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Isolation and Identification of Mosquito (*Aedes aegypti*) Biting Deterrent Fatty Acids from Male Inflorescences of Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg)

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ABSTRACT: Dried male inflorescences of breadfruit (*Artocarpus altilis*, Moraceae) are burned in communities throughout Oceania to repel flying insects, including mosquitoes. This study was conducted to identify chemicals responsible for mosquito deterrence. Various crude extracts were evaluated, and the most active, the hydrodistillate, was used for bioassay-guided fractionation. The hydrodistillate and all fractions displayed significant deterrent activity. Exploratory GC-MS analysis revealed more than 100 distinctive peaks, and more than 30 compounds were putatively identified, including a mixture of terpenes, aldehydes, fatty acids, and aromatics. A systematic bioassay-directed study using adult *Aedes aegypti* females identified capric, undecanoic, and lauric acid as primary deterrent constituents. A synthetic mixture of fatty acids present in the most active fraction and individual fatty acids were all significantly more active than *N*,*N*-diethyl-*m*-toluamide (DEET). These results provide support for this traditional practice and indicate the potential of male breadfruit flowers and fatty acids as mosquito repellents.

KEYWORDS: *mosquito repellent, capric acid, undecanoic acid, hendecanoic acids, lauric acid, hydrodistillation, smoke*

INTRODUCTION

Breadfruit, *Artocarpus altilis* (Parkinson) Fosberg, is a tropical staple food crop traditionally cultivated throughout Oceania (Melanesia, Micronesia, and Polynesia).[1] Cultivation and distribution of breadfruit were traditional practices of the peoples of the Pacific, who transported root cuttings from island to island by canoe. In 1787, the famous ship HMS Bounty was involved in a breadfruit expedition with a mission to distribute the plant throughout the Caribbean. Breadfruit now grows in the wet tropics, including the Caribbean, South America, South/South-East Asia, and parts of Africa, encompassing many countries that are most afflicted by insect-vectored diseases such as malaria, yellow fever, and dengue fever.[1,2] In addition to its value as a staple food crop, the breadfruit tree provides a variety of secondary products including timber, latex, and medicine.[1] One of the documented secondary uses practiced in Vanuatu, Hawaii, and perhaps other regions throughout Oceania was to burn the dried male inflorescence to repel flying insects such as mosquitoes.[1,3,4] The dried inflorescences are well suited for this purpose as they can be suspended by the peduncle and burned in a manner analogous to modern pyrethroid-based mosquito coils.

Preliminary studies supported the efficacy of this traditional practice and indicated that extracts made from male inflorescences of *Artocarpus altilis*, and the smoke produced when they are burned, deter mosquitoes from feeding in a live mosquito bioassay system.[5] However, the underlying chemical constituents responsible for this biting deterrent activity have not been previously investigated. The current study describes comparison of smoke extracts, solvent extracts, and a hydrodistillate followed by exploratory screening and systematic bioassay-guided fractionation to determine candidate phytochemicals with insect repellent activity in male breadfruit flowers.

MATERIALS AND METHODS

Collection of Plant Material. Male inflorescences were collected from mature breadfruit trees (*A. altilis* (Parkinson) Fosberg) maintained at the National Tropical Botanical Garden's Breadfruit Institute in the Kahanu Garden, Hana, Maui, HI (Figure 1A,B). Inflorescences were collected from several trees representing a diverse set of cultivars and were pooled prior to extraction. The inflorescences were dried in the sun for approximately 4 days until dry (Figure 1C). Once dried, the inflorescences were shipped to the Okanagan Campus of the University of British Columbia (UBC) for extraction and to the University of Mississippi National Center for Natural Products Research (NCNPR) and British Columbia Institute of Technology for analysis.

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Figure 1. (A) Mature breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) tree; (B) developing male inflorescence of *A. altilis*; (C) mature, dried male inflorescences of *A. altilis*; (D) K&D mosquito deterrence bioassay setup; (E) apparatus used for smoke extractions.

Extraction of Plant Material. *Smoke Extraction.* Smoke extracts were collected by burning intact, dried breadfruit inflorescences (Figure 1E) in a 1 L filter flask with compressed air continually adjusted to maintain the ember. The resulting smoky air was passed through a series of two filter flasks, each containing dichloromethane (300 mL in the first flask, 100 mL in the second flask; Fisher Scientific, Ottawa, ON, Canada) and water (100 mL in the first flask, 25 mL in the second flask). The extracts from both flasks were combined and then separated into the aqueous and dichloromethane portions using a 2 L separation funnel. The water and dichloromethane were removed from the extracts under vacuum in a rotary evaporator at 95 and 50 °C, respectively. The resulting extracts are designated "smoke".

Solvent Extracts. (a) Dichloromethane Extraction. A solvent extract was prepared by adding 5 g of dried ground breadfruit inflorescence to 50 mL of dichloromethane in a 50 mL glass centrifuge tube (Fisher Scientific) and placed on a rotary shaker for 2 h at 60 rpm. The extract was then centrifuged at 4500 rpm for 10 min and the supernatant collected. Another 25 mL of dichloromethane was added to the material, and it was again placed on the shaker for 30 min at 60 rpm. The extract was centrifuged and the supernatant collected and added to the first collection. The combined supernatant was filtered through no. 2 Whatman filter paper using a Büchner funnel and the dichloromethane removed under vacuum in a rotary evaporator at 50 °C.

(b) Ethyl Acetate Partition of Aqueous Extract. This extract was prepared by refluxing 100 g of dried ground breadfruit inflorescence in 1 L of deionized water for 2 h. The solution was boiled in a round-bottom flask with a Clevenger (lighter than water) style collector and a 30 cm condenser attached. Very little hydrodistillate accumulated in the collector, so the liquid in the collector was combined with the water containing the breadfruit tissue. The solution was filtered through no. 2 Whatman filter paper using a Büchner funnel. The liquid portion was then combined with 1 L of ethyl acetate (Fisher Scientific) in a separatory funnel and shaken vigorously. The partitioning was repeated, and the two ethyl acetate extracts were combined. The ethyl acetate layer was removed, dried with $MgSO_4$, and evaporated by rotary evaporation at 50 °C.

(c) Hydrodistillation. Volatile components of the male *A. altilis* inflorescences were extracted using a hydrodistillation apparatus (open system) with a Clevenger (lighter than H_2O) style collector containing 7 mL of pentane. Batches of approximately 300 g of dried ground

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breadfruit inflorescences were added to 5 L of water in a 12 L round-bottom flask. The mixture was boiled for 10 days, the pentane layer from the collector being collected and replaced periodically. Upon combination of the pentane layers, the solvent was removed under a flow of nitrogen gas. In latter assays, the pentane extract was placed in the freezer overnight, where some constituents crystallized. The liquid was decanted, and the crystals were kept separate for analysis. Residual pentane was removed under the flow of nitrogen gas at room temperature, and the resulting extract is designated hereafter "crystals".

Fractionation. A Horizon column chromatography system was used to fractionate the hydrodistillate extract (Biotage, Inc., Charlottesville, VA, USA) equipped with a flash collector and fixed-wavelength (254 nm) detector. The column used was a 40 mm × 150 mm, 40−63 μm particle size, Biotage 40 + M silica column. The column was equilibrated with 396 mL of 50:49:1, hexane/dichloromethane/methanol prior to loading a samplet (Biotage 40 + M) that had 100−300 mg of sample applied in a small volume of dichloromethane (2 mL) followed by evaporation of the dichloromethane under nitrogen gas. The samples were separated using an isocratic elution with 1728 mL of the same mobile phase. Fractions were collected in 18 mm tubes containing 24 mL/tube. Fractions were combined based on TLC using 250 μm silica gel plates (Analtech, Newark, DE, USA) with the same mobile phase and visualized by application of Godin reagent[6] with gentle heating to reveal terpenoid components in the fractions. Fractions were combined into seven composite fractions on the basis of their similarity on TLC plates hereafter referred to as fractions A−G.

Gas Chromatography−Mass Spectrometry of Raw Hydrodistillate. The hydrodistillate was analyzed on a 5975C series GC-MSD (Agilent, Mississauga, ON, Canada) equipped with a 30 m × 0.25 mm i.d., 5% phenyl methyl siloxane HP-5MS capillary column (Agilent) operated using the following parameters: oven conditions, initial temperature of 80 °C for 1 min, increased by 2 °C/min to 114 °C, and held for 1 min, then increased by 0.5 °C/min to 118 °C and held for 1 min, then increased by 2 °C/min to 185 °C, then finally increased to 310 °C at a rate of 5 °C/min and held for 2 min (total run time of 88.5 min). Injection parameters were as follows: initial inlet temperature of 250 °C with an injection volume of 1 μL (splitless); mass spectrometer detector, solvent delay of 3 min, low/high mass ranges of 40/550, ion source temperature of 280 °C, MS quad temperature of 150 °C, and MS source temperature of 230 °C. The hydrodistillate was injected neat, and peaks were identified on the basis of their match with spectra using the NIST98 chemical database.

FAME Analysis. Two milligrams of each extract, fractions A−G, crystals, and standards was dissolved in 2 mL of diethyl ether at room temperature overnight with a solution of diazomethane in diethyl ether (2 mL) for methylation.[7] Solvent and residual diazomethane were removed under a stream of N_2, and the samples were redissolved in diethyl ether for GC analysis.

GC-FID analysis of the methylated extracts and fractions was performed on a Varian CP-3800 GC (Agilent Technologies, Mississauga, ON, Canada). The GC was equipped with a 60 m × 0.25 mm i.d., 0.25 μm film thickness, DB-23 fused silica capillary column (Agilent Technologies) operated using the following conditions: the injector temperature was set at 270 °C, column temperature was initially set at 130 °C and held for 1 min followed by ramping from 130 to 170 °C at 6.5 °C/min, again followed by ramping from 170 to 215 °C at 2.8 °C/min, held for 12 min, and followed by a final ramp from 215 to 230 °C at 40 °C/min and held for 3 min; injection volume was set at 1 μL (split 20:1); 3.0 mL/min constant flow of He; and the FID temperature was set at 300 °C. Fatty acid methyl esters present in the samples were specifically identified by injection of methylated fatty acid standards (Sigma-Aldrich, St. Louis, MO, USA) and subsequent comparison of retention times of standards with those of unknowns.

Gas Chromatography−Mass Spectrometry of Methylated Samples. Methylated extracts, crystals, and fractions A-G were analyzed by GC-MS on a Varian CP-3800 GC coupled to a Varian Saturn 2000 MS/MS (Agilent Technologies). The GC was equipped with a 30 m × 0.25 mm i.d., 0.25 μm film thickness, DB-5 fused silica capillary column (Agilent Technologies) operated using the following conditions: injector temperature, 240 °C; column temperature, 60−240 °C at 3 °C/min, then held at 240 °C for 5 min; carrier gas, He; injection volume, 1 μL (splitless). MS ionization energy was set to 70 eV.

NMR Instruments. The hydrodistillate, fractions A−G, and crystals were dissolved in $CDCl_3$ at approximately 20 mg/mL, and their ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian ANOVA spectrometer (Varian, Palo Alto, CA, USA).

Mosquito Feeding-Deterrent Bioassay. In vitro bioassays were conducted as previously described.[8] Mosquitoes used in tests were *Aedes aegypti* (Liverpool), originally obtained from colonies at the Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, and maintained in colonies at the Beltsville Agricultural Research Center, USDA, ARS, Beltsville, MD. The larvae were reared by feeding larvae Hikari Cichlid Gold Fish Food (Kamihata Fish Ind. Ltd., Himeji, Japan). Adults were held under a 12:12 h (light/dark) photoperiod at 26 °C and 50% relative humidity and fed with a cotton pad moistened with 10% aqueous sucrose solution.

Adult female mosquitoes used in tests were between 5 and 10 days old and were kept without sucrose or water pads for 24 h prior to testing. The *Ae. aegypti* used in a given test session were all loaded into specially constructed feeding chambers, K&D modules,[8] at one time with five adult females/cell and tested within 1−2 h (Figure 1D). Treatments were replicated 10−20 times, representing a total of 50−100 mosquitoes. Tests were performed in an Air Science USA hood, with an air movement velocity of approximately 3.1−3.4 m^3/min, temperature of 26−28 °C, and relative humidity of 32−60%. A six-celled reservoir was connected to and heated by a Lauda E100 constant-temperature (38 °C) water circulator (Wobser GMGH and Co., Konigshofell, Germany).

Prior to each test, the upper surface of each reservoir was coated with a thin layer of high-vacuum silicone grease (Dow Corning Corp., Midland, MI, USA), and the cells were then filled (approximately 6 mL capacity) or topped-off with an aqueous solution of citrate−phosphate−dextrose−adenine (CPDA) to which ATP was added on the day of testing to give a concentration of 10^{-3} M ATP along with a food color dye (Esco Foods, San Francisco, CA, USA) to facilitate determination of mosquito feeding and engorgement. The filled cells were then covered with an Edicol collagen membrane strip and then with a just-treated organdy cloth (G Street Fabrics, Rockville, MD, USA) strip. Organdy strips were stretched lengthwise in a hood and treated using a pipet with first the control and then other treatments in the order of their randomly assigned positions. After solvent from the last applied treatment had evaporated (approximately 30 s), a Teflon separator was placed over the treated organdy strip, and together the separator and strip were placed on top of the reservoir.

K&D modules containing five adult females/cell were placed on the Teflon separator atop each reservoir; the sliding floors of the K&D modules were opened, allowing mosquitoes access to the treated organdy cloth and membrane-covered cell; and the number of mosquitoes biting (proboscis inserted through the cloth) and/or observed to be engorged within each cell at the end of a 3 min exposure period was recorded. Mosquitoes were used only once in a test and then frozen and discarded.

After completion of each test, the Teflon separator was rinsed in water for reuse; the treated organdy cloth and collagen membrane were removed and discarded; the reservoir cells were topped off, if needed, and any excess feeding solution on the surface of the reservoir was wicked off with tissue paper; and a new collagen membrane was applied. Only tests in which three or more females fed on the control cell were analyzed, as lower feeding rates were considered to be indicative of a substandard feeding readiness of that group of mosquitoes.

Experimental Design. Initial studies were conducted to determine the optimal extraction method for further analysis. Four extracts (two smoke extracts, dichloromethane, and ethyl acetate) were evaluated in the K&D bioassay with an application rate of 100 μg/cm^2. Each run of the bioassay also included DEET (positive control), applied at 4.7 μg/cm^2 (25 nmol/cm^2), and ethanol (negative control).

Table 1. Evaluation of *Artocarpus altilis* Inflorescence Extracts, Hydrodistillate Fractions, Fatty Acid Blends, and Pure Fatty Acids as *Ae. aegypti* Feeding Deterrents in the K&D Bioassay

			concentration			statistical significance[a]	
expt	treatment	*n*	$\mu g/cm^2$	$nmol/cm^2$	proportion not biting[b]	vs ethanol	vs DEET
1	ethanol	20	N/A	N/A	0.38 ± 0.049	N/A	***
	DEET	20	4.7	25	0.94 ± 0.024	***	N/A
	smoke						
	dichloromethane	20	100	N/A	0.74 ± 0.044	***	***
	aqueous	20	100	N/A	0.52 ± 0.05	*	***
	dichloromethane	20	100	N/A	0.56 ± 0.05	*	***
	ethyl acetate extract	6	100	N/A	0.86 ± 0.035	N/A[c]	N/A[c]
2-1	ethanol	18	N/A	N/A	0.34 ± 0.048	N/A	***
	DEET	18	4.7	25	0.79 ± 0.041	***	N/A
	hydrodistillate	18	10	N/A	0.82 ± 0.038	***	–
	ethyl acetate extract	18	1	N/A	0.54 ± 0.044	***	***
	ethyl acetate extract	18	10	N/A	0.66 ± 0.048	***	*
	ethyl acetate extract	18	100	N/A	0.74 ± 0.05	***	–
2-2	ethanol	10	N/A	N/A	0.38 ± 0.049	N/A	***
	DEET	10	4.7	25	0.86 ± 0.035	***	N/A
	hydrodistillate	10	1	N/A	0.7 ± 0.046	***	***
	hydrodistillate	10	10	N/A	0.96 ± 0.029	***	*
	hydrodistillate	10	100	N/A	1 ± 0	***	**
3-1	ethanol	20	N/A	N/A	0.32 ± 0.047	N/A	***
	DEET	20	4.7	25	0.89 ± 0.031	***	N/A
	hydrodistillate	20	10	N/A	0.99 ± 0.01	***	*
	fraction A	20	10	N/A	0.74 ± 0.044	***	*
	fraction D	20	10	N/A	0.87 ± 0.034	***	–
	crystals	20	10	N/A	0.82 ± 0.038	***	–
3-2	ethanol	12	N/A	N/A	0.35 ± 0.048	N/A	***
	DEET	12	4.7	25	0.8 ± 0.04	***	N/A
	hydrodistillate	12	10	N/A	0.88 ± 0.033	***	–
	fraction B	12	10	N/A	0.8 ± 0.04	***	–
	fraction C	12	10	N/A	0.85 ± 0.036	***	–
3-3	ethanol	20	N/A	N/A	0.3 ± 0.061	N/A	***
	DEET	20	4.7	25	0.9 ± 0.03	***	N/A
	hydrodistillate	20	10	N/A	0.95 ± 0.022	***	–
	fraction E	20	10	N/A	0.92 ± 0.027	***	–
	fraction F	20	10	N/A	0.88 ± 0.033	***	–
	fraction G	20	10	N/A	1 ± 0	***	*
4	ethanol	10	N/A	N/A	0.28 ± 0.045	N/A	N/A
	fraction G	10	4.7	25	0.96 ± 0.02	***	N/A
	synthetic G	10	10	N/A	1 ± 0	***	N/A
	blend 1	10	10	N/A	1 ± 0	***	N/A
	blend 2	10	10	N/A	0.48 ± 0.072	*	N/A
	blend 3	10	10	N/A	0.64 ± 0.048	***	N/A
5	ethanol	20	N/A	N/A	0.25 ± 0.043	N/A	***
	DEET	20	4.7	25	0.71 ± 0.045	***	N/A
	blend 1	20	N/A	25[d]	0.92 ± 0.027	***	***
	capric acid	20	4.3	25	0.85 ± 0.036	***	**
	undecanoic acid	20	4.7	25	0.91 ± 0.029	***	***
	lauric acid	20	5.0	25	0.82 ± 0.038	***	*

[a]Levels of significance of contrasts are denoted as follows: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; – = no significant difference. [b]Variation presented with the mean represents the standard error [c]Statistical comparison not made due to different sample sizes. [d]8.33 $nmol/cm^2$ of each of the three acids for a total of 25 $nmol/cm^2$

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Once optimized protocols were established, dose response experiments were conducted for the ethyl acetate and hydrodistillate at 1, 10, and 100 $\mu g/cm^2$. The crystals and fractions A–G were evaluated at 10 $\mu g/cm^2$ on the basis of the effective rates observed from ethyl acetate extract and the hydrodistillate. The hydrodistillate was included (10 $\mu g/cm^2$) as a second positive control for all further studies. Bioassays were conducted with 5 mosquitoes per cell and 20 cells per treatment in a completely randomized design. Statistical analysis was done using R to conduct contrast statements between treatments and the controls.

Fatty Acid Activity Analysis. Following preliminary extract analyses, commercially purchased fatty acids identified in the most active fraction, G, namely, capric acid (Sigma-Aldrich, St. Louis, MO, USA), undecanoic acid (Fluka, St. Louis, MO, USA), lauric acid (Fluka), myristic acid (Sigma-Aldrich), palmitic acid (Sigma-Aldrich), linoleic acid (Sigma-Aldrich), and α-linolenic acid (Sigma-Aldrich) were evaluated together and in three blends for potential antifeeding activity. Blend 1 was composed of the three shorter chained saturated fatty acids (capric acid, undecanoic acid, and lauric acid). Blend 2 contained the two longer chain saturated fatty acids (myristic acid and palmitic acid). Blend 3 contained the two unsaturated fatty acids (linoleic acid and α-linolenic acid). All blends were made from equal proportions of the included chemicals by weight and evaluated at 10 $\mu g/cm^2$. Fraction G was included in each run of the bioassay at a rate of 10 $\mu g/cm^2$ as the positive control, and ethanol was included in all bioassays as the negative control. Finally, the fatty acids that comprised blend 1, capric acid, undecanoic acid, and lauric acid, were evaluated individually alongside DEET, all at 25 $nmol/cm^2$.

RESULTS AND DISCUSSION

Across all experiments, the rate of insects not feeding in the positive control DEET was between 71 and >90% (Table 1). Rates of insects not feeding in the negative control, ethanol, were consistently between 20 and 30% (Table 1). In preliminary experiments, the aqueous and dichloromethane extracts of smoke residue, ethyl acetate extract, and dichloromethane extract were compared at 100 $\mu g/cm^2$. None of these extracts were as effective as DEET, but all treatments resulted in significantly fewer mosquitoes feeding than in the negative control. The ethyl acetate extract could not be directly compared to the others due to unequal replication resulting from a loss of sample, but was selected for a dose response trial at application rates of 1, 10, and 100 $\mu g/cm^2$ due to the promising preliminary data (Table 1). The deterrent activity of the ethyl acetate extract was dose dependent with a logarithmic relationship ($y = 0.0434 \ln(x) + 0.5467$, $R^2 = 0.9868$). In subsequent studies, the ethyl acetate extract was compared with a hydrodistillate extract at 1, 10, and 100 $\mu g/cm^2$. The hydrodistillate extract at 10 $\mu g/cm^2$ was significantly more active than the DEET control or the other extracts. The activity of the hydrodistillate was also dose dependent; however, the response was saturated above 10 $\mu g/cm^2$. The hydrodistillate at an application rate of 10 $\mu g/cm^2$ was selected for use in the remaining experiments.

An exploratory GC-MS analysis of the hydrodistillate detected a complex mix of over 100 peaks. Forty-three of these peaks had a relative concentration based on peak area above 0.1%. Comparison of the MS data to the NIST chemical database allowed for the putative identification of 30 peaks and revealed the presence of a diverse mixture of terpenes, aldehydes, fatty acids, and aromatic compounds (Table 2). NMR analysis of the hydrodistillate was indicative of aliphatic carboxylic acids and confirmed the prominence of fatty acids in the extract. The presence of fatty acids in the extract was further confirmed, and individual fatty acids were identified by methylation of the hydrodistillate followed by GC-FID of the resulting fatty acid methyl esters (FAMEs).

Table 2. Chemical Composition of Hydrodistillate of the Male Inflorescences of Breadfruit (*Artocarpus altilis*)

compound assignment[a]	MW	% relative abundance	compound class
5-methyl-2-furancarboxaldehyde	110	2.86	aldehyde
6-methyl-5-hepten-2-one	126	0.13	ketone
benzyl alcohol	108	0.16	aromatic
benzeneacetaldehyde	120	0.46	aldehyde
heptanoic acid	130	1.17	carboxylic acid
p-cymene	132	0.13	aromatic
linalool	154	0.10	terpene
nonanal	142	0.42	aldehyde
2,3-dimethylhydroquinone	138	0.10	aromatic
terpineol	154	0.22	terpene
α-muurolene	204	0.10	terpene
1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene	204	0.13	aromatic
2-isopropyl-5-methyl-9-methylenebicyclo{4.4.0}dec-1-ene	204	0.32	terpene
1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl) naphthalene	204	0.21	aromatic
copaene	204	0.45	terpene
myristic acid	228	1.28	fatty acid
methyl 9-methyltetradecanoate	256	0.13	ester
14-pentadecenoic acid	240	0.36	fatty acid
2-hydroxycyclopentadecanone	240	0.48	ketone
pentadecanoic acid	242	1.58	fatty acid
palmitoleic acid	254	0.10	fatty acid
palmitic acid	256	48.40	fatty acid
9,17-octadecadienal	264	2.25	aldehyde
9,12,15-octadecatrien-1-ol	264	2.06	alcohol
linoleic acid	280	11.20	fatty acid
16-methylheptadecanoic acid	284	0.16	fatty acid
linoelaidic acid	280	17.96	fatty acid

[a]Compounds were tentatively identified by comparison to the NIST98 chemical database. Only compounds with a probability of matching mass spectra >0.9 are shown.

The hydrodistillate was separated into seven fractions on the basis of their similarity on TLC using Godin reagent. All seven fractions applied at 10 $\mu g/cm^2$ displayed significant mosquito-deterrent activity; however, fraction G was the most active, resulting in none of the mosquitoes feeding at an application rate of 10 $\mu g/cm^2$. NMR analysis of fraction G suggested that it contained aliphatic carboxylic acids. Methylation followed by GC-FID analysis confirmed the presence of the FAs and was used to identify the most abundant compounds on the basis of peak area. The identified FAs in fraction G included five saturated fatty acids, capric acid (C10:0), undecanoic acid (C11:0), lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0), and two unsaturated FAs, linoleic (C18:2n6C) and α-linolenic acid (C18:3n3).

The seven fatty acids identified in fraction G were evaluated in four mixtures. The first group was a synthetic version of fraction G and included a mixture of all seven fatty acids, the second group comprised the shorter chained saturated fatty acids (C10, C11, and C12), the third group consisted of the longer chain saturated fatty acids (C14 and C16), and the fourth group contained the two unsaturated fatty acids (C18:2n6 and C18:3n3). The synthetic version of fraction G

resulted in greater activity than the original fraction; however, the fatty acids in the synthetic mixture were present in equal parts, and therefore the synthetic mix was not chemically identical to the original fraction. The three fatty acid blends all exhibited significant mosquito-deterrent activities, but the shorter chained fatty acids that made up group 1 were by far the most active and resulted in 100% biting deterrence. When the three fatty acids in blend 1 were evaluated individually, all three exhibited significantly greater deterrent activity than DEET when applied at equal molar concentrations (Table 1). Of the three fatty acids, undecanoic acid (C11:0) was the most active, followed by capric acid (C10:0), and then lauric acid (C12:0).

The results of the current study provide evidence that support the efficacy of the traditional practice of burning male inflorescences of breadfruit to repel flying insects[1,3,4] and indicate that the inflorescences could provide a valuable secondary product from the tree. The activity displayed by the solvent extracts suggests that the underlying chemical constituents responsible for the observed mosquito-deterrent activity are volatile compounds present in the inflorescences rather than resulting from thermal decomposition during combustion. The chemical composition of the most active extract, the hydrodistillate, was a complex mixture and included a number of terpenoids, ketones, and fatty acids, all of which provide promising avenues for further investigation (Table 2). Numerous terpenoids with mosquito-deterrent activity have been isolated from a taxonomically diverse array of plants, making this class of chemicals likely candidates as the active constituents. Some established examples include spathulenol, intermedeol, and callicarpenal from *Callicarpa* spp., Verbenaceae,[9] and nepetalactone from *Nepeta cataria*, Lamiaceae.[10] As such, fractionation of the breadfruit hydrodistiollate was conducted on the basis of the separation of the terpenoid components of the extract. However, whereas the terpenoid constituents may contribute to the deterrent properties of the inflorescences, fractionation of the extract based on the terpenoid profile did not effectively separate out the activity. Rather, it resulted in a series of fractions that all retained mosquito-deterrent properties (Table 1).

Fatty acids were a significant portion of the phytochemical composition of the hydrodistillate and all of the fractions. Several fatty acids have previously been identified as effective insect deterrents[7,11,12] and are the primary active compounds in *Jatropha curcas*, another plant traditionally used as a natural insect repellent.[7] The three most active fatty acids identified in fraction G, capric acid, undecanoic (hendecanoic) acid, and lauric acid, have all previously been identified as highly effective mosquito repellents.[11,12] A large-scale insecticide and insect-repellent screening program conducted during the 1940s and 1950s reported that all three of these compounds exhibited repellent activity against the yellow fever vectoring mosquito, *Ae. aegypti* L., as was shown in the present study, as well as the malaria vectoring mosquito, *Anopheles quadrimaculatus* Say.[11] When applied to the skin, capric acid and undecanoic acid (also referred to as hendecanoic acid[11]) were among the 66 most effective of the 4274 compounds tested against *Ae. aegypti*, effectively deterring mosquitoes from biting subjects for >300 min. In a similar assay against *An. quadrimaculatus*, undecanoic acid was among the 137 most effective and capric acid was among the 526 most effective compounds of the 3918 tested, deterring this species of mosquito from feeding for 91−150 and 61−90 min, respectively.[11] In further assays evaluating mosquito-repellent activity when applied to cloth, capric acid, undecanoic acid, and lauric acid were all among the 573 most effective compounds of the 6283 tested against *Ae. aegypti*, providing repellent activity for >21 days after application.[11] The compounds were less effective against *An. quadrimaculatus*; however, this was true for the majority of compounds evaluated in the program.[11] Three of the four less effective fatty acids found in fraction G, palmitic acid, myristic acid, and linoleic acid, were also evaluated in this screening program and resulted in no noticeable repellent activity in any of the mosquito assays in which they were included.[11] Overall, these data support the relative deterrent activities observed in the present study, suggest that the compounds identified in the breadfruit extracts are also effective against other species of mosquito such as *An. quadrimaculatus*, and indicate that breadfruit extracts could be effective when applied to skin or cloth.

The aforementioned screening program also evaluated the efficacy of some of these fatty acids as insecticides and repellents against a variety of other insects.[11] Capric acid exhibited high levels of toxicity to fleas, ticks, and chiggers at several stages of development. Whereas capric acid was found to be relatively nontoxic to lice and mosquito larvae[11] in this study, other researchers have reported toxicity to *Culex restuans* larvae at 150 ppm,[13] and an LD_{50} of 14 ppm against *Aedes triseriatus* larvae.[14] Repellent activity of capric acid was also observed toward fleas and ticks.[11] Undecanoic acid was less effective than capric acid as an insecticide, but exhibited some toxicity toward ticks and chiggers. Similar to capric acid, undecanoic acid exhibited excellent repellent activity against fleas and ticks. In a separate study, significant repellent activity was observed for undecanoic acid and lauric acid against house flies and horn flies.[12] Lauric acid was found to exhibit relatively high toxicity to *An. quadrimaculatus* larvae, causing 95−100% mortality at 10 ppm,[11] and has a reported LD_{50} of 7 ppm for *Ae. triseriatus* larvae.[13] The natural blend of these fatty acids found in breadfruit inflorescence hydrodistillate may endow it with a wide range of repellent/insecticidal activities and with further research could be utilized as a valuable agricultural byproduct.

Overall, this study provides evidence that the male inflorescences of breadfruit can be utilized as an effective mosquito deterrent and could provide a valuable secondary product from the breadfruit tree for local use or as a cash crop. This is of particular importance as breadfruit is adapted to the wet tropics, where mosquito-borne infectious diseases such as malaria, dengue fever, and yellow fever are the most destructive and cause untold economic and human loss.[2] Furthermore, the three shorter chained fatty acids identified here were found to be more effective mosquito feeding deterrents than DEET and could provide a viable alternative to DEET-based insect repellents. Further research is needed to explore the chemical diversity within the breadfruit inflorescences, develop breadfruit-based insect repellents, investigate the underlying mode of action of the active constituents, and evaluate the potential of the identified fatty acids as commercial insect repellents.

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The authors declare no competing financial interest.
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