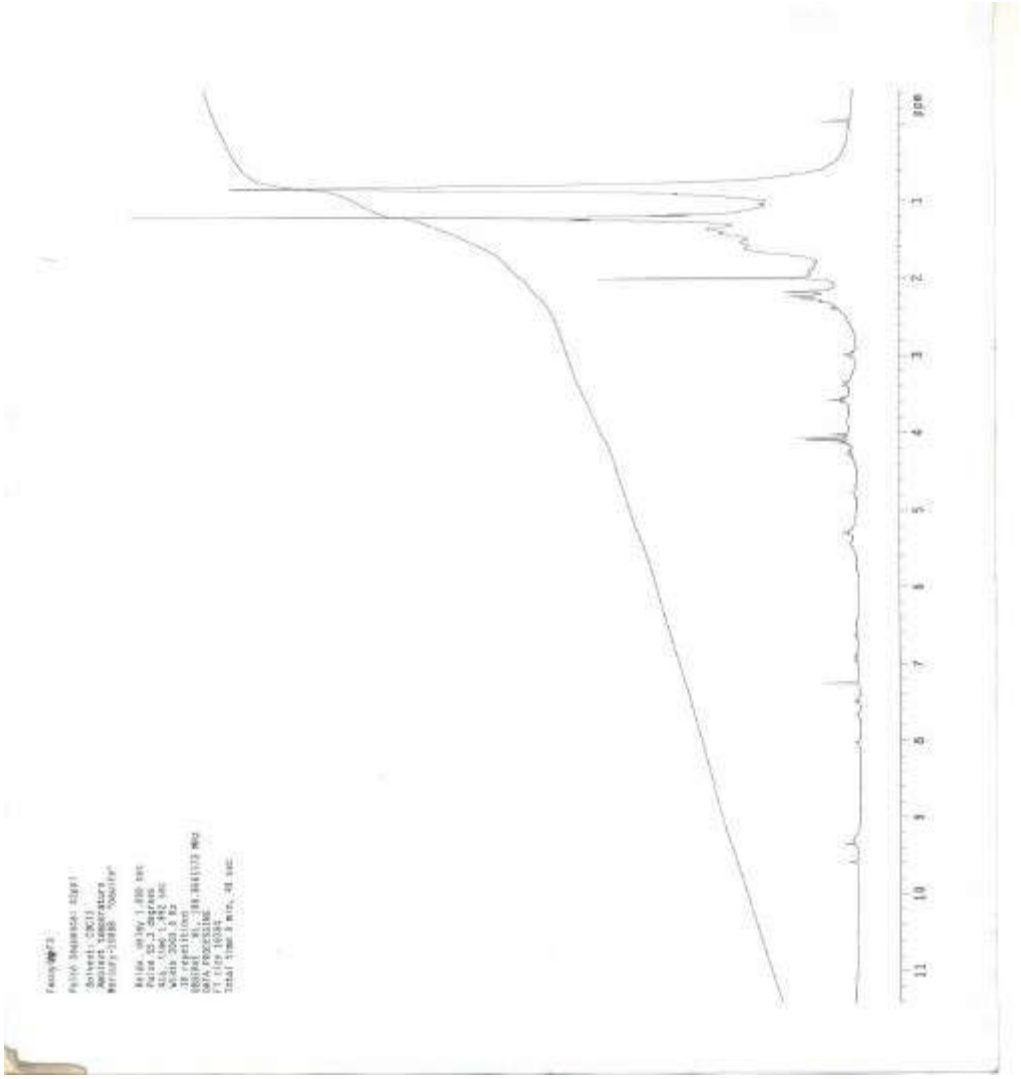

Fig. 4: Deduced structure quinone from fractions F3 of *A. danielli*

Table 1: Infrared and ultraviolet spectra of *A. danielli* ethereal fractions

Pet. Ether	IR (cm^{-1})	UV(nm) (absorbance)
extract	3448, 2934, 2718 1751, 1655 1460, 1393, 1180 937, 772	230 (3.00)
F1	3352, 2925, 1721 1440, 1380, 1235, 1054 894, 718	215 (3.00)
F2	3350, 2937, 2542, 2140, 1921 1621, 1440, 1380, 1235, 1089, 1040, 809	215 (3.00)
F3	3448, 2933, 2736, 2244 1717, 1460, 1368, 1174	215 (3.00)
F4	3350, 2937, 2536, 2414, 2140, 1921 1717, 1669, 1650, 1560 225 (2.80) 1447, 1374, 1070 876, 809, 774, 730	215 (3.00)

Generally, a wavelength of 2949-2919 cm^{-1} and 1460-1326 cm^{-1} were also recorded for the fractions which confirmed C-H bonds with methyl groups ($-CH_3$) or methylene groups ($-CH_2$). Measurements of wavelengths at 1927-1707 cm^{-1} showed the presence of aromatic groups (C=C) as also observed by Hershenson [18]. Harborne [13] reported that infrared spectrum could be used to identify functional groups in a compound by their characteristic vibration frequencies. Infrared spectrum of all the fractions showed peaks at1700-1630 cm^{-1}. Measurement of frequencies in the range of 1800-1630 cm^{-1} showed the presence of carbonyl groups (C=O) and quinone compounds were reported within these range by Brand and Eglinton [17], Harborne [13], Osawa *et al*., [4], Weng and Gordon [5] and Weng *et al*., [20]. Quinones chelated with α-hydroxyl group showed adsorption spectra between 1675-1647 cm^{-1} and 1675-1661 cm^{-1} [4] and ultraviolet spectra of F2 and F7 were within this range thus confirming the presence of quinone compounds in *A. danielli* active fractions. Osawa *et al*., [4] and Harborne [13] reported quinones as polyphenols.

The proton NMR spectra of purified F3 showed the presence of one aromatic quinoid ring proton with chemical shift at 7.2 ppm (Fig. 3). Proton NMR shows peaks for quinoid hydrogen at 7 ppm [20]. Fraction 3 showed a singlet peak at 1.0 ppm, 1.2 ppm indicating the presence of methylene groups as part of an aliphatic side chain and at 1.6 ppm showing the presence of methyl groups as part of an aliphatic side chain. It showed other peaks at 2.0 ppm, 2.3 ppm showing the presence of methyl groups attached to an aromatic structure. The presence of an aromatic structure implies the presence of a benzene ring structure. Harborne [13] reported the range of 2.25-2.50 ppm for structure of aromatic compounds with benzene ring attached to methyl group ($Ar-CH_3$), while 6.6-8 ppm for structure of aromatic compounds with benzene ring attached to a hydrogen (Ar-H). Odukoya *et al*., [12] reported the presence of a quinoid proton at 6.23 ppm, 2 methyl groups at 2.13 ppm and a mono-prenyl side chain at 3.07 ppm in the proton NMR of *A. danielli*. Carbon-13 NMR of fraction F3 shows the presence of thirty-five carbon atoms in the purified fraction. Twenty-eight carbon atoms are within the range of 0-60 ppm characteristic of aliphatic carbon atoms. While the remaining six carbon atoms are aromatic. Carbonyl carbons (ketonic) are reported within 160-230 ppm [21]. Ultraviolet spectra of *A. danielli* showed the presence of conjugation systems (double bonds) and polyphenols. Figure 4 shows the structure of deduced compound from fraction F3 of *A. danielli*. All the findings with TLC, UV, IR and NMR confirm a benzene ring-quinone with methyl and hydroxyl groupings as the active component in fraction F3 of *A. danielli*. This study therefore confirms of quinone compound as an active component in *A. danielli*.

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