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Chemical composition and larvicidal activity against *Aedes aegypti* L. (Diptera: Culicidae) of essential oils from leaves, stalks and roots of the *Croton nepetaefolius* Baill (Euphorbiaceae)

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Abstract
Croton nepetaefolius Baill is the aromatic plant native to northeast of Brazil popularly known as "marmeleiro sabiá". It is used in folk medicine as a stomachic, a carminative and for the treatment of intestinal colic. A total of 26 compounds were obtained by hydrodistillation and identified by GC-MS and CG-FID in the three essential oils samples, being 5 monoterpenes (36.9%), 7 sesquiterpenes (31.8%) and 1 Arylpropanoid (25.7%) in the essential oil of leaves, 6 monoterpenes (16.6%), 8 sesquiterpenes (14.1%) and 2 arylpropanoids (68.6%) in the essential oil of stalks and 7 monoterpenes (5.98%), 4 sesquiterpenes (4.52%) and 2 arylpropanoids (84.9%) in the essential oil of roots. The main constituents in the essential oil of leaves, stalks and roots are the monoterpene 1, 8-cineole (26.16%) and the arylpropanoids elemicin (60%) and methyl eugenol (46.31%), respectively. Essential oil of the leaves have been demonstrated larvicidal activity (LC_{50} 77.6 mg/mL), while the essential oils of the stalks (LC_{50} 134.9 mg/mL) and roots (LC_{50} 110 mg/mL) were considered not active. These results can be explained by the presence in the leaves essential oil from *C. nepetaefolius* of the monoterpenes sabinene, 1,8-cineole and terpinen-4-ol and sesquiterpenes *β*-caryophyllene and germacrene D natural products which have been reported to be active against *A. aegypti*.

Keywords: *Croton nepetaefolius*, essential oils, *Aedes aegypti*

1. Introduction
Dengue is an acute febrile disease that affects humans and constitutes a serious public health problem worldwide. It is estimated that 50 to 100 million people become infected by dengue each year in more than 100 countries in all continents, the transmission to man is done by *Aedes* mosquitos, especially species *aegypti* and *albopictus* [1, 2]. The vector control is based on the use of larvicides, such as, temephos (organophosphate insecticide) and the repetitive use of these insecticides in several countries has made the *A. aegypti* increasingly resistant, which is a major barrier to control the mosquito that transmits dengue. Thus, resistance to pesticides has guided research to find new methods to control *A. aegypti* [3, 4].
Thus, in view of the serious epidemiological picture reported and considering the absence of a specific treatment, it is essential to identify natural products that have repellent, larvicidal and insecticidal properties or act as adjuvants in the control of dengue arboviruses.
In recent years, search for efficient natural compounds with larvicidal activity and low environmental toxicity has increased, so natural products have been a promising alternative for pest control. Essential oils are outstanding candidates, since they are in some cases, active against *A. aegypti* [5], readily available, and economically viable. A study for determining the potency of larvicidal activity of natural products revealed that compounds considered highly active showing LC_{50} <50 mg/mL, compounds active showing LC_{50}<100 mg/mL and compounds not active showing LC_{50}>100 mg/mL [6].
The larvicidal activity of essential oils against *A. aegypti* can be attributed to many factors, among which we can mention: lipophilicity of terpenes and phenylpropanoids, because the association between these compounds and protein deactivation/enzyme inhibition the increase of transmembrane absorption of lipophilic drugs.

Double bonds are important in the larvicidal activity because hydrogenation of these bonds decreases the lipophilic character of these compounds, restricting their passage through the larvae cuticle [6]. Constituents present in essential oils affect biochemical processes, which specifically disrupts the endocrinologic balance of insects and another factor is the interference with GABA-gated chloride channels in insects caused by essential oils compounds [6].

Croton is a genus included in plant family Euphorbiaceae widespread in northeastern Brazil. Its use in popular medicine are related to cancer treatment, constipation, diabetes, digestive problems, dysentery, external wounds, fever, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain, ulcers, and weight-loss [7]. *C. nepetaefolius* is extensively used in folk medicine as a sedative and antispasmodic agent, also used as stomachic, carminative and intestinal pain, the latter being scientifically proven [8].

Larvicidal activity of essential oils from Northeastern Brazilian plants showed good results for *C. zehntneri*, essential oils from leaves, stalks, inflorescences and phenylpropanoid derivative *E*-anethole were tested at different concentrations against instar III larvae of *A. aegypti* and showed LC_{50} 56.2, 51.3, 57.5 and 69.2 mg/mL, respectively [9]. Essential oils from leaves, stalks and inflorescences of *C. jacobinensis* were tested at different concentrations against *Aedes aegypti* and showed LC_{50} 79.3, 117.2, 65.8 mg/ml, respectively [10]. Essential oils from aerial parts of *C. argyrophylloides*, *C. sonderianus* and *C. nepetaefolius* showed LC_{50} 94.6, 54.5 and 66.4 mg/mL and essential oils from leaves and *C regelianus* showed LC_{50} 84 and 24.22 mg/mL, respectively [11]. Therefore, the present study was aimed to show the chemical composition and the larvicidal activity against *A. aegypti* of essential oils from leaves, stalks and roots from *C. nepetaefolius*.

2. Methodology

2.1 Plant material

Leaves, stalks and roots of *C. nepetaefolius* were collected during 2004 in Caucaia, State of Ceará, Northeast of Brazil. A voucher specimem (33.582) was deposited at the Herbário Prisco Bezerra, at the Biology Department, at Federal University of Ceará, Brazil.

2.2 Extraction of the essential oils

The fresh leaves (400 g), stalks (1.250 g) and roots (600 g) of *C. nepetaefolius* were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours to afford 1.2% (w/w), 0.04% (w/w) and 0.89% (w/w), respectively. The yields (w/w) were calculated based on the fresh weight of the plant materials. After being filtered and dried over anhydrous sodium sulfate, the isolated oils were stored in sealed glass vials, which were maintained under refrigeration before analysis.

2.3 Gas chromatography with flame ionization detector

GC-FID for the quantitative analysis was carried out on a Shimadzu GC-17A gas chromatograph using a dimethylpolysiloxane DB-5 fused silica capillary column (30 mm x 0.25 mm, film thickness 0.25 mm). H_2 was used as the carrier gas at a flow rate of 1 mL/ min and 30 psi inlet pressure; split, 1:30; temperature program: 35-180 °C at 4 °C/ min, then heated at a rate of 17 °C/ min to 280 °C and held isothermal for 10 min; injector temperature, 250 °C; detector used FID, detector temperature, 250 °C.

2.4 Gas Chromatography-Mass Spectrometry

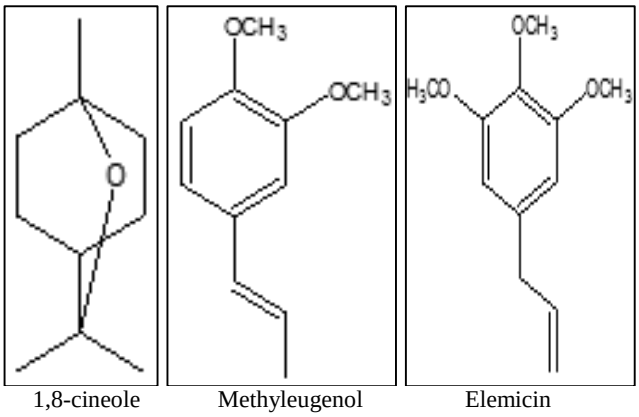
GC-MS for the analysis of the volatile constituents was carried out on a Hewlett-Packard Model 5971 GC/MS using a non-polar DB-5 fused silica capillary column (30 mm x 0.25 mm i.d., 0.25 mm film thickness); carrier gas helium, flow rate 1 mL/min and with split ratio 1:1. The injector temperature and detector temperature were 250 ºC and 200 ºC, respectively. The column temperature was programmed from 35 ºC to 180 ºC at 4 ºC/min and then 180 ºC to 250 ºC at 10 ºC/min. Mass spectra were recorded from 30 – 450 *m/z*. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library MS [12] searches using retention indices as a preselection routine, as well as by visual comparison of the fragmentation pattern with those reported in the literature [13].

2.5 Larvicidal bioassay

Essential oils were placed in beakers and dissolved in 20 mL H_2O/DMSO 1.5% (v/v) at concentrations of 50-500 mg/mL, followed by the addition of 50 larvae at the third-instar. For each experiment, both positive (Temephos at 3.22 mg/mL) and negative (distilled water containing 1.5% DMSO) control assays were carried out. Mortality was recorded after 24 h of exposure, during which no nutritional supplement was added. The experiments were carried out at 28 ± 2 °C. Each test was performed in triplicate. Data were evaluated through regression analysis. From regression line, the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *A. aegypti* [12].

3. Results and Discussion

The essential oils extracted from the leaves, stalks and roots of *C. nepetaefolius* were analyzed by GC/MS and GC/FID the constituents identified and quantified (Table 1). A total of 26 compounds organized in order of elution in a DB-5 column was identified in the three essential oils samples, with the main constituents being the monoterpene 1,8-cineol (leaves) and the arylpropanoids elemicina (stalks) and methyleugenol (stalks and roots) (Figure 1).



1,8-cineole Methyleugenol Elemicin

Fig 1: Major constituents present in the essential oils of leaves, stalks and roots of *C. nepetaefolius*

Table 1: Chemical composition of essential oil from leaves, stalks and roots of *C. nepetaefolius.*

Compounds	*Retention Indices	Leaves (%)	Stalks (%)	Roots (%)
α-Pinene	925		2.39	0.64
Camphene	954		0.92	0.28
β-Pinene	956		2.32	0.67
Sabinene	975	1.39		
1,8-cineole	1031	26.16		0.93
Linanool	1097		1.41	0.20
Camphor	1146		4.82	2.83
α-Terpineol	1167	6.37		0.43
Terpinen-4-ol	1177	1.13		
Myrtenol	1196	1.92	4.82	
δ-Elemene	1338	3.09		
Isoledene	1376		1.36	
α-Copaene	1377		3.70	
Methyleugenol	1385		8.66	46.31
α-Gurjunene	1410		1.40	1.81
β-Caryophyllene	1419	6.95		0.64
α-Santalene	1421		1.60	
α- Bergamotene	1437		0.71	
α-Humulene	1455	0.76		
α-Curcumene	1481		0.80	1.74
Germacrene D	1485	8.05	2.64	
Bicyclogermacrene	1488	5.56		
δ-Cadinene	1523		2.40	0.33
Spathulenol	1553	4.54		
Elemicin	1557	25.75	60.00	38.68
Caryophyllene oxide	1556	2.92		
TOTAL (%)	94.59		99.95	95.49

*Experimentally determined Kováts retention indices.

The essential oils were evaluated for their larvicidal potential against *A. aegypti* dengue transmitting mosquito larvae using temephos (O, O '- (thiodi-4, 1-phenylene) bis (O, O-dimethyl phosphorothioate) as control. Essential oils tested showed LC_{50} values of 77.6 (leaves), 134.9 (stem) and 110 mg/mL (roots), respectively.

The essential oil of the leaves was obtained with a yield of 1.2%, and a total of 13 constituents (94.5%) were identified, being 5 monoterpenes (36.9%), 7 sesquiterpenes (31.8%) and 1 Arylpropanoid (25.7%). The essential oil of the stalks presented yield of 0.04%, identifying 16 constituents (99.9%), 6 monoterpenes (16.6%), 8 sesquiterpenes (14.1%) and 2 arylpropanoids (68.6%), meanwhile, the chemical composition of roots essential oil, obtained with 0.89% yield, is being reported for the first time, showed the presence of 13 constituents (95.4%), 7 monoterpenes (5.98%), 4 sesquiterpenes (4.52%) and 2 arylpropanoids (84.9%).

Chemical composition of essential oil from leaves of *C. nepetaefolius* collected in Caucaia State of Ceará, showed that main constituents being the monoterpene 1,8-cineol and arylpropanoid elemicin and was considered active (LC_{50} 77.6 mg/mL), while results previously published reports the phenylpropanoid methyleugenol as major component of essential oils from leaves and aerial parts of *C. nepetaefolius*, which have been reported to be active against *A. aegypti* with LC_{50} 84 mg/mL (leaves) [15] and LC_{50} 66.4 mg/mL (aerial parts) [16], the differences in larvicidal activity can be explained by the differences on chemical composition of essential oils from leaves and aerial parts of *C. nepetaefolius*, which can be attributed to the environmental factors and influenced by the edaphoclimatic conditions.

Therefore, the essential oils of the leaves from *C. nepetaefolius* was more active (LC_{50} 77.6 mg/mL) and the essential oils of the stalks (LC_{50} 134.9 mg/mL) and roots (LC_{50} 110 mg/mL) were considered not active. These results can be explained by the presence in essential oils of the leaves from *C. nepetaefolius* of the monoterpene hydrocarbon sabinene (LC_{50} = 21.2 mg/mL), oxygenated monoterpenes 1,8-cineole (LC_{50} = 47.9 mg/mL) and terpinen-4-ol (LC_{50} = 64.76 mg/mL) and sesquiterpenes hydrocarbons β-caryophyllene (LC_{50} = 88.3 mg/mL) and germacrene D (LC_{50} = 18.78 mg/mL) which have been reported to be active against *A. aegypti* [6].

The results showed a possible relationship between larvicidal activity and presence of monoterpenes and sesquiterpenes in the essential oil from leaves of *C. nepetaefolius*, since these substances can serve to increase the transmembrane absorption of lipophilic drugs which can kill larvae of *A. aegypti* [17] and that may also act synergistically [18].

4. Conclusion

Essential oil of the leaves from C. *nepetaefolius* have been demonstrated larvicidal activity, which can be explained by the presence of the monoterpenes sabinene, 1, 8-cineole and terpinen-4-ol and sesquiterpenes β-caryophyllene and germacrene D**,** natural products which have been reported to be active against *A. aegypti*, since they serve to increase the transmembrane absorption of lipophilic drugs and kill larvae of *A. aegypti*. So essential oil of the leaves from *C. nepetaefolius* can be used as a natural insecticide against *A. aegypti*, since this essential oil are active, biodegradable, and nontoxic to the environment.

5. Acknowledgment

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