JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of Extraction Methods for Marker Compounds in the Essential Oil of Lemon Grass by GC

BRIAN T. SCHANEBERG† AND IKHLAS A. KHAN*,†,‡

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, Mississippi 38677, and Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677

A gas chromatography flame ionization detection method for the quantification of bioactive marker compounds (neral, geranial, geraniol, limonene, citronellal, and β-myrcene) in the essential oil of *Cymbopogon citratus* (lemon grass) was developed. Four procedures for the extraction of essential oils from *C. citratus* were compared including solvent extraction, steam distillation extraction, accelerated solvent extraction, and supercritical fluid extraction. Solvent extraction by sonication with nonpolar solvents showed comparable results to the steam distillation method. Several commercial products prepared from *C. citratus* and *Cymbopogon flexuosus* were analyzed and compared.

KEYWORDS: Lemon grass; *Cymbopogon citratus*; *Cymbopogon flexuosus*; extraction; GC-FID

INTRODUCTION

Cymbopogon citratus (Nees) Stapf. (Gramineae), commonly know as lemon grass, is widely used as an essential ingredient in Asian cuisine due to its sharp lemon flavor. In India, a tea prepared from lemon grass is used as a sedative for the central nervous system (*1*). The essential oil of lemon grass has also been used to treat a wide variety of health conditions such as acne, athlete's foot, excessive perspiration, flatulence, muscle aches, oily skin, and scabies (*2*). Bioactivity studies have shown that various components in the essential oil possess antimicrobial (*3*, *4*), antifungal (*5*, *6*), antibacterial (*7*), and mosquito repellent activity (*8*).

The essential oil of lemon grass is mainly comprised of citral (on average, 65−80%). Citral is a mixture of bioactive isomers neral (**1**) and geranial (**2**) (**Figure 1**). Other isolated active components are limonene (**3**), citronellal (**4**), β-myrcene (**5**), and geraniol (**6**) (*9*). The essential oils of lemon grass have found a market in the aroma and massage therapy industry and can be purchased in stores (*2*). High-quality lemon grass essential oil is composed primarily of citral (>75%) (*9*). Lemon grass essential oil product quality was determined by the gas chromatography flame ionization detection (GC-FID) method developed herein.

Because the climate in Mississippi is suitable for growing lemon grass as a commodity, an analysis method to determine citral (**1** and **2**) content in the plant must be developed. As a volatile isomeric mixture, citral content can be determined by GC-FID. Optimization of a procedure for the extraction of the essential oil must then be conducted.

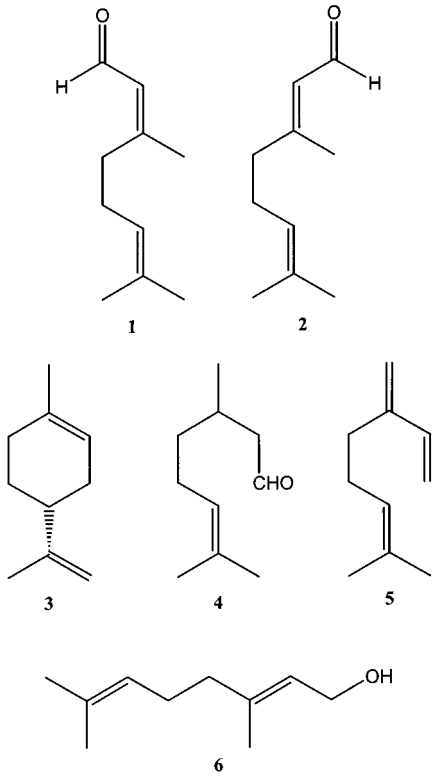

Figure 1. Structures of the marker compounds detected in the essential oil of *C. citratus*.

Steam distillation is the primary method for extracting the essential oil from plants for commercial products. The advantage over traditional extraction methods involving solvent is the absence of nonvolatiles in the extract such as chlorophyll and

* To whom correspondence should be addressed. Tel: (662)915-7821. Fax: (662)915-7989. E-mail: ikhan@olemiss.edu.
† Research Institute of Pharmaceutical Sciences.
‡ School of Pharmacy.

10.1021/jf011078h CCC: $22.00
Published on Web 02/02/2002

Table 1. Concentration of Marker Compounds Detected in *C. citratus*

method[a]	mg/mL[a]	markers in extract (%)	citral in markers (%)	SD (%) ($n = 3$)[b]
		Solvent Sonication		
hexanes	2.74 (1.17)	37.72	86.83	1.27
dichloromethane	1.86 (0.44)	14.02	83.93	3.60
acetone	1.44 (0.25)	12.26	80.65	2.59
methanol	0.22 (1.31)	0.69	79.48	4.20
		Accelerated Solvent		
hexanes	0.13 (2.41)	5.98	74.98	2.60
dichloromethane	0.25 (0.81)	13.22	43.28	0.91
acetone	0.34 (2.75)	6.18	67.10	2.73
methanol	0.20 (1.52)	5.23	27.54	1.53
		Supercritical Fluid		
no modifier				
1 h	0.63 (0.18)	9.73	69.95	1.65
10% hexanes modifier				
1 h	0.48 (0.52)	10.35	85.84	2.37
2 h	0.64 (0.09)	12.73	72.11	0.12
4 h	1.22 (0.05)	11.75	78.53	0.80
30% hexanes modifier				
2 h	1.08 (0.09)	16.99	77.14	3.50
10% dichloromethane modifier				
2 h	0.89 (0.62)	37.13	73.43	4.68
30% dichloromethane modifier				
2 h	0.80 (0.12)	7.80	72.15	0.13
		Steam Distillation		
10 g, 2 h	3.03 (0.54)	58.32	78.19	0.54
10 g, 6.5 h	0.84 (0.12)	29.35	81.07	0.13
20 g, 9.5 h	0.57 (0.82)	21.84	92.81	4.32

[a] The standard deviation for GC method validation of a single extract is given in parentheses ($n = 3$). [b] The standard deviation for the validation of the extraction procedures ($n = 3$).

fatty acids. This means that no cleanup procedure is needed, which may lead to the loss of product.

Steam distillation may not be the most convenient method for the analysis of essential oil components in plant material, specifically in lemon grass. The essential oils can also be extracted by other methods that utilize less plant material and shorter extraction times, such as solvent extraction with sonication, supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE). This study compares solvent extraction, SFE, and ASE with steam distillation in the analysis of active compounds in lemon grass as well as the quantification of active compounds in commercial lemon grass essential oils.

MATERIALS AND METHODS

Chemicals. Citral, geraniol, (*R*)-(+)-limonene, citronellal, β-myrcene, and the internal standard capric acid methyl ester were purchased from Sigma (St. Louis, MO). Solvents (hexanes, dichloromethane, acetone, and methanol) were high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Fair Lawn, NJ).

Commercial Products. Lemon grass essential oil products were purchased from retailers on the Internet and from a local herbal store.

Plant Material. *C. citratus* (Nees) Stapf. was grown in the greenhouse of the University of Mississippi, University, MS. Voucher specimens of the samples are deposited at the National Center for Natural Products Research, University, MS.

Internal Standard Solution. The internal standard was prepared by diluting 860.00 mg of capric acid methyl ester in 500 mL of dichloromethane. The final concentration was 1.72 mg/mL.

Steam Distillation. The essential oils were extracted with an extraction/distillation apparatus from Kimble/Kontes (Article No. 523010-0000, Vineland, NJ). The plant material was placed in a round-bottom flask, and Nanopure water was added until it was covered. Amounts of samples and times for extraction are listed in **Table 1**. Hexanes were used to remove the essential oil from the water layer. The hexanes layer was removed and then dried with anhydrous sodium sulfate. The dried solution was diluted to 10 mL in a volumetric flask with the internal standard solution. The samples were then analyzed by GC-FID (**Table 1**).

Solvent Extraction. Extraction took place in a 50HT Aquasonic Sonicator (VWR Scientific Products, West Chester, PA). Approximately 2.5 g of plant material was placed into a 50 mL screw-capped polypropylene centrifuge tube (Falcon tubes). The solvent (hexanes, dichloromethane, acetone, or methanol), about 20 mL, was added to the tube. Sonication of the samples was carried out for 30 min. Upon completion of the sonication, the emulsion was centrifuged for 8 min at 3000 rpm in a Marathon 21K/Br centrifuge (Fisher Scientific, Fair Lawn, NJ) and the supernatant was then decanted into a flask. This process was repeated twice. The combined supernatants were dried in a vacuum applying no heat, thus minimizing the loss of any volatiles. Each dried sample was then dissolved in 10 mL of internal standard solution and analyzed by GC-FID (**Table 1**).

SFE. SFE was performed in an Isco SFX 2-10 Supercritical Fluid Extractor with two Isco model 260D syringe pumps (Isco, Inc., Lincoln, NE). Approximately 2.5 g of plant material was used for each extraction. Once placed in an extraction cell, the sample was extracted with supercritical CO_2. The pressure was maintained at 80 atm at 50 °C, with flow rates fluctuating between 0.2 and 1.5 mL/min. All extractions were collected in HPLC grade dichloromethane.

The first extraction involved supercritical CO_2 with 10% hexanes as modifier for 1, 2, and 4 h three times each; 30% hexanes as modifier, 10% dichloromethane as modifier, and 100% CO_2 were also tested. Extraction experiments were performed in triplicate. The collected samples were concentrated en vacuo and weighed. Each sample was dissolved in 10 mL of internal standard solution and analyzed by GC-FID.

ASE. Extraction was accomplished with a Dionex ASE 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA). The pressure was held at 1000 psi with a constant temperature of 40 °C. Each sample (approximately 5.0 g) was extracted three times with 15 mL of solvent with a static time of 10 min. Four solvents were tested including hexanes, dichloromethane, acetone, and methanol (HPLC grade). The extracts (45 mL) were diluted to 50 mL. Five milliliters of the diluted extract was placed in a 10 mL volumetric flask containing 17.2 mg of

Table 2. Regression Equations and Correlation Coefficients (R^2) for the Lemon Grass Standards

standard	R^2	regression equation[a]
β-myrcene	1.000	$y = 1.27(x) - 0.0199$
limonene	1.000	$y = 1.45(x) - 0.0355$
citronellal	1.000	$y = 1.07(x) - 0.0347$
neral	1.000	$y = 1.17(x) + 0.0813$
geraniol	0.989	$y = 1.30(x) - 0.0752$
geranial	1.000	$y = 1.20(x) - 0.0187$

[a] x = amount ratio.

capric acid methyl ester and then diluted with dichloromethane. Once GC-FID analysis was finished (**Table 1**), the samples were concentrated en vacuo, and the weight was recorded.

GC-FID Conditions. Chromatograms were obtained on an HP5890 GC-FID, equipped with an HP7673 GC/SFC Injector (Agilent Technologies, Palo Alto, CA). HP Chemstation software was used for analysis. The column flow rate was 1 mL/min (Helium carrier gas), and the split ratio was 1:100. A DB-1 Column from Agilent (15 m × 0.25 mm ID, 0.25 μm film thickness) was used for all separations. The program started at 90 °C for 4.5 min. The temperature was increased at 5 °C/min to 110 °C, then at 30 °C/min to 200 °C. This was held for 5 min. Then, the temperature was increased at 50 °C/min to 300 °C and held for 2 min; the total run time was 20.5 min. Injector and detector temperatures were kept constant at 250 and 300 °C, respectively. The sample volume injected was 2 μL.

Calibration Curve. Five concentration levels (17.0−0.17 mg/mL) of the standard compounds, all being diluted with the internal standard solution, were prepared. The solutions were transferred to GC vials and each injected in triplicate. The percentage of neral and geranial in the citral standard was determined based on the area percent of each individual peak in the citral standard (neral 38% and geranial 62%). A five-point calibration curve was created for each reference standard by performing linear regression on the amount ratio vs response ratio. The regression equations and correlation coefficients (R^2) were recorded (**Table 2**), and 0.001 mg/mL was determined as the limit of detection for all compounds.

Method Validation. The precision of the GC method was confirmed by injecting each sample in triplicate, and a standard deviation less than 5.00% was achieved (**Table 1**, column 2). Each extraction method was carried out three times ($n = 3$), and the standard deviation was less than 5.00% (**Table 1**, column 5). The accuracy of the method was determined by spiking an essential oil free sample of *C. citratus* (407.4 mg) with 16.8 mg of citral. The plant material used for this experiment was extracted exhaustively (five times) with hexanes and monitored by GC for the absence of citral. Extraction then took place following the solvent extraction procedure with hexanes. The extract was diluted in a 10 mL volumetric flask with the dichloromethane internal standard solution (1.72 mg/mL) and analyzed by GC-FID.

Sample Preparation and Analysis of Commercial Products. The samples were prepared by recording the weight of the product (about 70 mg) in a tared 10 mL volumetric flask. The samples were then diluted with the internal standard solution. All commercial products were injected in triplicate. Excel was used to calculate the percent of the marker compounds and the standard deviations (**Table 3**).

RESULTS AND DISCUSSION

Data obtained by different extractions (percentage of **1**−**6** in an extract and citral percentage in the marker compounds detected) of *C. citratus* are listed in **Table 1**.

Solvent Extraction by Sonication. Different solvents were tested for an exhaustive extraction of *C. citratus* (hexanes, dichloromethane, acetone, and methanol). Each solvent was tested in triplicate to ensure reproducibility. The percentage of marker compounds detected in each extract was then determined by GC-FID. The hexanes extract contained the highest percentage of all measured marker compounds (37.72%). Lower percentages of marker compounds were detected in the dichloromethane, acetone, and methanol extracts (14.02, 12.26, and 0.69%, respectively). Citral content of the measured marker compounds was determined and compared. A citral percentage greater than 75.00% was desirable (*9*). The hexanes extract had the highest percentage of citral (86.83%) in the measured marker compounds. Dichloromethane, acetone, and methanol extracts also had citral concentrations above 75.00% (83.93, 80.65, and 79.48%, respectively) in the measured marker compounds. Of the four solvents tested for extraction by solvent sonication, hexanes extracted the highest concentration of measured marker compounds, and the hexanes extract contained the highest concentration of citral.

ASE. The dichloromethane extract contained the highest concentration of marker compounds detected (13.22%). Marker compound percentages were below 7.00% in the acetone, hexanes, and methanol extracts (6.18, 5.98, and 5.23%, respectively). The hexanes extract contained the highest concentration of citral (74.98%) in the measured marker compounds. Citral concentrations were below 70.00% in the acetone, dichloromethane, and methanol extracts (67.10, 43.28, and 27.54%, respectively). Of the four solvents tested for ASE, none extracted a citral percentage higher than 75.00% in the measured marker compounds. Each solvent was tested in triplicate to ensure reproducibility.

SFE. Extraction with 10% dichloromethane as the modifier gave the highest percentage of markers in the extract (37.13%). Citral concentration in the measured marker compounds was the greatest with 10% hexanes modifier for 1 h (85.84%). Extracts obtained by using 10% hexanes modifier for 4 h and 30% hexanes modifier for 2 h had citral concentrations above 75.00% (78.53 and 77.14%, respectively) in the measured marker compounds. All other SFE extracts had citral percentages below 75.00%. Each modifier was tested in triplicate to ensure reproducibility.

Steam Distillation. Percentages were compared by the time allowed for the extraction of essential oils. The highest percentages of marker compounds were present in the distillate after 2 h (58.32%) and decreased as the extraction time increased to 9.5 h (21.84%). The opposite was shown for the citral content. After 9.5 h, the citral content in the measured marker compounds was the greatest (92.81%) and the lowest after 2 h (78.19%). Each steam distillation time was tested in triplicate to ensure reproducibility.

Comparison. The GC chromatogram of the hexanes extract obtained by sonication is shown in **Figure 2**, and the region between 1 and 5 min was expanded in **Figure 3**. The concentration of geraniol (**6**) was the next highest to citral (neral and geranial) of the measured marker compounds. Limonene (**3**) was not detected in this sample.

The citral percentage in the measured marker compounds was highest in the solvent extracts obtained by sonication with hexanes (86.83%) when compared to SFE and ASE. Solvent sonication with dichloromethane yielded a slightly lower value of 83.93%. Extraction by SFE using 10% hexanes as modifier over 1 h also produced an extract containing greater than 80.00% citral in the measured marker compounds. Citral percentages were below 80.00% by all other solvent extractions tested.

The percentage for citral in the marker compounds obtained by solvent sonication with hexanes was comparable to the steam distillation extracts. This method had advantages over steam distillation. Less plant material was needed for analysis, and the extraction time was shorter. Solvent extraction with hexanes could be used to aid in the development of lemon grass as a

Table 3. Marker Compounds Present in Lemon Grass Essential Oil Products (%)

	β-myrcene	limonene	citronellal	neral	geraniol	geranial	citral	total
P1	2.16	4.44	0.69	27.05	3.75	36.64	63.69	74.73
	(0.23)[a]	(0.14)	(0.50)	(0.02)	(0.09)	(0.01)		(0.01)
P2	ND	1.91	ND	28.65	4.80	37.20	65.85	72.56
		(0.40)		(0.11)	(0.14)	(0.05)		(0.04)
P3	ND	1.89	ND	29.04	5.00	37.26	66.30	73.19
		(0.11)		(0.10)	(0.10)	(0.08)		(0.08)
P4	1.81	3.22	ND	20.30	3.00	29.95	50.25	58.28
	(0.50)	(0.35)		(0.06)	(0.11)	(0.05)		(0.07)
P5	1.59	2.23	2.62	30.03	4.26	42.41	72.44	83.14
	(0.50)	(0.50)	(0.32)	(0.32)	(0.28)	(0.32)		(0.08)
P6	0.29	3.05	1.23	27.11	5.86	35.36	62.47	72.90
	(0.21)	(0.36)	(0.19)	(0.22)	(0.27)	(0.12)		(0.10)
P7	0.27	3.80	1.21	27.08	5.74	34.41	61.49	72.51
	(0.40)	(0.50)	(0.44)	(0.02)	(0.42)	(0.08)		(0.03)
P8	0.23	2.65	0.70	22.05	5.00	31.82	53.87	62.45
	(0.11)	(0.12)	(0.06)	(0.05)	(0.08)	(0.04)		(0.05)
P9	2.24	2.37	1.82	29.71	3.88	40.29	70.00	80.30
	(0.25)	(0.15)	(0.46)	(0.03)	(0.16)	(0.02)		(0.04)

[a] Standard deviation in parentheses ($n = 3$). ND = not detected.

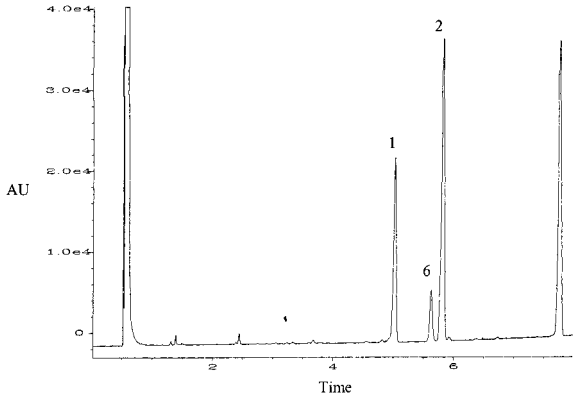

Figure 2. Chromatogram of the hexanes sonication extract of lemon grass.

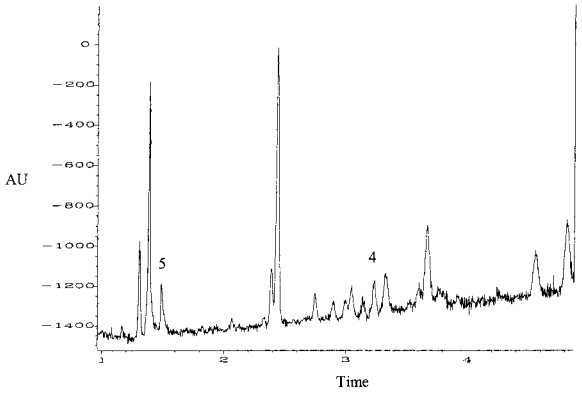

Figure 3. Expansion of 1–5 min from **Figure 2**.

cash crop in Mississippi, in which a strain of lemon grass is grown to achieve a desired concentration of citral present in the essential oil.

As previous studies have shown, the amount of citral remains constant over time, even after the plant material has been dried (*9*). A volatility and spiking study were both completed for the solvent sonication with hexanes method. After extraction with hexanes, the extract was analyzed. Then, the solvent was evaporated, redissolved in dichloromethane, and analyzed again. The concentration of essential oil marker compounds remained constant. Additionally, a spiking experiment was performed. Because citral is the compound of importance, a plant sample of *C. citratus*, which had been exhaustively extracted and monitored for the absence of citral, was spiked with this compound. After solvent extraction with hexanes, the citral recovery was >99.00%.

Product Analysis. The quality of some commercial lemon grass products was tested by the GC method (**Table 3**). Again, >75.00% citral content was the standard for high-quality lemon grass essential oil products (*9*). Three of the products were extracted from *Cymbopogon flexuosus*, which is also commercially used (P1−P3), five were extracted from *C. citratus* (P4−P8), and the species was unknown for P9. Two of the *C. flexuosus* products were produced by the same company with different batch numbers (P2 and P3) but were obtained from two different market sources. P1 and P5 were purchased from the same company, but P1 was derived from *C. flexuosus*, and P5 was derived from *C. citratus*.

The results of the commercial products are collated in **Table 3**. All six of the marker compounds were found in P1 and P5−P9, while β-myrcene (**5**) and citronellal (**4**) were not detected in P2 and P3. Citronellal (**4**) was not present in P4. Citral was the major component in each product. None of the products met the high-quality standard of >75.00% citral concentration in the measured marker compounds. P5 and P9 had the highest concentration of citral, 72.44 and 70.00%, respectively.

The quantity of marker compounds in each product was different. Differences in quantities are likely due to the environment in which the lemon grass was grown (soil, temperature, sunlight, etc.) and due to slight variations in the species. Excessive rainfall will actually decrease the content of citral in the essential oil (*9*). The manufacturing of the product and the age of the product may also have an effect on the marker compound concentrations. The difference between species could not be determined.

ACKNOWLEDGMENT

The authors thank Dr. C. Burandt, NCNPR, for providing the authentic plant material for this study.

LITERATURE CITED

(1) Nair, E. V. G. Essential Oil of East Indian Lemongrass: Present Position in India and Scope of its Development. *Cultiv. Util. Med. Aromat. Plants* **1977**, 204−206.

(2) Lawless, J. *Illustrated Encyclopedia of Essential Oils*: *The Complete Guide to the Use of Oils in Aromatherapy & Herbalism*; Element Books: Rockport, MA, 1995; pp 56−67.

(3) Ibrahim, D. Antimicrobial Activity of the Essential Oil of the Local Serai, *Cymbopogon citratus*. *J. Biosci.* **1992**, *3* (1−2), 87−90.
(4) Onawunmi, G. O. Evaluation of the Antimicrobial Activity of Citral. *Lett. Appl. Microbiol.* **1989**, *9* (3), 105−108.
(5) Wannissom, B.; Jarikasem, S.; Soontorntanasart, T. Antifungal Activity of Lemon Grass Oil and Lemon Grass Oil Cream. *Phytother. Res.* **1996**, *10* (7), 551−554.
(6) Viollon, C.; Chaumont, J.-P. Antifungal Properties of Essential Oils and Their Main Components Upon *Cryptococcus neoformans*. *Mycopathologia* **1994**, *128* (3), 151−153.
(7) Onawunmi, G. O.; Yisak, W. A.; Ogunlana, E. O. Antibacterial Constituents In the Essential Oil of *Cymbopogon citratus* (DC.) Stapf. *J. Ethnopharmacol.* **1984**, *12* (3), 279−286.
(8) Govere, J.; Durrheim, D. N.; Du Toit, N.; Hunt, R. H.; Coetzee, M. Local Plants as Repellents Against *Anopheles arobiensis*, in Mpumalanga Province, South Africa. *Central African J. Med.* **2000**, *46* (8), 213−216.
(9) Guenther, E. *The Essential Oils*: *Volume IV*; D. Van Nostrand Company, Inc.: New York, 1950; pp 20−40.

Received for review August 10, 2001. Revised manuscript received November 21, 2001. Accepted December 13, 2001. This work was funded in part by the United States Department of Agriculture, ARS Specific Cooperative Agreement No. 58-6408-7-012 and Alcorn State University in Mississippi through the Natural Products Initiative.

JF011078H