Extraction and Identification of Antioxidants from the Spice *Aframomum danielli*

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ABSTRACT: Successive extractions with diethyl ether and methanol of the whole seeds of the spice *Aframomum danielli* yielded diethyl ether extract (ADEE), 13.9%, and methanol extract (ADM), 3.4%, respectively. Similarly, reextraction of the defatted seeds of *A. danielli* successively with diethyl ether and methanol yielded extracts DFADEE (7.9%) and DFADM (6.7%), respectively. When these extracts were added to refined peanut oil (PNO) at 200 ppm, they showed good antioxidative effects. The percentage antioxidant effectiveness (AE) values were as follows: DFADM (87.3) > ADM (85.3) > ADEE (83.4) = *tert*-butyl hydroquinone (83.4) on day 20 of storage in an oven maintained at 65 ± 1°C. Generally, antioxidant extracts prepared from *A. danielli* were also more effective than butylated hydroxytoluene and α-tocopherol in stabilizing refined PNO. Antioxidant components of *A. danielli* were tentatively identified as phenolic compounds of the trihydroxy type with reducing properties. All extracts prepared from *A. danielli* showed strong ultraviolet-absorbing characteristics, and methanol was a good extracting solvent.

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KEY WORDS: *Aframomum danielli,* African spice, antioxidant effectiveness, lipid oxidation, natural antioxidants, phenolic compounds, protection factor.

Off-odor, off-flavor, and other deteriorative changes generally lead to product rejection and loss of revenue in the food industry. As far as fats and oils are concerned, the problems associated with lipid oxidation can be countered by the application of antioxidants. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (TBHQ) are presently used in the food industry to stabilize oils against oxidation (1,2). However, adverse reports regarding these synthetic antioxidants exist in the literature (3–5). Therefore, in considering these reports and the present trends toward naturally preserved food products, herbs and spices have been targeted as sources of natural antioxidants (6–8). However, these reports involve several spices and herbs that are of commercial value (9), and there are few reports on the antioxidative activities of spices and herbs that have hitherto been of no commercial value. Therefore, the present work was carried out to isolate and identify the antioxidant compounds of the spice *Aframomum danielli*. *Aframomum danielli* belongs to the family Zingiberaceae, which is closely related to *Ammomum* Roxb. from tropical Asia. *Elletaria cardamomum* (L.) Maton, the source of commercial cardamoms, also belongs to the family Zingiberaceae (10).

MATERIALS AND METHODS

Spice and antioxidative screening procedure. The spice *A. danielli* was obtained locally in Ibadan, Nigeria. Prior to utilization, the seeds were cleaned of extraneous matter, pulverized, and sieved (60 mesh). Thereafter, the spice powder was added at 1, 2, 4, and 10% levels to refined peanut oil (PNO) (45 g) in 50-mL beakers. After careful mixing, they were transferred to an oven maintained at 65 ± 1°C for an accelerated storage study for up to 20–30 d. Control beakers contained only refined PNO, and some had synthetic antioxidants, BHT, or TBHQ (food-grade antioxidants). Peroxide values (PV) were determined every 5 d on the withdrawn samples by AOCS Official Method Cd 8-53 (11). The rate of oxidation was determined by using the procedure reported earlier (12). The percentage antioxidant effectiveness (AE) was calculated from the equation:

$$\text{AE} = \frac{\text{PV of control} - \text{PV of test sample}}{\text{PV of control}} \times 100\% \qquad [1]$$

The ability of *A. danielli* to stabilize PNO was also ascertained by using the oven weight gain method. The method described by Olcott and Einset (13) was modified as follows: 1.0 g sample of PNO (PV, 2.0 meq O_2/kg oil) was weighed into a clean 2-in. (50-mm) glass mycological Petri dish (Borosil Glass Works Ltd., Mumbai, India). Different amounts of petroleum ether extract of *A. danielli* powder (0.1, 1.0%), the defatted material (1, 10%), or synthetic antioxidant (Table 1) were then added to the Petri dishes, mixed carefully, and stored at 65 ± 1°C as described before. Each sample was taken out of the oven for weighing every 24 h, cooled to room temperature (13), weighed, and returned to the oven. Increase in weight was recorded for up to 50 d, and

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TABLE 1
Monomolecular Rate Constant of Peanut Oil Treated with the Whole Spice Powder of *Aframomum danielli*[a]

Treatment	Rate constant ($K_m \times 10^3$) at 65 ± 1°C
Refined peanut oil (PNO) (control)	18.67 ± 0.10[d]
PNO + TBHQ, 0.02%	2.82 ± 0.20[a]
PNO + *A. danielli,* 1.0%	17.61 ± 0.04[c]
PNO + *A. danielli,* 2.0%	17.32 ± 0.05[b,c]
PNO + *A. danielli,* 4.0%	16.62 ± 0.04[b]
PNO + *A. danielli,* 10.0%	16.82 ± 0.11[b]
SE_m (12 df)	± 0.21

[a]Values are means ± SD for three samples; any two means with different superscripts (a–d) are significantly different ($P \leq 0.05$) SE_m = standard error of mean values; df = degrees of freedom.

the induction period was taken as the time required for the sample to gain 0.5% in weight to the nearest day. The protection factor (PF) was calculated from the equation:

$$PF = \frac{\text{day test attained 0.5\% weight gain}}{\text{day control sample attained 0.5\% weight gain}} \quad [2]$$

The data were subjected to two-way analysis of variance, followed by Duncan's new multiple range test for segregating differences between means (14).

Extraction of antioxidative components of A. danielli. The method described by Chang *et al*. (6) was modified as follows: *Aframomum danielli* powder (10 g) was refluxed with diethyl ether (100 mL) for 2 h at 60°C, cooled, and filtered. The filtrate was rotary-evaporated at 25°C (92–100 rpm/15 in. pressure, Rotavapor Model RE 121, Buechi Laboratoriums-Technik AG, Flawil, Switzerland) to get the antioxidant extract, ADEE. Similarly, the residue was successively reextracted with methanol (100 mL), allowed to cool to room temperature, filtered, followed by rotary evaporation at 40°C to yield another antioxidant extract, ADM. Residue obtained after fat extraction (i.e., after petroleum ether extraction for fat content determination, also known as the defatted material) (13.0 g) was also similarly reextracted with diethyl ether and methanol successively to yield antioxidant extracts DFADEE and DFADM, respectively. The extracts so obtained from *A. danielli* were added to refined PNO at 200 ppm, along with BHT and TBHQ as synthetic antioxidants, to get a comparative antioxidant effect assessment. Storage of treated PNO and periodic PV determinations were carried out as described earlier. Slower formation of peroxides was used as a measurement of the antioxidant activity of each extract (6).

Identification of antioxidant components. The methods described by Daniels and Martin (15) and Pratt and Miller (16) for identifying antioxidant activity [in accordance with the method of Duve and White (17)] was modified as follows: thin-layer chromatography (TLC) plates, laboratory-coated with silica gel G (Ranbaxy Laboratories Ltd., SAS Nagar, Punjab, India) to 0.3 mm thickness, were spotted with 20 µL of each extract (from 1% solution in the respective solvent used for antioxidant extract preparation from *A. danielli*). The plates were then developed in the upper phase of chloroform/ethanol/acetic acid (98:2:2). Thereafter, the plates were sprayed with a solution of 9 mg of β-carotene dissolved in 30 mL chloroform, to which 20 mg of linoleic acid and 60 mL ethanol were added. The sprayed plates were exposed to sunlight of bright intensity (during the noon–afternoon hours) for 6 h. The retention of color of β-carotene (for antioxidant effect) or fading of orange color of β-carotene (for pro-oxidant or no antioxidant effect) were taken as corresponding to the relative antioxidative activity of each extract (18,19); TBHQ was used as a control antioxidant, which retained the orange color.

Furthermore, TLC tests to identify the chemical composition of the antioxidant extracts from *A. danielli* were carried out as follows: Spray 1: A 1% solution of potassium ferricyanide in water and a 1% solution of ferric chloride in water were sprayed on the developed TLC plates, which gave a blue color to indicate that these extracts may be phenolic compounds (20). Spray 2: Ferric chloride (2 g), dissolved in 100 mL ethanol, sprayed onto the developed TLC plates produced blue coloration for some spots, which indicates the probable presence of trihydroxy phenolic compounds (21). Spray 3: Ammoniacal silver nitrate solution was prepared by mixing 30 mL ammonium hydroxide and 70 mL water. Silver nitrate (3.4 g) in 100 mL water was added to the solution and sprayed on the developed chromatograms. After heating for 10 min at 105°C, brown, black, and gray spots were produced as evidence of reducing compounds (17). Separate TLC plates, developed in a similar fashion, were also exposed to iodine vapors to show the representative number of TLC spots in each extract.

Ultraviolet (UV)-visible absorption spectra of extracts were recorded on a UV-visible double-beam recording spectrophotometer (model UV-240; Shimadzu Corporation, Kyoto, Japan) from 0.0067% solutions of the extracts in methanol. Infrared (IR) spectra were also recorded on a Fourier-transform IR spectrophotometer (model Bruker IFS 25; Bruker, Karlshrue, Germany) with the Nujol mull technique in a sodium chloride cell over the range of 600–4000 cm^{-1}.

RESULTS AND DISCUSSION

Screening of A. danielli *for antioxidative effect*. Addition of whole ground powder of *A. danielli* at levels of 1, 2, 4, and 10% to refined PNO revealed only a marginal antioxidative effect of the spice compared to a synthetic antioxidant TBHQ (Table 1). Also, in the oven weight gain method, the petroleum ether extract of *A. danielli* and the defatted material did not show a good antioxidant effect, although it showed an induction period of 190 h and a PF of 0.79 (Table 2). Thus, pro-oxidant effects were found with the petroleum ether extract of *A. danielli* at 0.1 and 1.0% level and with 1.0 and 10% levels of the defatted powder of the spice when added to refined PNO (Tables 1 and 2). However, the pro-oxidant effect of *A. danielli* at high concentrations is not unusual because primary antioxidants are known to be effective at low concentrations, while at higher levels they may become pro-oxidants (22).

TABLE 2
Induction Periods of Peanut Oil Treated with the Petroleum Ether Extract and Defatted Spice Powder of *A. danielli*

Treatment	Storage at 65 ± 1°C		
	Induction period (h)	Protection factor[a]	Remarks
Refined PNO (control)	240 ± 17^{p}	1.0	—
Refined PNO + BHT, 0.02%	313 ± 8^{q}	1.31 ± 0.07	Antioxidant
Refined PNO + *A. danielli* extract[b], 0.1%	167 ± 5^{m}	0.70 ± 0.04	Prooxidant
Refined PNO + *A. danielli* extract[b], 1.0%	95 ± 4^{l}	0.40 ± 0.01	Prooxidant
Refined PNO + *A. danielli* defatted powder[c], 1.0%	238 ± 8^{p}	0.99 ± 0.07	No effect
Refined PNO + *A. danielli* defatted powder[c], 10.0%	190 ± 6^{n}	0.79 ± 0.04	Prooxidant
SE_m (35 df)	± 4		

[a]Calculated from the equation: Protection factor = (day test attained 0.5% weight gain)/(day control sample attained 0.5% weight gain).
[b]Petroleum ether extract.
[c]Residual powder after petroleum ether extraction of *A. danielli* powder. Values are means ± SD for six samples; any two means with different superscripts (l–q) are significantly different ($P < 0.05$). Abbreviations: SE_m, standard error of mean values; df, degrees of freedom; PNO, peanut oil; BHT, butylated hydroxytoluene; for other abbreviation see Table 1.

TABLE 3
Monomolecular Rate Constant of PNO Treated with Diethyl Ether and Methanol Extracts of Whole Spice and Defatted Spice Powder of *A. danielli*

Treatment	Rate constant ($K_m \times 10^3$) at 65 ± 1°C
Refined peanut oil (PNO) (control)	35.90 ± 0.29^{p}
PNO + TBHQ, 0.02%	2.82 ± 0.20^{m}
PNO + BHT, 0.02%	2.18 ± 0.14^{l,m}
PNO + ADEE, 0.02%[a]	7.83 ± 0.20^{n}
PNO + ADM, 0.02%[b]	1.08 ± 0.13^{l}
PNO + DFADEE, 0.02%[c]	10.59 ± 0.23^{o}
PNO + DFADM, 0.02%[d]	1.31 ± 0.20^{l}
SE_m(14 df)	± 0.44

[a]ADEE = diethyl ether extract of *A. danielli*.
[b]ADM = methanol extract of *A. danielli*.
[c]DFADEE = diethyl ether extract of defatted powder of *A.danielli*.
[d]DFADM = methanol extract of defatted powder of *A. danielli*. Values are means ± SD for three samples; any two means with different superscripts (l–p) are significantly different ($P < 0.05$); SE_m = standard error of mean values; df = degrees of freedom. For other abbreviations see Table 2.

Antioxidant effects of A. danielli *extracts*. Extraction of the powder of *A. danielli* with different solvents, however, revealed the antioxidative effects of the spice. At 200 ppm, the order of percentage AE of the various extracts from *A. danielli* (Figs. 1–4) after 20 d, when added to refined PNO, at 65 ± 1°C was: DFADM (87.3) > ADM (85.3) > ADEE (83.4) = TBHQ (83.4). With BHT, the order was DFADM (87.3) > ADM (85.3) > ADEE (83.4) > BHT (79.6). The K_m values given in Table 3 indicate a significant antioxidant effect for ADM and DFADM, comparable to BHT and TBHQ. The effect of ADEE and DFADEE was considerably lower than for BHT and TBHQ, although a significant antioxidant effect was observed for the extracts compared to the control. To our knowledge, this is the first report of antioxidant activity of *A. danielli*. The yields and colors of antioxidant extracts from the spice *A. danielli* by different solvents are shown in Table 4. A yield of 13.9% was obtained when *A. danielli* was extracted with diethyl ether (ADEE), but a lower yield (3.4%) was obtained with methanol (ADM). The extracts DFADEE and DFADM, prepared from the defatted material by successive extraction with diethyl ether and methanol, respectively, gave different yields and colors (Table 4).

The differences found for the antioxidant activities between whole spice petroleum ether extracts and of defatted material and those of diethyl ether and methanol extracts could be explained as follows. The antioxidant principles present in *A. danielli* are soluble in slightly polar solvents, such as diethyl ether and methanol. The antioxidant principles are not extracted by petroleum ether, and therefore, there is no

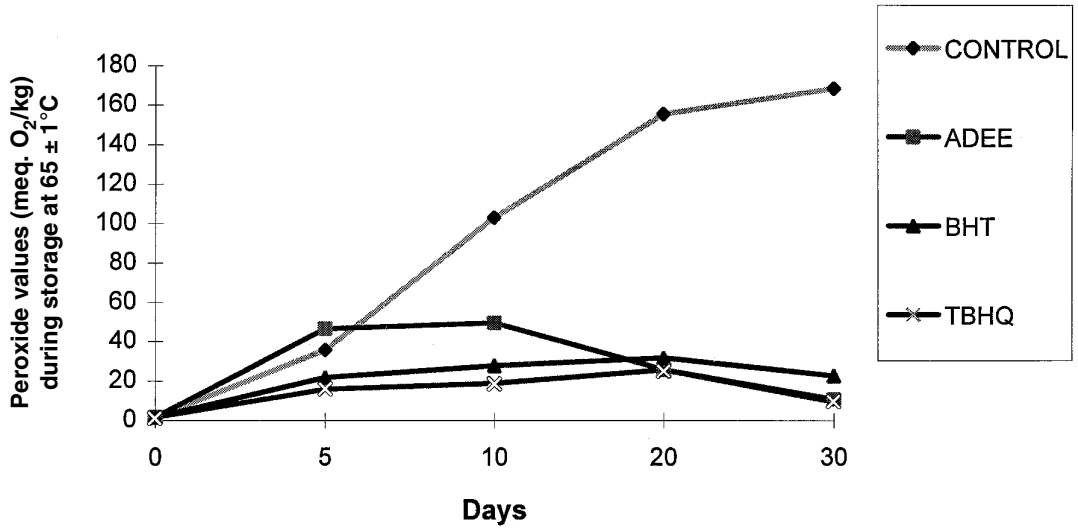

FIG. 1. Peroxide values of peanut oil treated with diethyl ether extract of *Aframomum danielli* (ADEE). BHT, sample treated with butylated hydroxytoluene; TBHQ, sample treated with *tert*-butyl hydroquinone.

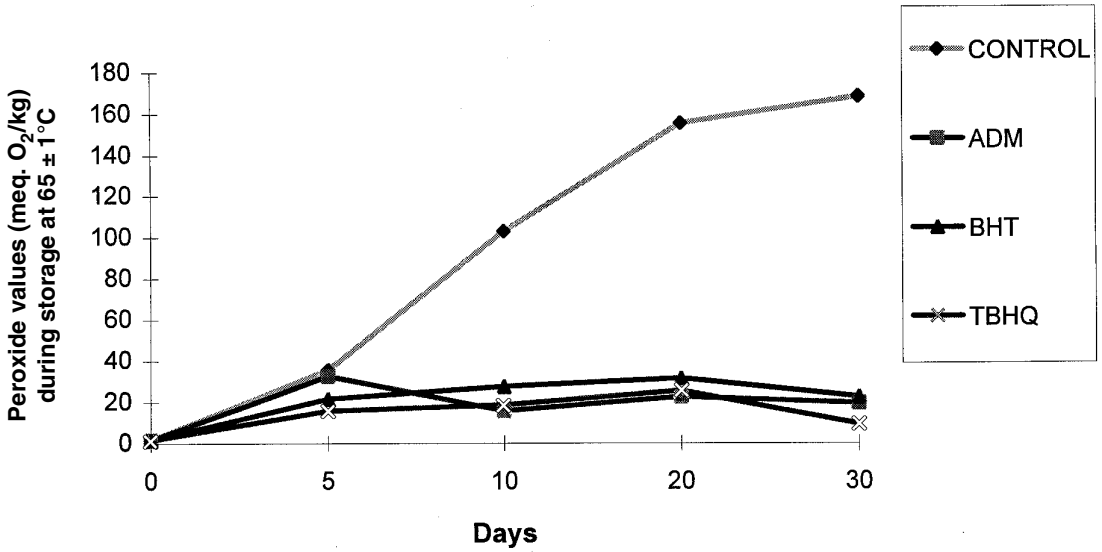

FIG. 2. Peroxide values of peanut oil treated with methanol extract of *Aframomum danielli* (ADM). For other abbreviations see Figure 1.

antioxidant activity for this extract. However, for the defatted material, although the antioxidant is present, it is present inside the cellular compartments of the material, and the oil cannot reach inside and extract it. Also, for extraction of the antioxidant principles from the spice *A. danielli*, a nonpolar solvent such as petroleum ether (or a vegetable oil) may not be suitable. Diethyl ether and methanol, which are more polar solvents, extracted the antioxidant principles effectively, and therefore, there is a difference in antioxidant effect for the different extracts.

Tentative identification of antioxidant components. TLC patterns and R_f values of the components of antioxidant extracts from *A. danielli* are shown in Table 5. With R_f 0.0, the antioxidant components of ADM and DFADM were tentatively identified to be phenolic compounds with reducing properties. The specific extinction coefficients in methanol for antioxidant extracts ADEE, DFADEE, ADM, and DFADM are shown in Table 6. The extracts had strong UV absorption at 228 nm (ADEE, DFADEE) and 210 nm (ADM, DFADM), and IR wavenumbers of 2560–4000 cm^{-1} indicated possible phenolic hydroxyl groups in the compounds present in the extract, suggesting the presence of phenolic compounds in the extracts, similar to the specific spray reagents. According to Shahidi and Wanasundara (23), natural antioxidant extracts, such as ADEE, DFADEE, ADM, and DFADM from *A. danielli,* are primarily plant polyphenolic compounds that may occur in several parts of the plant. In line with our present work, additional studies have thus been

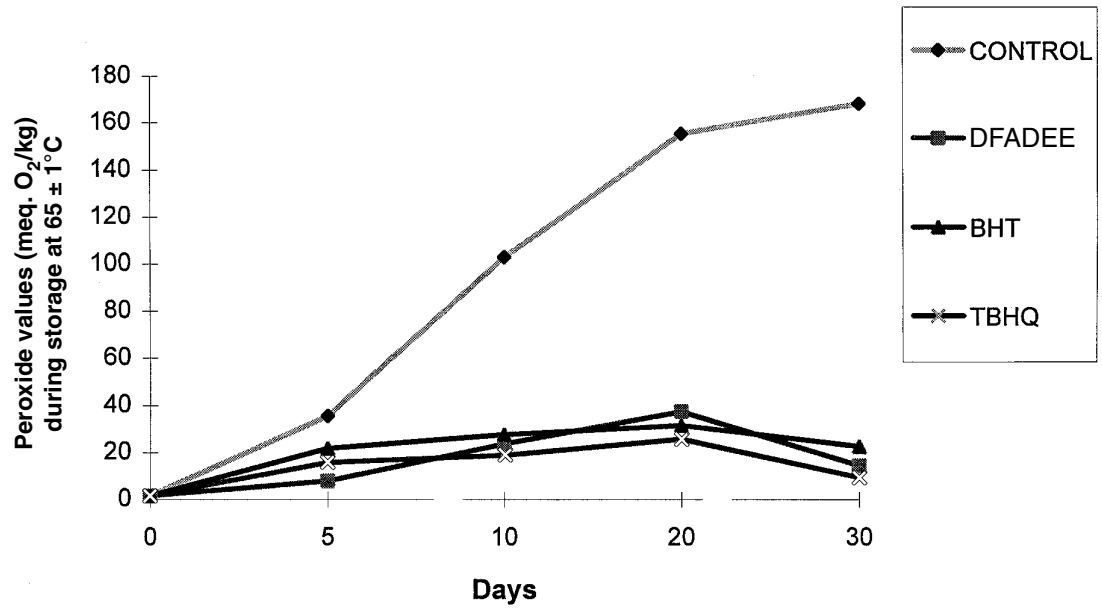

FIG. 3. Peroxide values of peanut oil treated with diethyl ether extract of defatted powder of *Aframomum danielli* (DFADEE). For abbreviations see Figure 2.

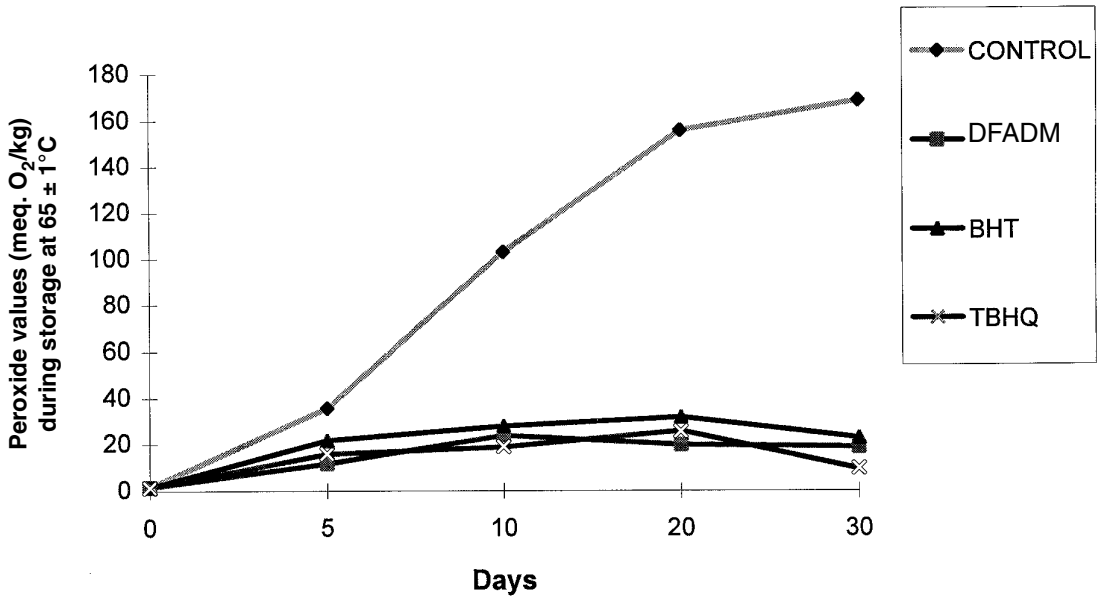

FIG. 4. Peroxide values of peanut oil treated with methanol extract of defatted powder of *Aframomum danielli* (DFADM). For abbreviations see Figure 2.

TABLE 4
Yields and Colors of Antioxidant Extracts Isolated from *A. danielli* with Different Solvents

Extract[a]	Solvent	Yield (% dry basis)	Appearance of extract	Starting material
ADEE	Diethyl ether	13.9 ± 0.10	Reddish-yellow liquid	*A. danielli* whole powder
ADM	Methanol	3.4 ± 0.20	Dark-brown powder	*A. danielli* whole powder
DFADEE	Diethyl ether	7.9 ± 0.10	Light-yellow powder	*A. danielli* defatted material
DFADM	Methanol	6.7 ± 0.15	Light-brown powder	*A. danielli* defatted material

[a]For abbreviations see Table 3.

TABLE 5
Thin-Layer Chromatography Patterns, R_f Values and Tentative Identification of Antioxidant Components of *A. danielli*

Extract[a]	No. of compounds[b]	R_f value	Antioxidant component[c]	Phenolic component[d]	Trihydroxy phenolic type[e]	Reducing component[f]
ADEE	11 (4)	0.43	+	+	–	–
ADM	2 (1)	0.00	+++	+	+	+
DFADM	4 (1)	0.00	+++	+	+	+
DFADEE	8 (2)	0.43	+	+	–	–

[a]ADEE, DFADEE, antioxidant diethyl ether extract from *A. danielli* whole powder and defatted material; ADM, DFADM, successive extracts obtained with methanol from *A. danielli* whole powder and from defatted material after diethyl ether extraction.
[b]Components found with iodine vapor, and number in parentheses is the compound number with the R_f value given alongside the table to show antioxidant activity.
[c–f]Detected by specific spray reagents (Refs. 18–20).

TABLE 6
Ultraviolet Spectral Characteristics of Antioxidant Extracts from the Spice *A. danielli*

Extract[a]	A[b] (nm)	B[c]	C[d]	A	B	C	D[e] (nm)
ADEE	206	1.48	113.9	228	1.64	126.2	228
DFADEE	206	1.00	149.9	220	1.11	166.4	228
ADM	210	2.34	350.8	282	0.69	103.5	210
DFADM	210	2.34	350.8	282	0.63	94.5	210

[a]ADEE, DFADEE: diethyl ether extracts from *A. danielli* whole powder and defatted material; ADM, DFADM: successive extracts obtained with methanol from *A. danielli* whole powder and defatted material after diethyl ether extraction.
[b]A: wavelength of maximum absorption.
[c]B: optical density.
[d]C: extinction coefficient of 1% solution in 1-cm cell pathlength.
[e]D: wavelength of maximum extinction coefficient.

carried out to identify natural phenolics that possess antioxidant activity (24–26).

Our present findings of the antioxidative effects of *A. danielli* should therefore rekindle more research into the application of natural antioxidants for lipid stabilization.

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