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Content, Composition, and Bioactivity of the Essential Oils of Three Basil Genotypes as a Function of Harvesting

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A study was conducted to evaluate the effect of cut on biomass productivity, oil content, composition, and bioactivity of *Ocimum basilicum* L. (cvs. German and Mesten) and *Ocimum sanctum* L. (syn. *O. tenuiflorum* L.) (cv. Local) in Mississippi. Yields of basil herbage and essential oil were high and comparable to those reported in the literature. Essential oil content of *O. basilicum* cv. German varied from 0.40 to 0.75%, the oil content of cv. Mesten varied from 0.50 to 0.72%, and the oil content of cv. Local (of *O. sanctum*) ranged from 0.17 to 0.50% in air-dried basil. Herbage and essential oil yields of cvs. German and Mesten of *O. basilicum* increased with the second and then again with the third cut, whereas herbage and oil yields of cv. Local of *O. sanctum* increased with the third cut relative to the previous cuts. Overall, essential oil yields were 115, 123, and 51 kg/ha for the cvs. German, Mesten, and Local, respectively. The major oil constituents of cvs. German and Mesten (of *O. basilicum*) were (−)-linalool (30–40%) and eugenol (8–30%), whereas the major oil constituents of cv. Local (of *O. sanctum*) were eugenol (8–43%) and methylchavicol (15–27%). Essential oils from both species grown in Mississippi showed in vitro activity against *Leishmania donovani* (IC_{50} = 37.3–49.6 μg/mL), which was comparable to the activity of commercial oil (IC_{50} = 40–50 μg/mL). Minor basil oil constituents (+)-δ-cadinene, 3-carene, α-humulene, citral, and (−)-*trans*-caryophyllene had antileishmanial activity, whereas other constituents were ineffective. None of the oil was cytotoxic to mammalian cells.

KEYWORDS: Basil; *Ocimum basilicum*; *Ocimum sanctum*; *Ocimum tenuiflorum*; antimicrobial; antileishmanial; essential oils

INTRODUCTION

The genus *Ocimum* L. includes approximately 150 species (*1*), with a great variation in phenotype, oil content, composition, and possibly bioactivity (*2*). Holy basil [*Ocimum sanctum* L. (syn. *Ocimum tenuiflorum* L.)] and sweet basil (*Ocimum basilicum* L.) are the two basil species that are considered to be promising essential oil crops. The basil essential oil contains pleasant aroma and is known to possess antimicrobial (*3–6*) and insecticidal (*7, 8*) activities. Recently, essential oil of African basil (*Ocimum gratissimum* L.) was found to have in vivo antimalarial activity (*9*) and in vitro antileishmanial activity (*10*). However, there are no reports on the antimalarial or antileishmanial activity of essential oils from sweet or holy basil. Due to its pleasant aroma and antimicrobial activity, basil essential oil is a major aromatic agent with applications in various industries such as the food, pharmaceutical, cosmetic, and aromatherapy industries. The traditional and most widely used method for basil extraction is via steam distillation of the whole aboveground basil herbage (stems, leaves, and flowers) (*11–13*). When grown for essential oil production, basil is harvested in full bloom, because the content and the composition of the oil are optimal at that stage (*11, 13*). Depending on the climate, basil could be harvested one to three times during the cropping season (*11*). There is a great variation of essential oil composition (and aroma) among basil cultivars currently on the international market. One of the oils with the finest aroma is obtained from European basil that contains linalool and methylchavicol as the main components (*14*). A different chemotype, the Reunion basil, is characterized by high levels of methyl-

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chavicol, whereas the tropical chemotype of basil is known to have methyl cinnamate as the main component of the essential oil. Another basil chemotype that is high in eugenol is grown in North Africa, Russia, Eastern Europe, and parts of Asia (*14*). There is a significant interest in basil as a new high-value essential oil crop in Mississippi and other states in the southeastern United States. However, there are no reports on basil essential oil content, composition, and bioactivity from plants grown in there.

The hypothesis of this study was that cut (subsequent harvesting) would alter productivity, essential oil content, composition, and bioactivity of sweet (*O. basilicum*) and holy (*O. sanctum*) basils. Furthermore, this study compares the chemical composition and bioactivity of basil oil produced in Mississippi to commercially available basil oils produced in other parts of the world. This is the first study on alterations in basil oil content, composition, and bioactivity depending on the cut. Also, this is the first study on oil content, composition, and bioactivity of field-grown sweet (*O. basilicum*) and holy (*O. sanctum*) basil cultivars from the southeastern United States.

MATERIALS AND METHODS

Plant and Growth Conditions. A randomized complete block design with four replications was used for the field experiment. The experiment was conducted in 2006 at the North Mississippi Research and Extension Center (NMREC) in Verona, MS, using sweet basil (*O. basilicum* L. cvs. German and Mesten) and holy basil (*O. sanctum* L. cv. Local). Certified basil seeds (from Richter's, Ontario, Canada, and from the Research Institute for Roses and Medicinal Plants in Kazanluk, Bulgaria) were used for the production of seedlings. Basil seedlings from the three cultivars were produced in a greenhouse in March, in 48-cell plastic trays filled with growth medium Metromix 300 (The Scotts Co., Marysville, OH). Basil plants were grown in the greenhouse for 40 days under natural light with a day temperature of 22–25 °C and a night temperature of 18 °C. Throughout seedling production, basil plants were irrigated once every 24 h and fertilized weekly with 1.8 g of 20−20−20 N−P_2O_5−K_2O dissolved in 300 mL of water.

Basil seedlings were transplanted into the field in May 2006 in prepared raised beds covered with black plastic to reflect horticultural practices in this part of the country. Soil samples were collected from the basil experiment site prior to land preparation and analyzed for extractable nutrients. The soil of the experimental site was Quitman sandy loam (fine-loamy, siliceous, semiactive, thermic, Aquic Paleudult) with 1.05% organic matter, 5% clay, 57% silt, and 37% sand, pH of 6.5, and the concentrations of available nutrients (in kg/ha): P, 50.4; K, 62; Ca, 1935; Mg, 97; Zn, 1.3; S, 164; and Na, 141.

Land preparation consisted of disking four times and the formation of raised beds (15 cm high and 75 cm wide across the top) using a press-pan-type bed shaper. The bed shaper machine was also used to cover the beds with black plastic mulch and to place a drip-tape irrigation tube in the middle of the bed under the plastic 4–5 cm below the soil surface. Research plots were 1 m × 6 m with 40 plants per plot. Basil transplants were transplanted on two rows on each bed, with 30 cm in-row and between-row spacing.

Basil plants were irrigated weekly as needed through the subsurface drip irrigation tape and fertilized weekly with totals for the cropping season of 120, 80, and 100 kg/ha of N, P_2O_5, and K_2O, respectively, using irrigation water via the drip tape. No pests or diseases were observed on any of the basil cultivars, and the black plastic mulch provided efficient weed control over the growing season. However, two of the common weeds for the area, yellow nutsedge (*Cyperus esculentus*) and purple nutsedge (*C. rotundus*), punched through the plastic and had to be removed by hand several times during the growing season. Basil plants were cut (harvested) three times over the growing season (June 9, July 26, and October 22), each time when plants reached full bloom. Plants were hand-harvested by cutting the herbage at 15 cm above the soil; fresh weight was recorded. Then the plants were dried at temperatures up to 40 °C, dry weight was recorded, and the plants were stored until further analyses. Cutting at around 15 cm above soil surface allowed basil plants to form new branches from the sleeping buds on the stem and the very tender small branches below 15 cm that were not harvested.

Basil essential oil was extracted via steam distillation using a modified Clevenger collector apparatus (*15*) from Scientific Glass. The distillations were carried out on a sample size of 150 g of dry aboveground material (stems, leaves, and flowers) with a distillation time of 120 min. The oil yield was calculated as the volume (milliliters) of oil per weight (gram) of fresh basil tissue. In addition, six commercially available basil oils were purchased from ATL Canada (Ontario, Canada). The six commercial basil oils originated from Bulgaria, India, Italy, The Seychells, and the United States.

GC-MS Analysis. Chemical standards, basil oil from the field experiment, and commercial basil oil samples were analyzed by GC-MS on a Varian CP-3800 GC coupled to a Varian Saturn 2000 MS/MS. The GC was equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm, with a film thickness of 0.25 μm) operated using the following conditions: injector temperature, 240 °C; column temperature, 60–240 at 3 °C/min, then held at 240 °C for 5 min; carrier gas, He; injection volume, 1 μL (splitless); MS mass range, 40−650 *m/z*; filament delay, 3 min; target TIC, 20000; prescan ionization time, 100 μs; ion trap temperature, 150 °C; manifold temperature, 60 °C; and transfer line temperature, 170 °C.

Quantitative Analysis. Commercial standards (1*S*)-(−)-α-pinene, camphene, eugenol, methylchavicol, methyleugenol, (−)-*trans*-caryophyllene, 1,8-cineole, 3-carene, and citral were purchased from Aldrich (St. Louis, MO), whereas (−)-linalool, (−)-camphor, (+)-δ-cadinene, α-humulene, eucalyptol, (−)-bornyl acetate, and methyl cinnamate were purchased from Fluka (Buchs, Switzerland). With five concentration points, an external standard least-squares regression for quantification was used. All 16 analytes were used to formulate separate calibration curves. Linearity was imposed by using response factors and regression coefficients independently. The response factors were calculated using the equation RF = DR/*C*, where DR is the detector response in peak area (PA) and *C* is the analyte concentration (Supporting Information).

The chromatograms of each of the oils from the field experiments and the commercial basil oil samples were compared to the standard injections. The target peaks were confirmed by both retention time and mass spectra. Confirmed integrated peaks were then used to determine the percentage of each chemical constituent in the essential oil. The RF of the target chemical constituent was used to determine the "percent in oil" for each sample using the equation PA/RF/*C* = % (peak area/response factor/concentration) in oil.

Assay for in Vitro Antileishmanial Activity. Antileishmanial activity of the extracts and the purified constituents (procured from commercial sources) was tested in vitro on a culture of *Leishmania donovani* promastigotes. In a 96-well microplate assay, the extracts or compounds with appropriate dilution were added to the *Leishmania* promastigotes culture (2×10^6 cells/mL). The plates were incubated at 26 °C for 72 h, and growth of *Leishmania* promastigotes was determined by Alamar Blue assay (*16*). Pentamidine and Amphotericin B were used as the standard antileishmanial agents. IC_{50} (the concentrations causing 50% inhibition in growth) and IC_{90} (the concentrations causing 90% inhibition in growth) values for each extract and compound were computed from the growth inhibition curve.

Antimicrobial and Antimalarial Activity and Assay for Cytotoxicity. Antimicrobial and antimalarial activities were tested by a previously described procedure (*17*). Essential oils as well as pure compounds were tested for in vitro cytotoxicity against mammalian kidney fibroblasts (VERO) and kidney epithelial cells (LLC-PK11) by Neutral Red assay (*18*) as described earlier (*17*).

Data analyses of oil content, dry herbage, and essential oil yields data sets were performed using one-way ANOVA in Quattro Pro.

RESULTS AND DISCUSSION

Overall, herbage yields of cvs. German and Mesten of *O. basilicum* increased with the second and then with the third cut, whereas herbage yields of cv. Local of *O. sanctum* increased with the third cut relative to the first or second (**Table 1**). This

Table 1. Effect of Genotype and Harvest (Cut) on Dry Matter Yields (DMY) of Herbage, Essential Oil Content, and Yields of Basil

species, measurement	first cut	second cut	third cut
***O. basilicum* cv. German**			
DMY, kg/ha	3348c[a]	4929b	8245a
oil content, %	0.41b	0.77a	0.75a
oil yield, kg/ha	13.7c	40.0b	61.8a
***O. basilicum* cv. Mesten**			
DMY, kg/ha	3126c	4837b	9627a
oil content, %	0.50b	0.72a	0.75a
oil yield, kg/ha	15.6c	34.8b	72.2a
***O. sanctum* cv. Local**			
DMY, kg/ha	5201b	5225b	6427a
oil content, %	0.22b	0.17b	0.48a
oil yield, kg/ha	11.4b	8.9b	30.8a

[a] Means with the same letter within a row and a species are not significantly different at $P \leq 0.05$.

increase with the second or third cut might be due partially to the fact that basil has established root systems and developed lower stem (that is not cut) at the time of the first harvest, and plants need fewer resources for building various plant parts. Hence, most of the plant resources are used to develop new branches and leaves. Whereas first-cut yields of *O. basilicum* were lower than yields of *O. sanctum*, the third-cut yields were higher than yields of *O. sanctum*.

The essential oil content of cvs. German and Mesten of *O. basilicum* increased in the second and third harvests relative to the first harvest, whereas the essential oil content of cv. Local from *O. sanctum* increased with the third harvest relative to the first or second harvest (**Table 1**). There were no differences in essential oil content between the two cultivars of *O. basilicum*, whereas cv. Local of *O. sanctum* contained less oil than the two *O. basilicum* cultivars. Oil yields, as a function of herbage yields and oil content, followed the same trends. To our knowledge, this is the first report on herbage yields and essential oil content increase with subsequent cuts (harvests) of the three cultivars and the two species. Our results confirm previous reports of differences in oil content between *O. basilicum* and *O. sanctum* (*19*). The increase in essential oil content of the three cultivars from *O. basilicum* and *O. sanctum* with subsequent cuts might be due to changes in environmental conditions, most probably increased temperature during the plant establishment after the first and second harvests (data not shown). Also, research has demonstrated that some stress factors, such as chemical elicitors (*20, 21*) and heavy metals (*22*), could increase the production and accumulation of secondary metabolites in aromatic crops. In this study, cutting of basil might act as a stress factor that promotes the synthesis and accumulation of essential oil in basil with subsequent cuts. Overall, the essential oil contents of the three basil cultivars in this study were similar to the oil content reported for the same species grown in other regions of North America (*19*).

Basil essential oil content was reported to range from 0.07 to 1.37% (*23, 24*), and different cultivars would normally have different oil contents (*24*). However, a screening of a large number of basil accessions in the United States demonstrated that the essential oil content varied from 0.04 to 0.7% (*26*). Most studies on basil essential oil content support the latter findings (*26*). Hence, our results suggest that *O. basilicum* cultivars grown in Mississippi may accumulate a relatively high concentration of essential oil, compared to the findings of Simon et al. (*26*). This could be due to the higher temperatures in Mississippi during the growing season compared to the temperatures in Indiana (*26*).

Overall, basil essential oil from the herbage (aboveground plant parts) of the *O. basilicum* cvs. German and Mesten contained linalool, camphor, δ-cadinene, α-pinene, α-humulene, camphene, eucalyptol, eugenol, isobornyl acetate, methylchavicol, methyleugenol, and *trans*-caryophyllene (**Table 2**). Basil essential oil of *O. sanctum* cv. Local contained the same constituents, with the exception of camphor, camphene, and isobornyl acetate, which were not detected in the oil of *O. sanctum*. The major constituents of *O. basilicum* cultivars were linalool (30–40%) and eugenol (8–30%), whereas the major constituents of *O. sanctum* cultivar were eugenol (8–43%) and methylchavicol (15–27%) (**Table 2**). *O. sanctum* cv. Local contained <1% of linalool in the essential oil, which supports previous findings (*27*). In the latter study (*27*), the major constituents of that specific chemotype of *O. sanctum* (syn. *O. tenuiflorum*) were methyleugenol, eugenol, (*E*)-cinnamyl acetate, and β-caryophyllene.

The two cultivars Mesten and German of *O. basilicum* had similar oil compositions. The linalool content of *O. basilicum* cultivar oil from the third cut was higher relative to the linalool content in oil from the first cut. The third cut has also increased the concentration of isobornyl acetate in cv. Mesten but not in cv. German (**Table 2**).

The third cut increased the concentration of α-humulene, eugenol, methylchavicol, and *trans*-caryophyllene in *O. sanctum* cv. Local relative to the first cut, whereas the second cut decreased the concentration of eugenol relative to the first or third cut, respectively. The concentration of other oil constituents was not influenced by the cut (subsequent harvest). Previous research has demonstrated that method of harvesting (primary vs secondary branches vs cutting biomass at 30 cm above ground) can alter the concentration of major oil constituents of *O. sanctum* oil (*27*). Our results extend this understanding in a way that the subsequent cut can also alter holy basil oil compositon.

The essential oil composition of the commercially available basil samples (that were purchased and analyzed under the same conditions following the methods in this study) varied greatly (**Table 3**). For instance, the major constituent of basil oil from Bulgaria and "linalool" type basil oil was linalool; the major constituents of basil oil from India were linalool and *trans*-caryophyllene; the major constituent in "sweet" basil was methylchavicol; the major constituents in oil from Italy were linalool and isobornyl acetate; and the major constituent in "lemon" basil oil was citral. We found a wide range of variation in oil constituents in the six commercially available basil oil types from Bulgaria, Italy, India, The Seychelles, and the United States. Overall, the compositions of the essential oil of basil cultivars grown in Mississippi were similar to those of the purchased and analyzed in this study commercial basil oils, suggesting the oil from basil grown in Mississippi could be marketed in the same way as basil oils already on the world market. Furthermore, the oil compositions of the three cultivars in this study were within the range of oil constituents of marketable basil oils from India, France, Australia, and The Seychelles as reported previously (*28*).

Our results on the essential oil composition of the three cultivars are similar to other reports on cv. Mesten of *O. basilicum* and cv. Local of *O. sanctum* (*19*). However, whereas the latter authors (*19*) found linalool concentration in cv. Mesten to be up to 57% and in most instances above 50%, in this study, the linalool concentration of oil from this cultivar grown in Mississippi was much lower, around 30–40%. Also, whereas eugenol was one of the major oil constituents of cv. Mesten

Table 2. Chemical Constituents[a] of Basil Essential Oil as a Function of Genotype and Cut (Subsequent Harvest) in Percent of Total Oil

	% analyte in total oil (mean ± standard deviation)								
	cv. German			cv. Local			cv. Mesten		
compound[b]	first	second	third	first	second	third	first	second	third
(−)-linalool	30.7 ± 3.68	36.5 ± 5.10	38.8 ± 1.27	0.310 ± 0.0316	0.378 ± 0.0114	0.191 ± 0.0315	37.0 ± 3.47	29.4 ± 12.8	40.3 ± 0.886
(−)-camphor	0.598 ± 0.237	0.737 ± 0.312	0.825 ± 0.0480				0.356 ± 0.195	0.531 ± 0.168	0.499 ± 0.127
(+)-δ- cadinene	3.43 ± 0.163	0.585 ± 0.353		0.168 ± 0.0194					0.602 ± 0.262
(1*S*)-(−)-α-pinene	0.294 ± 0.107	0.268 ± 0.163	0.213 ± 0.145	0.371 ± 0.173		0.355 ± 0.120	0.290 ± 0.169	0.433 ± 0.0810	0.307 ± 0.0821
α-humulene	0.315 ± 0.143	0.524 ± 0.0351	0.453 ± 0.0325	0.976 ± 0.0849	1.34 ± 0.123	1.99 ± 0.425	0.415 ± 0.0539	0.668 ± 0.138	0.482 ± 0.0288
camphene	0.140 ± 0.0108	0.0754 ± 0.0673	0.196 ± 0.186				0.108 ± 0.0791	0.196 ± 0.0194	0.166 ± 0.083
citral									
eucalyptol	2.66 ± 0.243	4.29 ± 0.839	2.23 ± 0.265	5.45 ± 0.633	3.68 ± 2.15	4.62 ± 0.384	3.35 ± 0.211	3.11 ± 0.596	2.70 ± 0.433
eugenol		8.24 ± 3.53	28.0 ± 2.72	18.0 ± 6.85	7.78 ± 3.21	42.5 ± 5.12	9.68 ± 0.439	11.9 ± 3.16	30.2 ± 3.67
(−)-bornyl acetate	0.878 ± 0.243	1.21 ± 0.0589	0.930 ± 0.284				1.05 ± 0.0473	1.35 ± 0.410	1.85 ± 0.313
methylchavicol				14.7 ± 1.30	27.2 ± 4.75	20.9 ± 1.94	0.0481 ± 0.0121	0.0648 ± 0.0501	
methyleugenol		0.117 ± 0.00456		0.210 ± 0.149					
(−)-*trans*-caryophyllene	0.118 ± 0.0144	0.176 ± 0.0366	0.176 ± 0.00797	0.490 ± 0.0587	0.575 ± 0.0553	1.36 ± 0.197	0.119 ± 0.00835	0.196 ± 0.0214	0.178 ± 0.0188

[a] All compounds were identified by comparison of mass spectrometry and retention index data with those of commercially available standards. Quantification was performed using response factors determined from commercially available standards. [b] 1,8-Cineole, 3-carene, and methyl cinnamate were not detected in any of the samples.

Table 3. Chemical Constituents[a] of Commercial Basil Essential Oils, in Percent of Total Oil

	% analyte in total oil (mean ± standard deviation)					
compound[b]	Bulgaria	India	Italy	lemon	linalool	sweet
(−)-linalool	37.1 ± 11.4	7.80 ± 0.192	4.14 ± 0.482	0.169 ± 0.0290		0.773 ± 0.0473
(−)-camphor			0.0333 ± 0.00294		62.1 ± 3.82	0.802 ± 0.0412
(+)-δ- cadinene	0.342 ± 0.0362	0.179 ± 0.0563		0.308 ± 0.0196		0.273 ± 0.0143
(1*S*)-(−)-α-pinene	0.115 ± 0.0277	0.186 ± 0.0511	0.00564 ± 0.00257	0.135 ± 0.0502		0.239 ± 0.0692
3-carene						0.186 ± 0.00253
α-humulene	0.256 ± 0.0331	0.0857 ± 0.0956		0.433 ± 0.0225		0.0324 ± 0.000678
camphene			0.0160 ± 0.00222			0.118 ± 0.0700
citral				44.7 ± 2.63		
eucalyptol	2.97 ± 0.645	0.0424 ± 0.00108	0.00761 ± 0.00476			0.367 ± 0.533
eugenol						
(−)-bornyl acetate			17.9 ± 2.75			0.258 ± 0.00588
methylchavicol	0.381 ± 0.0883	0.227 ± 0.00525	0.169 ± 0.0281	0.0744 ± 0.00948	0.149 ± 0.0171	71.3 ± 23.0
(−)-*trans*-caryophyllene	0.119 ± 0.0135	11.1 ± 0.144		1.74 ± 0.0967		0.0433 ± 0.00716

[a] All compounds were identified by comparison of mass spectrometry and retention index data with those of commercially available standards. Quantification was performed using response factors determined from commercially available standards. [b] 1,8-Cineole, methyl cinnamate, and methyleugenol were not detected in any of the samples.

essential oil in our study from Mississippi, eugenol was found in trace amounts in the study in Atlantic Canada (*19*), and whereas eugenol and methylchavicol were the major oil constituents of cv. Local of *O. sanctum* in our study in Mississippi, the major constituents of the same cultivar in the study in Atlantic Canada (*19*) were carene, methylchavicol, elemene, and α-humulene, depending on the location (*19*). The differences in oil constituents of the same cultivars from *O. basilicum* and *O. sanctum* grown in this study in Mississippi and in Atlantic Canada (*19*) could be due to differences in environmental conditions during the growing season at the two locations, most probably temperature, soil characteristics, and production system. Summers in Atlantic Canada are cool, moist, and short, whereas summers in Mississippi are hot and long, allowing for three cuts of basil. Furthermore, the soil type and nutrient availability in the two studies were different. In addition, the production systems were different: black plastic mulch was utilized in our study in Mississippi; no mulch was used in the study in Atlantic Canada (*19*). Our results on the variation of essential oil constituents from this study versus the essential oil constituents from the same basil cultivars grown in different environments demonstrated a significant effect of the environment on the essential oil composition of basil, supporting previous research on *O. sanctum* (*1*).

Some researchers (*14*) grouped basil cultivars in three types in accordance with the main essential oil component. If this classification of the latter study (*14*) is followed, the essential oil of cvs. German and Mesten would be type 1 chemotype with linalool as the main component, but it can also be type 2, known as European basil. However, cv. Local of *O. sanctum* major constituents were eugenol and methylchavicol, and this would not fit in the classification reported previously (*14*). Overall, the essential oil composition of cvs. Mesten and German of *O. basilicum* in this study were comparable to the oil composition of 10 basil cultivars as reported previously (*14*). However, the latter authors (*14*) did not characterize oil types from *O. sanctum*.

All of the essential oils were tested for antimicrobial, antimalarial, antileishmanial, and cytotoxic activities. No antimicrobial or antimalarial activity was observed. However, all of the essential oils were found to inhibit the growth of *L. donovani* promastigotes with IC_{50} values in the range of 37–50 μg/mL and IC_{90} values in the range of 88–90 μg/mL (**Table 4**). A difference in the oil composition of cultivars does not seem to affect the antileishmanial activity of basil oils significantly. The effectiveness of the oils from Mississippi cultivars was also very similar to the effectiveness of commercially obtained oils as shown in **Table 4**. None of the samples of

Table 4. In Vitro Antileishmania Activity of Basil Essential Oils against *Leishmania donovani* Promastigotes and Their Cytotoxicity to Mammalian Kidney Cells

	L. donovani promastigotes		kidney fibroblast	kidney epithelial cells
sample	IC_{50}[a] (μg/mL)	IC_{90}[b] (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
O. basilicum cv. German[c]	48.3 ± 2.8	88.3 ± 2.8	NC[f]	NC
O. sanctum cv. Local[c]	37.3 ± 4.6	90.0 ± 4.6	NC	NC
O. basilicum cv. Mesten[c]	49.6 ± 0.6	90.0 ± 0.0	NC	NC
Basil Bulgaria (Commercial oil)[d]	40	>100	NC	NC
Basil sweet (commercial oil)[d]	50	90	NC	NC
pentamidine[e]	1.46 ± 0.51	4.98 ± 1.1	NC	NC
amphotericin B[e]	0.09 ± 0.01	0.35 ± 0.12	7.5	0.6

[a] Concentration of the oil causing 50% inhibition. [b] Concentration of the oil causing 90% inhibition. [c] Values are mean ± SD of samples from three harvests. [d] Values are mean of two samples. [e] Positive control. [f] No cytotoxicity up to 40 μg/mL.

Table 5. In Vitro Antileishmania Activity of Chemical Constituents Present in Basil Essential Oils against *Leishmania donovani* Promastigotes and Their Cytotoxicity to Mammalian Kidney Cells

	L. donovani		kidney fibroblasts	kidney epithelial cells
constituent	IC_{50} (μg/mL)	IC_{90} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
(−)-linalool	NA[a]	NA	NC[b]	NC
(+)-camphor	NA	NA	NC	NC
(+)-δ-cadinene	4	7	NC	NC
(1*S*)-(−)-α-pinene	NA	NA	NC	NC
1,8-cineole	NA	NA	NC	NC
3-carene	27	>40	NC	NC
α-humulene	19	37	NC	NC
camphene	NA	NA	NC	NC
citral	19	37	NC	22.4
eucalyptol	NA	NA	NC	NC
eugenol	NA	NA	NC	NC
(−)-bornyl acetate	NA	NA	NC	NC
methylchavicol	NA	NA	NC	NC
methyl cinnamate	NA	NA	NC	NC
methyleugenol	NA	NA	NC	NC
(−)-*trans*-caryophyllene	19	35	NC	NC
pentamidine	1.2	6.1	NC	NC
amphotericin B	0.09	0.3	7.5	0.6

[a] No observable effect observed on growth of *L. donovani* promastigotes up to 40 μg/mL concentration of the pure constituent. [b] No cytotoxicity up to 40 μg/mL.

essential oils showed any cytotoxicity to mammalian kidney cells up to a highest concentration of 40 μg/mL. Among the pure constituents (obtained commercially), cadinene, 3-carene, α-humulene, citral, and *trans*-caryophyllene showed antileishmanial activity, whereas others were ineffective (**Table 5**). The antileishmanial effect of cadinene was at least 5 times more potent than the effects of 3-carene, α-humulene, citral, and *trans*-caryophyllene. None of these constituents were cytotoxic to mammalian cells up to a concentration of 40 μg/mL, except citral (**Table 5**). The antileishmanial effect of citral could be attributed to its cytotoxic action. The pure compounds did not show any antimicrobial or antimalarial activity. Leishmanianses are human diseases caused by protozoan parasites belonging to the genus *Leishmania* (*10, 29*). Although leishmaniases affect over 12 million humans and cause significant mortality, there are a limited number of drugs available for treatment of these diseases (*10*). Some eugenol- and linalool-rich whole essential oils were found to have significant antileishmanial activity (*10, 29*), opening the possibilities for the development of drugs with fewer side effects and higher efficacy, with plant natural compounds as principal components (*10*). Hence, the antileishmanial effect of basil oil constituents found in our study could contribute to new drug development for the control of these diseases.

It was interesting to note that the major constituents (−)-linalool, eugenol, and methylchavicol of *O. basilicum* and *O. sanctum* did not show any antileishmanial activity. The antileishmanial activity of essential oil preparations of cvs. Local, German, and Mesten and the commercial oil seems to be due to the presence of minor constituents such as cadinene, 3-carene, α-humulene, citral, and *trans*-caryophyllene; cadinene was the most potent of these. Although citral, in the purified form, showed a mild cytotoxic effect on kidney epithelial cells, it did not seem to contribute any toxicity to the essential oils, and the composition analysis showed that citral was present in negligible quantities which could not be quantified (LOQ = 0.0001 mg/mL). Results from this study indicate that both sweet (*O. basilicum*) and holy (*O. sanctum*) basils could be grown as essential oil crops in Mississippi and provide three cuts and high yields. Furthermore, the essential oil produced in Mississippi could be marketed on the international market as its composition and biological activity were similar to those of basil oils from other countries currently available on the international market.

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Supporting Information Available: Standard curve data of the commercial standards. This information is available free of charge via the Internet at http://pubs.acs.org.

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