

Chemical compositions, antioxidant, antimicrobial, and mosquito larvicidal activity of *Ocimum americanum* L. and *Ocimum basilicum* L. leaf essential oils

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Research Article

Keywords: Ocimum, Basil leaf, Antibacterial, Flavour, Aromatherapy

Posted Date: January 20th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2403403/v1>

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Abstract

Background: *Ocimum americanum* L. and *Ocimum basilicum* L. are highly valued aromatic medicinal plants. The leaves of *O. basilicum* and *O. americanum* are widely used as spices in traditional cuisine. Its essential oil is extensively used in the food, cosmetic, and pharmaceutical industries. The purpose of this study is to investigate the main chemical profile of *O. americanum* and *O. basilicum* leaf essential oils and to assess their effects on antibacterial, antioxidant, and larvicidal properties.

Methods: Essential oils were extracted from the leaves of *O. basilicum* and *O. americanum* using steam distillation in a Clevenger-type apparatus. The chemical constituents of the essential oils (EO) were analyzed by gas chromatography-mass spectrometry (GC-MS). DPPH, FRAP, and metal chelating techniques were used to assess the free radical scavenging ability of the oils. The extracted oil was also tested for its antibacterial activities by a disc-diffusion test and the broth microdilution method. Furthermore, the mosquito larvicidal activity was tested using standard protocols.

Results: Camphor (33.869%), limonene (7.215%), longifolene (6.727%), caryophyllene (5.500%), and isodene (5.472%) were identified as the five major compounds in *O. americanum* leaf essential oil. The essential oil yield was 0.4%, and citral (19.557%), estragole (18.582%), linalool (17.605%), and camphor (9.224%) were the major compounds found among the 37 chemical constituents identified in *O. basilicum* oil. *O. basilicum* exhibited more potent antioxidant activity in DPPH, FRAP, and ABTS tests than *O. americanum*. The zones of inhibition and MIC of the oils in the microdilution and disc diffusion methods were 8.00 ± 0.19 to 26.43 ± 2.19 mm and $3.12 - 100$ $\mu\text{g/mL}$, respectively. At 400 ppm, *O. basilicum* and *O. americanum* demonstrated larvicidal activity with mortality ratios of 73.60 ± 0.89 and $78.00 \pm 1.00\%$, respectively. Furthermore, after 30 min of exposure, the larval death rate was 73.60 ± 0.89 and $78.00 \pm 1.00\%$ for *O. americanum* and *O. basilicum* respectively.

Conclusions: These findings revealed that essential oils extracted from the leaves of *O. basilicum* and *O. americanum* exhibited reasonable antioxidant, antibacterial, and mosquito larvicidal potentials that can be used as alternative medicine in treating human health and larvicidal mosquito control.

Background

Plants are essential to maintaining the ecological dynamics of ecosystems. Furthermore, they offer numerous substances with potential healing properties [1, 2]. Medicinal plant extracts and essential oils are factories of valued natural bioactive compounds widely evaluated/used in the food, pharmaceutical, nutraceutical, and cosmetic industries due to their fragrance, flavor, and therapeutic abilities [3–6]. Terpenes and their oxygenated derivatives, benzoids, and phenylpropanoids are volatile, complex mixtures of essential oils [4, 7]. Generally, essential oils are often two or three compound mixtures in high quantities (> 20–95%); these are the major constituents, while others can be present only in very small amounts.

Free radicals like reactive oxygen species (ROS) are formed in redox processes, which are the leading cause of degenerative diseases like stroke, rheumatoid arthritis, diabetes, inflammation, aging, cancer, and neurological disorders in the human body [2, 8]. ROS have been connected with lipid oxidation, peroxidation, and DNA mutations of proteins [9]. Any substance that can remove ROS is called an antioxidant. Due to their resonance

stabilization effect, phenolic compounds can effectively scavenge free radicals [10]. Interest in the finding of new plant-based drugs with antioxidant properties has grown in modern times.

Nowadays, bacterial infections are on the rise. As with the use of antibiotics in bacterial treatment, the percentage of therapeutic drug failures is increasing due to the development of resistance in microbial strains. Notwithstanding medical advances in infectious diseases, chemotherapy and immunization remain serious health concerns [11]. The resistance of microorganisms towards medicines used as antimicrobial drugs imposes the necessity to investigate new medicines with potential antimicrobial action [12]. Further studies have focused on essential oils extracted from plants that contain various volatile bioactive constituents that can act as therapeutics in humans [6, 7].

Aedes aegypti L. is responsible for transmitting arboviruses that cause diseases such as yellow fever, dengue, chikungunya, and zika in tropical regions [13–15]. Rapid urbanization, a lack of infrastructure, and poor sanitation are ideal circumstances for the spread of this female *Aedes aegypti* mosquito, which is resistant to pesticides and commercialized repellents [13, 15]. Furthermore, the drawbacks of chemical pesticides, such as pollutants and harm to non-target species, have limited their widespread usage [16]. Therefore, it is necessary to develop alternative pesticides with high efficacy, minimal environmental harm, and low toxicity to humans for controlling mosquitoes. Currently, mosquito control programs focus more on eradicating mosquito larvae with plant-based agents [17]. In this context, essential oils extracted from plants have garnered much attention as possible sources of mosquito repellents and larvicides [18].

The genus *Ocimum* (Basil) comprises 160 species and is the major species in the Lamiaceae globally. *Ocimum* is called "the king of herbs" due to the presence of enormous applications in traditional medicine, pharmaceuticals, and the cosmetic industry [19, 20]. Several species of *Ocimum*, such as *O. basilicum*, *O. gratissimum*, *O. tenuiflorum*, *O. americanum*, *O. kilimandscharicum*, and *O. micranthum*, are cultivated for the isolation of high-value fragrance, flavor, and medicinal properties [21]. *Ocimum* has traditionally been used to treat a variety of ailments, including rheumatism, epilepsy, paralysis, diarrhea, sunstroke, influenza, high fever, gonorrhea, abdominal pains, mental illness, colds, and coughs, as well as having antipyretic, anti-emetic, stomatic, antihelmintic, and antimalarial activity [22]. Furthermore, *Ocimum* leaves have been investigated as a food and flavoring agent due to their aromatic properties [23]. Its essential oils and aromatic fragrance have numerous therapeutic potentials, especially antimicrobials, antioxidants, insecticidal, and nematocidal properties [24]. Interestingly, *Ocimum* essential oils were documented as rich in terpenoids and phenylpropanoids such as methyl cinnamate, linalool, thymol, camphor, citrol, eugenol, and geraniol, which are important active constituents that are variable and influenced by environmental factors [23–25].

Ocimum basilicum L. is an aromatic herb and main commercial crop with numerous biological uses [26]. This plant is used as a supplement in the perfume, pharmaceutical, food, aromatherapy, and cosmetic industries as its main constituent [27]. Essential oils and aromatic leaves have been used in plant-based healthcare since ancient times in traditional systems of medicine [28]. *Ocimum basilicum* is an aromatic fragrance distinguished by its chemotypes, especially methyl chavicol-rich, linalool-rich, eugenol-rich, methyl eugenol-rich, and methyl cinnamate-rich, which were documented in India [23]. Earlier studies have reported that essential oil extracted from *O. basilicum* exhibited potential antimicrobial, fungicidal, antioxidant, antiviral, anti-proliferative, anti-inflammatory, antispasmodic, and sedative properties [29].

Another renowned species of *Ocimum* is *Ocimum americanum* L. (syn. *O. canum* Sims.), which is commonly spread through India and tropical Africa. It is also known as "hairy basil. Preparations achieved from its aerial parts are repetitively used in folk remedies to treat insomnia and anxiety [30]. In Nigeria, *O. americanum* leaf decoction or infusion is used to control fever, coughs, colds, piles, and diabetes[31]. In Yoruba tribals, *O. americanum* is used to prepare soup due to its aroma and flavor [32]. *O. americanum* acetone extract inhibited neurotoxins that caused brain damage in rats [33]. With these regards, the current work was conducted to determine the chemical profile of the essential oils extracted from *O. basilicum* and *O. americanum* leaves and to investigate their antibacterial, antioxidant, and anti-mosquito larvacidal properties.

Methods

Plant material and extraction of essential oils

The fresh leaves of *O. basilicum* and *O. americanum* were collected from the agricultural field (Permission was obtained from the land owner), Coimbatore, Tamil Nadu, India. Identification was confirmed by Dr. Jagathes Kumar, Assistant Professor and Botanist, Department of Botany, Sri Vijay Vidyalaya College of Arts & Science. The voucher specimens (LO005 and LO006) were deposited in the herbarium center. The collected leaves of *O. basilicum* and *O. americanum* were shade dried at room temperature. The essential oil was then isolated from 500 g dried leaves through hydrodistillation for 3 hours using a Clevenger apparatus. The extracted oils were dried with anhydrous sodium sulfate (1.0 g), separated, and stored at 4°C for further use.

Chemical Constituents Of Essential Oils

After the separation of essential oil, the chemical composition of essential oil was determined by GC-MS by Clarus 680 GC-MS (PerkinElmer®) equipped with a capillary column (30m×0.25mm) of diphenyl dimethyl polysiloxane model Elite-5 MS. The oven temperature was slowly increased from 60 to 300°C at a level of 3°C min⁻¹. The temperatures of the injection and mass were set at 220 and 240°C, respectively. Essential oil (1.0 µL) was injected in a ratio of 1:200. The flow rate of helium gas was 1 mL/min. GC-MS was performed using the same chromatography and a mass spectrometer. The GC condition was also the same. Chemical constituents were identified by retention time and mass spectra in the Wiley and NIST libraries of GC-MS and literature.

Antioxidant activities of essential oils

Dpph Radical Scavenging Activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging potential of essential oils was measured as described by Blois [34] with minor changes. 300 µL essential oil of varying concentration (20–100 µg/mL) was mixed with DPPH solution (3 mL 0.1 mM). These oil mixtures were kept in the darkroom for 30 minutes, and the colour reduction by the essential oil of the stable DPPH radical was measured at 517 nm in a spectrophotometer. Ascorbic acid, rutin, and BHT were used as standards. DPPH was calculated using the below equation:

$$\% \text{ inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

The concentration of oil that reduced the DPPH solution by 50% (IC₅₀) was calculated.

Measurement Of Abts Radical Cation Assay

ABTS' radical cation decolourization potential of essential oil from *O. americanum* and *O. basilicum* was measured by Re et al. [35]. Total antioxidant activity (TAA) was validated as Trolox equivalent µM/g oil.

Frap Reducing Antioxidant Power Assay

The FRAP-reducing antioxidant power potential of *O. basilicum* and *O. americanum* essential oils was esteemed by Pulido et al. [36]. FRAP was indicated as mM Fe (II) equivalent/g oil.

Metal Chelating Activity

The chelation of ferrous ions by *O. basilicum* and *O. americanum* essential oils was assessed by Dinis et al. [37]. EDTA calculated the chelation efficacy of the oil, and results were measured as mg EDTA equivalent/g oil.

Anti-bacterial Activity

The anti-bacterial activity of *O. basilicum* and *O. americanum* essential oils was assessed by two tests: (i) the disc-diffusion test and (ii) the broth microdilution test (determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)).

Bacterial Strains

Antibacterial efficacy of *O. basilicum* and *O. americanum* oils were tested against gram-positive bacteria: *Bacillus subtilis* (MTCC-441), *Streptococcus pyogenes* (MTCC 1928), *Enterococcus faecalis* (MTCC 439), *Staphylococcus aureus* (MTCC-96), and gram-negative bacteria: *Escherichia coli* (MTCC-724), *Klebsiella pneumoniae* (MTCC-432), *Proteus vulgaris* (MTCC-426), *Salmonella paratyphi* (MTCC-735), *Aeromonas hydrophila* (MTCC-7646), *Pseudomonas aeruginosa* (MTCC 2453) and *Enterobacter aerogenes* (MTCC 39).

The Diameter Of The Inhibition Zone By Disc Diffusion

The antibacterial activity of *O. basilicum* and *O. americanum* essential oils was evaluated by the paper disc diffusion method, according to Mahendran and Ranjitha Kumari [38]. Muller-Hinton (Himedia, India) agar plates were prepared and stored at 20 ± 2°C. Test bacterial suspension 100 µL containing 10⁸ CFU/mL of each bacterial suspension was dispensed and uniformly spread above the sterile plates using a glass spreader. Then, 20 µL (5, 10, and 20 mg/mL) of each essential oil was loaded onto a sterile paper disc (6 mm). Oil-

loaded paper discs were placed on the surface of the inoculated plates, and then the plates were incubated at $37 \pm 2^\circ\text{C}$ for 24 h. Streptomycin (20 $\mu\text{g}/\text{disc}$) and cefotaxime (20 $\mu\text{g}/\text{disc}$) discs were used as a reference. The experiment was carried out in three plates (in triplicate) for each organism. The inhibition zone diameter was measured in millimeters (mm). The results were accounted as mean $\pm$ SD.

Broth Microdilution Method For Mic And Mbc

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *O. basilicum* and *O. americanum* oils were evaluated by the microdilution method according to Duarte et al. [39]. Briefly, 100 μL of two-fold serial diluted (100 to 0.781 $\mu\text{g}/\text{mL}$) oils of *O. basilicum* and *O. americanum* was deposited in each well in 96 well. Then, 100 μL of bacteria at 10^6 CFU were added to all wells. After incubating at 37°C overnight, 40 μL (200 $\mu\text{g}/\text{mL}$) of *p*-iodonitrotetrazolium violet (INT) was deposited to all wells for evaluating bacterial growth. Bacterial growth was shown by detecting the reduction of yellow to red formazan after 2 h of incubation at 37°C [40]. To analyze MBC, 10 μL of each bacterial culture was subcultured on Muller-Hinton plates for 24 h.

Mosquito Larvicidal Bioassay

The eggs of *Aedes aegypti* were acquired from the Center for Research in Medical Entomology, Madurai, India. The larvae were fed on yeast powder and dog biscuits in a 1:3 ratio. 10% sucrose with chicks for blood meal was nourished for adults. Mosquitoes were maintained at 70–85% relative humidity (RH) and $28 \pm 2^\circ\text{C}$ temperatures with 12h light.

The larval mortality was measured according to Panneer Selvam et al. [41] with slight modification. 25 larvae at the 3rd and 4th instar stages were separately kept in 200 mL paper cups containing 99 mL of water with 1 mL of oil (400, 200, 100, 50, and 25 PPM) added. Mortality was detected after 24 and 48 h. Toxicity and larvicidal activities were described using LC_{50} and LC_{90} data and probit analysis.

Statistical analysis

All the biological activities of both essential oils were repeated in triplicate, and the results were given as mean $\pm$ standard deviation, calculated using SPSS version 26. The IC_{50} value was investigated statistically with a Student's t-test by using a one-way analysis of variance (ANOVA). Finney's probit analysis estimated the LC_{50} and LC_{90} lethal concentrations.

Results

Chemical constituents of *O. basilicum* and *O. americanum* essential oils.

Hydro-distillation of *O. basilicum* and *O. americanum* yielded 0.6% (3.2 mL) and 0.4% (2.3 mL), respectively. The essential oils of *O. basilicum* and *O. americanum* were investigated, and their chromatographic profiles are shown in Figs. 1A&B. Their chemical compositions are depicted in Tables 1&2. Results exhibited that 37

and 34 compounds were identified, accounting for 81.141% and 100% of the oil, in *O. basilicum* and *O. americanum*, respectively. As shown in Table 1, the main components of *O. basilicum* were 3,7-dimethyl-, (Z)-(-citral) (19.557%), estragole (18.582%), linalool (17.605%), and camphor (9.224%).

Table 1
Chemical composition of *Ocimum basilicum* oil

Peak	Retention time (min)	Mass	Compounds	Area (%)
1	4.994	136	α -Pinene	1.010
2	5.264	136	Camphene	0.729
3	5.795	128	1-Octen-3-ol	0.771
4	5.850	136	β -Pinene	0.246
5	6.550	136	D-Limonene	1.287
6	6.600	154	Eucalyptol	0.251
7	6.770	136	α -Ocimene	0.391
8	6.990	136	3-Carene	0.235
9	7.700	154	<i>Cis</i> - β -Terpineol	2.715
10	8.596	152	Camphor	9.224
11	8.921	152	(-)-Myrtenol	0.781
12	8.981	152	(+)- trans-Limonene oxide	0.757
13	9.441	148	Estragole	18.582
14	10.071	152	2,6-Octadienal, 3,7-Dimethyl-, (Z)- (β – citral)	19.557
15	10.862	204	α - farnesene	0.250
16	11.137	166	4,8-dimethyl-nona-3,8-dien-2-one	0.479
17	11.452	154	(.+/-)-Lavandulol,	0.855
18	11.707	180	Geranyl vinyl ether	0.734
19	11.952	204	Cadrene	0.766
20	12.427	204	Caryophyllene	3.009
21	12.497	204	<i>trans</i> - α -Bergamotene	1.470
22	12.547	204	<i>isoledene</i>	0.685
23	12.662	204	α -Cubebene	0.560
24	12.847	204	α -Caryophyllene	0.793
25	13.153	204	Aromadendrene	1.311
26	13.298	204	(-)-Iso aromadendrene -(V)	2.268
27	13.378	204	Patchoulene	2.300

Peak	Retention time (min)	Mass	Compounds	Area (%)
28	13.553	204	Longifolene	0.309
29	13.623	204	(-)- α -panasinsen	0.199
30	13.833	222	Levomenol	4.888
31	14.273	222	(-)-Globulol	1.282
32	14.418	220	Caryophyllene oxide	0.219
33	14.708	166	Cyclohexane, Butylidene	0.761
34	15.193	222	Epiglobulol	0.443
35	15.484	222	α -Bisabolol	0.320
36	15.684	222	Veridiflorol	0.495
37	19.680	222	Ledol	0.209
Total				81.141%

The EOs obtained from *O. americanum* had 37 9 chemical compositions with camphor (33.86%), limonene (7.21%), longifolene (6.72%), veridiflorol (5.85%), isodene (5.472) and α -pinene (5.19%) as the main chemical components (Table 2 & Fig. 2).

Table 2
Chemical composition of *Ocimum americanum* oil

Peak	Retention time (min)	Mass	Compounds	Area (%)
1	5.039	136	α -Pinene	5.195
2	5.315	136	Camphene	4.674
3	5.710	204	α -farnesene	0.972
4	5.755	128	1-Octen-3-ol	0.398
5	5.870	136	β -Pinene	1.694
6	6.165	136	α -Phellandrene	0.773
7	6.625	136	Limonene	7.215
8	7.025	136	3-Carene	1.673
9	7.235	154	<i>Cis</i> - β -Terpineol	0.535
10	7.460	136	Cyclohexene	1.433
11	7.756	154	Eucalyptol	1.220
12	8.771	152	Camphor	33.869
13	8.856	154	Iso Borneol	0.388
14	8.971	154	Borneol	1.394
15	9.046	154	α -terpinenol	0.671
16	9.241	148	Estragole	0.937
17	9.326	152	(-)-Myrtenol	3.206
18	9.796	152	Octadienal	0.476
19	10.212	204	Naphthalene	0.691
20	11.727	204	Copaene	0.696
21	11.907	204	Cadrene	1.508
22	12.402	204	Caryophyllene	5.500
23	12.462	204	β -Caryophyllene	1.060
24	12.532	204	Aromadendrene	1.696
25	12.803	204	α -Caryophyllene	0.661
26	13.118	204	α -Cubebene	0.450
27	13.308	204	Longifolene	6.727

Peak	Retention time (min)	Mass	Compounds	Area (%)
28	13.403	204	Isoledene	5.472
29	13.558	204	Epizonarene	0.992
30	14.293	222	Veridiflorol	5.858
31	14.693	222	Humulane	0.541
32	15.038	222	α -cadinol	0.341
33	15.189	222	Ledol	0.512
34	15.369	222	γ -Muurolene	0.572
Total				100%

Antioxidant Activity

In the present research, the antioxidant activities of *O. basilicum* and *O. americanum* essential oils were determined by four different investigations, such as DPPH, FRAP, ABTS, and metal chelating assays.

In the present result, it was concluded that *O. basilicum* oil had more noticeable activity than *O. americanum* oil since its IC₅₀ value was lower (11.56 ± 0.89 μ g/mL) than that of *O. americanum* (13.42 ± 1.03 μ g/mL). However, the standards, which were ascorbic acid (3.72 ± 0.60 μ g/mL), rutin (4.65 ± 0.52 μ g/mL), and BHT (7.89 ± 0.49 μ g/mL), exposed the higher activity.

Trolox equivalent antioxidant capacity (TEAC) was also assessed by neutralizing the radical cation of ABTS. The ABTS activity observed for *O. basilicum* essential oil (2842.12 ± 10.39 μ M TEAC/g oil) was more potent than *O. americanum* (2085.07 ± 7.43 μ M TEAC/g oil). The results of FRAP and metal chelation activity of *O. americanum* and *O. basilicum* essential oils are shown in Table 3. In ferric reducing and chelating the metal ions, *O. basilicum* showed a higher FRAP (907.24 ± 18.29 mM Fe (II)/g oil) and metal chelating (189.16 ± 09.04 mg EDTA Eq/g oil) activity.

Table 3

ABTS^{•+} scavenging, FRAP, and metal chelating activities of essential oils obtained from *O. basilicum* and *O. americanum* leaves.

Sample	ABTS ^{•+} (μ M TEAC/g oil)	FRAP (mM Fe (II) Eq/g oil)	Metal chelating activity (mg EDTA Eq/g oil)
OA	2085.07 ± 7.43^c	851.32 ± 13.71^c	106.01 ± 12.07^c
OB	2842.12 ± 10.39^b	907.24 ± 18.29^b	189.16 ± 09.04^b
Ascorbic acid	4174.87 ± 12.65^a	2365.65 ± 11.87^a	312.65 ± 10.21^a

Values are means of three independent analyses $\pm$ standard deviation ($n = 3$). Mean values followed by different superscript letters indicate significant statistical differences ($p < 0.05$).

Antibacterial activity of *O. americanum* and *O. basilicum* essential oils

The zone of inhibition (ZI) of *O. basilicum* and *O. americanum* oils evaluated by the disc diffusion method is shown in Fig. 3.

The essential oils obtained from *O. basilicum* and *O. americanum* displayed strong antibacterial activity against tested pathogenic bacteria with a ZI of 8.00–26.43 mm. Remarkably, the highest antibacterial activity was observed in *P. vulgaris* with the ZI of *O. basilicum* essential oil (26.43 mm), followed by *O. americanum* (24.00 mm), comparable to streptomycin (20 $\mu\text{g}/\text{disc}$) 23.17 mm. Bactericidal activities against *S. paratyphi*, *E. aerogenes*, and *E. faecalis* ranged from 10.00 to 23.00 mm (Figs. 3A & B).

The occurrence of live microorganisms in wells, an indication of colony formation, was confirmed by the decreases in yellow INT to triphenyl formazan (pink); this colour was not detected in dead cells. The specific concentration of essential oil capable of preventing the development of bacteria was investigated using this technique. The MIC and MBC of *O. basilicum* and *O. americanum* oils are displayed in Table 4 and differ from 3.12 to 100 $\mu\text{g}/\text{mL}$ as detected in the microdilution method.

Table 4
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *O. americanum* and *O. basilicum* essential oils.

Microorganisms	<i>O. americanum</i>		<i>O. basilicum</i>		Cefotaxime	
	MIC value	MBC value	MIC value	MBC value	MIC value	MBC value
	($\mu\text{g}/\text{ml}$)	($\mu\text{g}/\text{ml}$)	($\mu\text{g}/\text{ml}$)	($\mu\text{g}/\text{ml}$)	($\mu\text{g}/\text{ml}$)	($\mu\text{g}/\text{ml}$)
<i>Staphylococcus aureus</i>	100.00	100.00	12.50	12.50	3.12	3.12
<i>Bacillus subtilis</i>	50.00	12.50	3.12	3.12	12.50	12.50
<i>Enterococcus faecalis</i>	100.00	100.00	12.50	12.50	4.88	3.12
<i>Streptococcus pyogenes</i>	50.00	50.00	6.25	6.25	3.12	2.4
<i>Escherichia coli</i>	9.76	12.50	3.12	3.12	3.12	3.12
<i>Salmonella paratyphi</i>	9.76	12.50	3.12	3.12	6.25	6.25
<i>Proteus vulgaris</i>	9.76	12.50	3.12	3.12	3.12	3.12
<i>Aeromonas hydrophila</i>	50.00	19.53	6.25	6.25	6.25	6.25
<i>Klebsiella pneumoniae</i>	50.00	50.00	6.25	6.25	3.125	3.12
<i>Enterobacter aerogenes</i>	100.00	100.00	12.50	12.50	6.25	6.25
<i>Pseudomonas aeruginosa</i>	50.00	12.50	6.25	6.25	12.50	12.50

Values are means of three independent analyses (n = 3).

Larvicidal Activity

The larvicidal efficiency of *O. basilicum* and *O. americanum* essential oils was studied. The essential oils had a notable larvicidal action, and the results are presented in Table 5.

Table 5

Larvicidal properties of leaf essential oils from *O. americanum* and *O. basilicum* against the larvae of *A. aegypti* after 12 and 24 h of exposure period

Sample	Mosquito life stages	Time	Concentration (ppm)	% mortality $\pm$ SD	LC ₅₀ (LCL-UCL) ^a	LC ₉₀ (LCL-UCL) ^a	χ^2 (df = 4) ^b
OA	Second instar	After 12 h	25	23.57 $\pm$ 0.42			
			50	33.88 $\pm$ 0.46			
			100	45.00 $\pm$ 1.00			
			200	55.80 $\pm$ 0.83			
			400	74.60 $\pm$ 1.14			
		After 24 h	25	23.56 $\pm$ 0.12	160.34(86.76-217.58)	627.43(457.69-1202.18)	5.406
			50	47.00 $\pm$ 0.69			
			100	64.30 $\pm$ 1.02			
			200	80.00 $\pm$ 1.50			
			400	91.54 $\pm$ 1.56	87.96 (12.70-163.11)	439.54(292.16-716.18)	10.45
	Third instar	After 12 h	25	11.80 $\pm$ 0.89			
			50	31.91 $\pm$ 1.53			
			100	45.00 $\pm$ 1.00			
			200	57.80 $\pm$ 1.30			
			400	73.60 $\pm$ 0.89	134.54(46.72-227.60)	1119.07(781.28-2828.96)	2.34
		After 24 h	25	21.20 $\pm$ 1.30			
			50	44.56 $\pm$ 1.28			
			100	58.20 $\pm$ 1.30			

Sample	Mosquito life stages	Time	Concentration (ppm)	% mortality $\pm$ SD	LC ₅₀ (LCL-UCL) ^a	LC ₉₀ (LCL-UCL) ^a	χ^2 (df = 4) ^b
OB	Second instar	After 12 h	200	75.60 $\pm$ 0.54			
			400	82.60 $\pm$ 0.89	73.73(16.54-148.55)	577.28(353.49-961.49)	2.39
			25	32.20 $\pm$ 0.44			
			50	44.60 $\pm$ 1.14			
			100	53.20 $\pm$ 1.09			
			200	67.20 $\pm$ 0.83			
	Third instar	After 12 h	400	76.40 $\pm$ 1.14	61.14(17.76-342.99)	1114.68(242.16-3126.01)	2.18
			25	41.60 $\pm$ 1.14			
			50	62.80 $\pm$ 1.92			
			100	71.40 $\pm$ 1.19			
			200	87.40 $\pm$ 1.14			
		After 24 h	400	93.20 $\pm$ 0.83	37.14(0.08-137.77)	286.55(30.43-548.49)	1.22
			25	27.40 $\pm$ 1.24			
			50	34.00 $\pm$ 0.87			
			100	56.76 $\pm$ 0.69			
			200	69.10 $\pm$ 1.88			
		After 12 h	400	78.00 $\pm$ 1.00	92.65(0.03-259.13)	950.72(495.34-3371.99)	1.56
			25	42.35 $\pm$ 1.20			
			50	64.50 $\pm$ 1.57			

Sample	Mosquito life stages	Time	Concentration (ppm)	% mortality $\pm$ SD	LC ₅₀ (LCL-UCL) ^a	LC ₉₀ (LCL-UCL) ^a	χ^2 (df = 4) ^b
			100	79.25 $\pm$ 1.65			
			200	86.00 $\pm$ 1.72			
			400	95.50 $\pm$ 1.89	31.43(0.41-111.35)	233.43(37.22-459.36)	1.09

LCL lower confidence level, UCL upper confidence level

^a95% Confidence interval

^b Degrees of freedom; χ^2 chi-square value

After 12 and 24 hours of exposure, larvicidal effects and mortality were observed in second and third-instar larvae. Table 5 demonstrated that the larval mortality of *A. aegypti* (second and third instars) increased after the treatment of *O. basilicum* and *O. americanum essential oils* at different concentrations (25–400 μ g/mL). The treatment of *O. basilicum* at 25 g/mL caused 41.60% and 42.35% mortality in second and third instar larvae, respectively, whereas it increased to 93.20% and 95.50% after a 24 h treatment period (Table 5).

After a 24 h exposure period, the LC₅₀ of *O. basilicum* appeared to be the most effective against *Aedes aegypti* (LC₅₀: 37.14 and 31.43 ppm, LC₉₀: 286.55 and 233.43 ppm for second and third instars, respectively), followed by *O. americanum* (LC₅₀ 87.96 and 73.73 ppm LC₉₀ 439.54 and 577.28 ppm).

Discussions

In *Ocimum* spp., essential oils are generally rich in estragole and 2, 6-octadienal, which was observed in our result for *O. basilicum* essential oil. The components were clearly different from the essential oil reported by Mohamed Abdoul-Latif et al. [20], who reported that linalool (41.2%) and estragole (30.1%) were the major compounds in *O. basilicum* from Djibouti in East Africa. The components were also different from those reported by Srivastava et al. [23], who found that methyl chavicol (51.2–58.0%), methyl eugenol (10.0–15.0%), meta-eugenol (4.5–9.3%), and camphor (4.5–5.7%) content are the main components in chemotype II from India. The results of the EO analysis introduced methyl chavicol (88.6%) as the major compound of *O. basilicum* [42]. In contrast to our findings, some researchers reported that the main constituents of *O. basilicum* oil are 2,6-octadienal, 3,7-dimethyl-, (Z)-(20.01%), citral (20.11%), and eugenol (26.76) [43].

As shown in Table 2, the GC–MS analysis identified 34 compounds, representing 100% of the total EO in *O. americanum*. Interestingly, *O. americanum* oil was rich in camphor (33.86%) and limonene (7.21%). In contrast to our findings, Mohamed Abdoul-Latif et al. [20] reported that the main compounds in *O. americanum* essential oil were carvotanacetol (38.5%) and estragole (27.5%). Other investigators presented eugenol (45.2%), methyleugenol (14.8%), and (E)-caryophyllene (30.2%) as major constituents from Rudrapur, India, in *O. americanum* [44]. Similarly to our result, camphor was a major chemical constituent in *O. minimum*, *O.*

canum, and *O. gratissimum* with different percentages [45–47]. These results suggested that the chemical composition of essential oils in aromatic medicinal plants can vary depending on environmental factors and EO extraction methods [7, 48]. Since the chemical profiles were quite different among the source of plant materials, it would affect the biological activity of the oils. Therefore, determining the chemical profiles of plant materials is essential for the quality control of the products.

Essential oils are capable natural scavengers that can diminish free radical generation. They are being offered as potential alternatives to artificial preservatives [48]. Essentially, essential oils react with the DPPH radical (deep violet colour) and alter it to hydrazine (DPPH-H discoloration). The amounts of discoloration indicate the scavenging ability of the sample [50]. This mechanism occurred due to the antioxidants, which donate electrons/hydrogen atoms to the stable radicals. Free radical scavenging is generally indicated as a percent of DPPH inhibition and the concentration essential for a 50% DPPH reduction (IC_{50}). In the present result, it was concluded that *O. basilicum* oil had more noticeable activity than *O. americanum* oil since its IC_{50} value was lower ($11.56 \pm 0.89 \mu\text{g/mL}$) than that of *O. americanum* ($13.42 \pm 1.03 \mu\text{g/mL}$). However, the standards, which were ascorbic acid ($3.72 \pm 0.60 \mu\text{g/mL}$), rutin ($4.65 \pm 0.52 \mu\text{g/mL}$), and BHT ($7.89 \pm 0.49 \mu\text{g/mL}$), exposed the higher activity. These responses are consistent with those of Hazrati et al. [7] and Rezzoug et al. [27], who reported that essential oils were less active than the standard antioxidants.

Commonly, the antioxidant ability of oils is associated with their major bioactive constituents [51]. In a previous study, essential oils such as camphor, α -pinene, terpinene-4-ol borneol, eucalyptol, p -cymene, and β -pinene were tested separately for antioxidant activity, which revealed that they had low free radical scavenging capacities [52]. Zengin and Baysal [53] found that linalool and α -terpineol have poor radical scavenging capacity. In fact, camphor, limonene, longifolene, caryophyllene, isodene, β -citral, estragole, and linalool were the most abundant chemical constituents in both *O. americanum* and *O. basilicum* in our study. This composition might clarify why oils display low antioxidant ability compared to synthetics. In numerous reports, the antioxidant capacity of essential oils is determined by the occurrence of phenolic chemical constituents and the synergistic effects of major chemical compounds that are consistent with our results [7, 54–56]. Essential oils are a mixture of many chemical constituents derived from secondary metabolism. Tohidi et al. [57] reported that oxygenated monoterpenes displayed higher antioxidant, antifungal, and antibacterial activities. However, minor chemical constituents act to play an important role, and it is not well known which constituents/combinations of them are accountable for their antioxidant activities [58].

Usually, essential oils and their chemical constituents are highly hydrophobic, which permits *Bacteria* to lose their cell constituents and cause cell death [59]. The antibacterial activity of both *O. basilicum* and *O. americanum* was studied in several bacteria. The agar disc method was cheap, simple, and reproducible [60]. The essential oil of *O. basilicum* offered superior antibacterial activity. MIC values of *O. basilicum* oil were lower than those of *O. americanum* oil. Similarly, observation was achieved from other essential oils [61, 62]. It was evidenced for all tested organisms that MBC was similar to MIC values, which showed the antibacterial ability of these essential oils. A similar observation was made in earlier research [60, 62].

The presence of oxygenated monoterpenes such as geraniol formate, geraniol, citronellyl formate, citronellol, and linalool (64.93–80.31%) as main constituents is responsible for this essential oils strong antibacterial activity against almost all susceptible microorganisms. According to specific studies, crude essential oils have

greater antibacterial activity than a single compound [63]. Essential oils' antimicrobial activity has been linked to their hydrophobic nature, which allows them to penetrate the gram-positive bacterial cell membrane and inactivate molecular mechanisms that cause cell death [4, 7, 64].

Table 5 demonstrated that the larval mortality of *A. aegypti* (second and third instars) increased after treating *O. basilicum* and *O. americanum* essential oils at different concentrations (25–400 ppm). The treatment of *O. basilicum* at 25 ppm caused 41.60% and 42.35% mortality in second and third-instar larvae, respectively, whereas it increased to 93.20% and 95.50% after a 24 h treatment period (Table 5). A similar tendency has been observed for both instars of *A. aegypti* at different concentrations of *O. americanum* treatment at 12 and 24 h. Mortality was dose-dependent in our study. Earlier reports are in agreement with our statement [65–67].

The results of the larvicidal ability revealed that the ratio of deaths is directly proportional to the oil concentration. This demonstrated that concentration plays a vital role in larvicidal activity. At the $P \leq 0.05$ level, the chi-square was significant. Essential oils isolated from plants have been documented as botanical pesticides [68]. *Ipomoea cairica* essential oil exhibited 100% mortality for *Anopheles subpictus*, *Culex tritaeniorhynchus*, and *Aedes albopictus* [69]. Tiwary et al. [70] perceived the larvicidal effects of linalool against *A. stephensi* ($LC_{50} = 58$ ppm), *C. quinquefasciatus* ($LC_{50} = 49$ ppm), and *A. aegypti* ($LC_{50} = 54$ ppm). Cheng et al. [71] observed that 36.0 to 86.8 $\mu\text{g/mL}$ essential oils were required to kill *A. aegypti*. Cavalcanti et al. [72] determined that the larvicidal efficacy of Brazil oils ranged from 60 to 69 $\mu\text{g/mL}$ against *Aedes aegypti*. Rahuman et al. [73] also perceived that *Feronia limonia* dried leaves were associated with higher activity against *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti*. The larvicidal efficacy of *Thymus vulgaris*, *Mentha arvensis*, *Cymbopogon citratus*, *P. graveolens*, and *O. basilicum* essential oils were investigated against *C. quinquefasciatus*. Cheng et al. [71] reported that 1,8-cineole and eugenol from *O. gratissimum* had LC_{50} 60 ppm against *A. aegypti* larvae. Similar activity was observed in *O. basilicum* and *O. americanum*. Earlier researchers had identified the efficiency of essential oils against mosquito larvae and adults [74].

Conclusions

We conclude that the results demonstrated that camphor, limonene, longifolene, caryophyllene, estragole, and linalool were the most abundant components in *O. basilicum* and *O. americanum*. This study discovered that oils had strong antibacterial and larvicidal activity but low antioxidant activity. The essential oils should be further evaluated to develop safe agents for larvicidal therapy. Further studies should be made to reveal the mode of action of these oils, which might help understand possible roles in human wealth.

Abbreviations

EO: Essential oil; GC-MS: Gas Chromatography-Mass Spectrometry; *O. basilicum*: *Ocimum basilicum*; *O. americanum*: *Ocimum americanum*; DPPH: 2,2-diphenylpicrylhydrazyl; FRAP: Ferric reducing antioxidant power; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; MIC: Minimal inhibitory concentration; MBC: minimum bactericidal concentration; LC_{50} & 90 : Lethal Concentration (50% or 90%); IC_{50} : Half-maximal inhibitory concentration (IC_{50}); PPM: parts per million; BHT: butylated hydroxytoluene; INT: iodonitrotetrazolium or 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium; ZI: Zone of Inhibition; EDTA: Ethylenediaminetetraacetic acid;

Declarations

Ethics approval and consent to participate

All methods complied with relevant institutional, national, and international guidelines and legislation. We furthermore declare that there is no ethical issue in our experiments.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are included within the manuscript and are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

Funding

Not applicable.

Authors' contributions

GM designed, and conceptualized the study, analyzed the data, drafted the report, and prepared the final manuscript. SV reviewed and edited the final manuscript. The author(s) read and approved the final manuscript.

Acknowledgments

We would like to express our gratitude to the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, and GM thankful to the Second Century Fund (C2F), Chulalongkorn University (CU), for C2F Postdoctoral Fellowship.

Authors' information

Not applicable.

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Figures

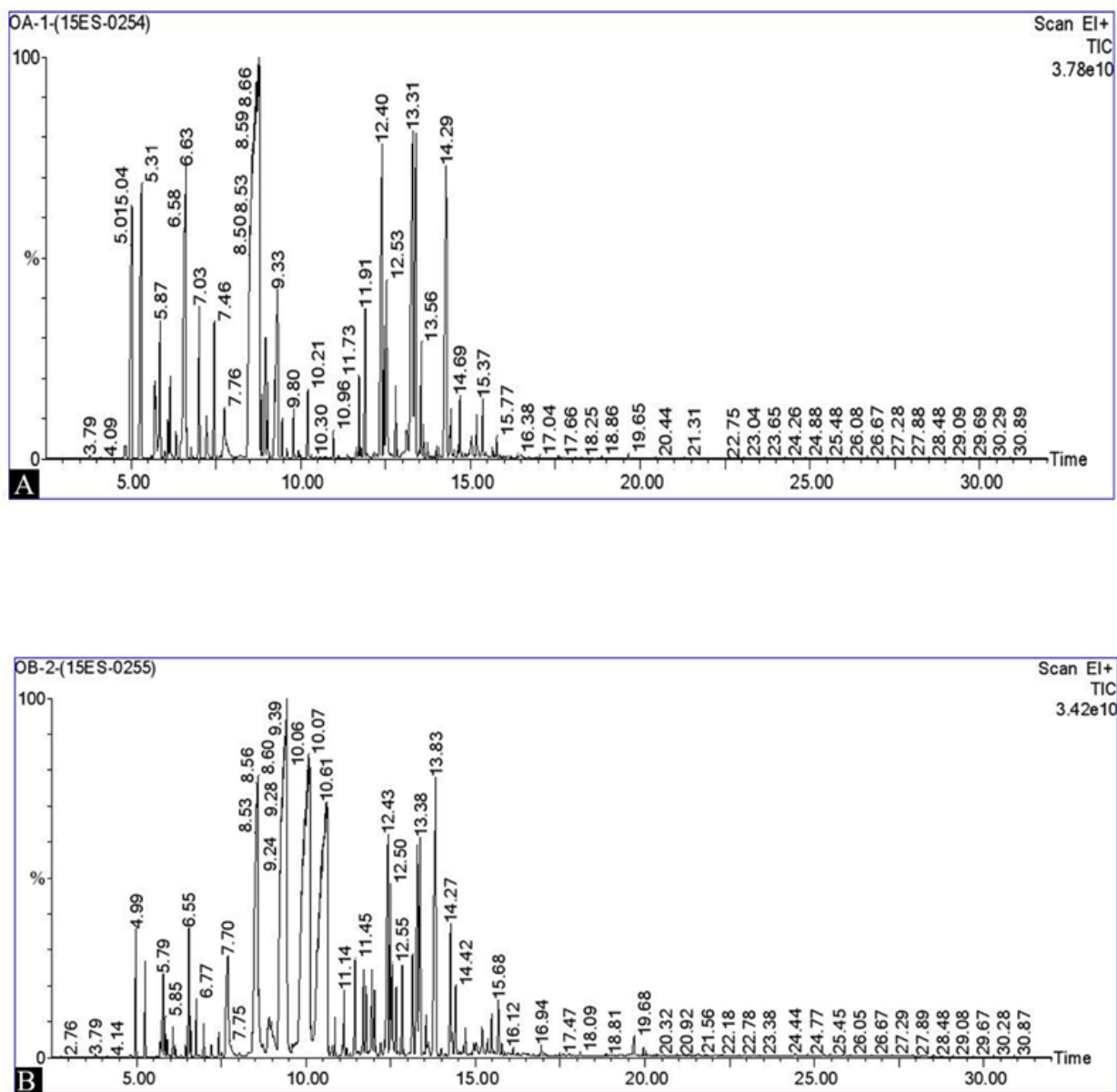
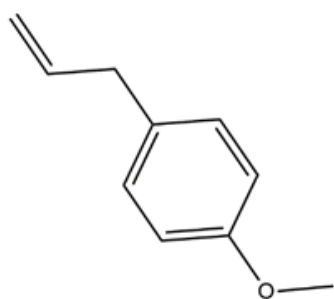


Figure 1

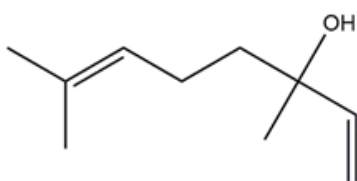
GC-MS chromatogram of leaves essential oils from *Ocimum* species.

A. *Ocimum americanum* L.

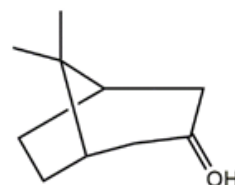
B. *Ocimum basilicum* L.



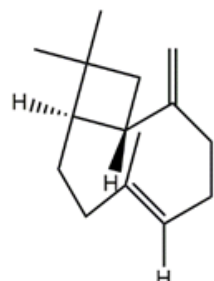
Estragole



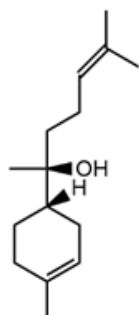
Linalool



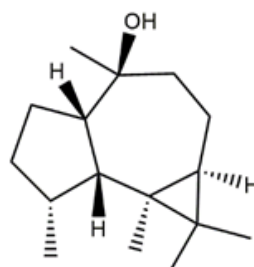
Camphor



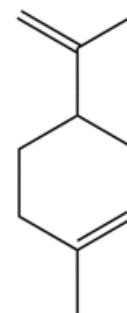
Caryophyllene



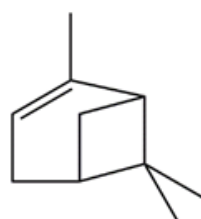
Levomenol



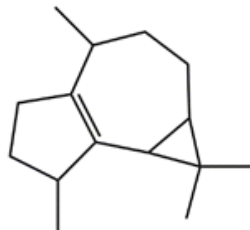
Veridiflorol



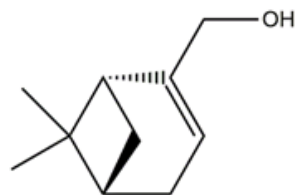
Limonene



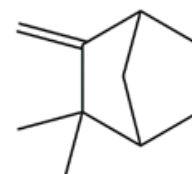
alpha-Pinene



Isolatedene



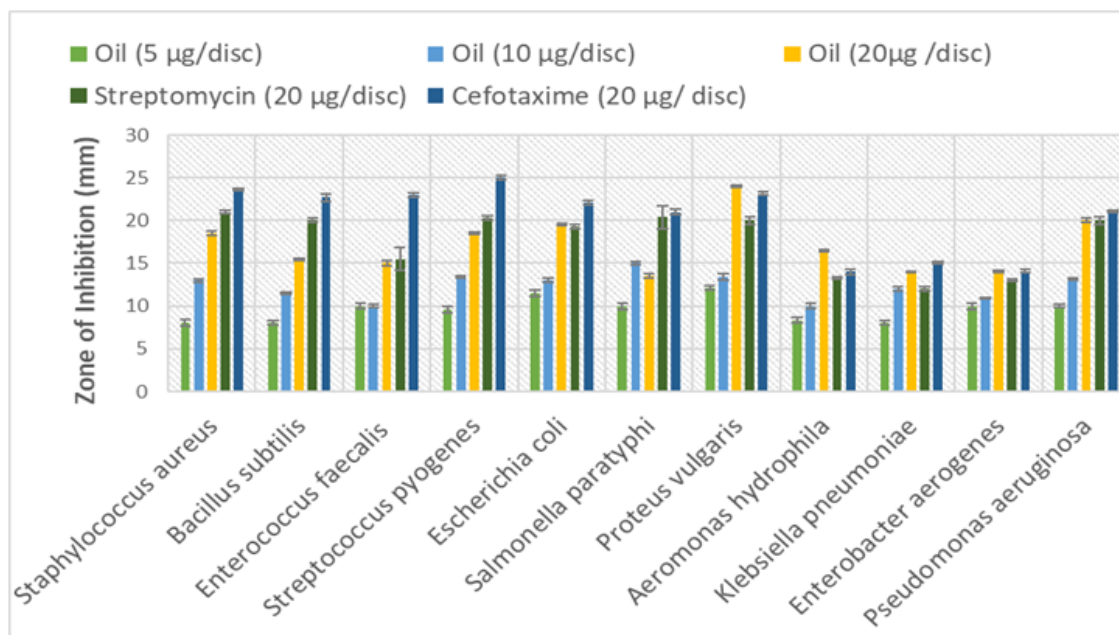
(-)-Myrtenol



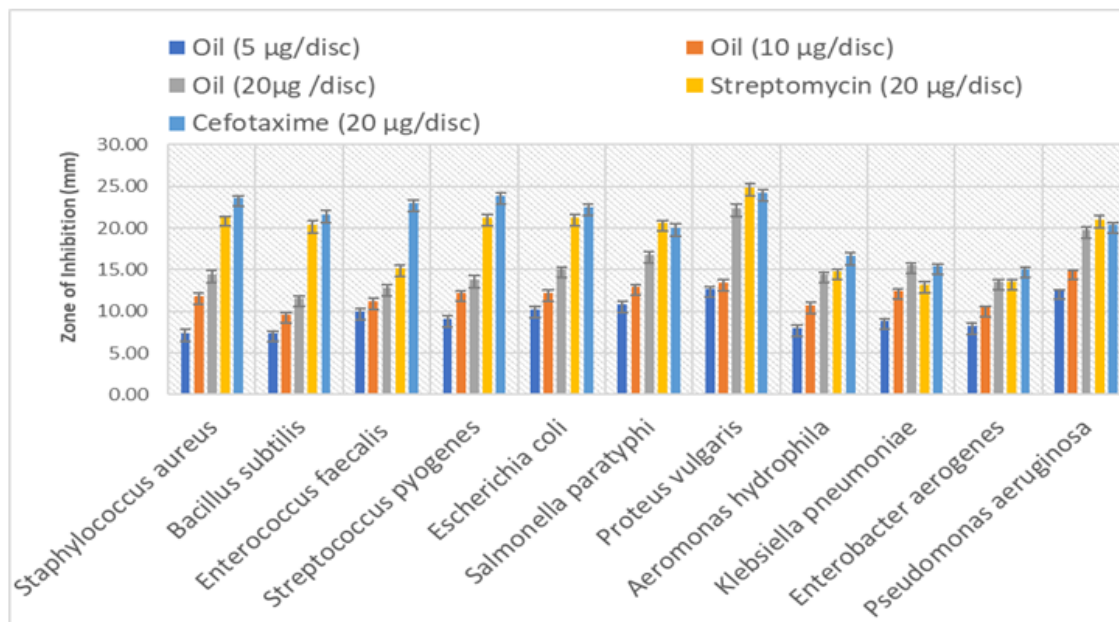
Camphene

Figure 2

Some main chemical structures from *O. americanum* and *O. basilicum*



A. Antibacterial activity of *O. americanum* leaves essential oil.



B. Antibacterial activity of *O. basilicum* leaves essential oil

Values are means of three independent analyses $\pm$ standard deviation ($n = 3$).

Figure 3

Antibacterial activity of essential oils of *O. basilicum* and *O. americanum* leaves