DOI: 10.15625/2615-9023/19644

CHEMICAL COMPOSITION AND LARVICIDAL ACTIVITY OF ESSENTIAL OIL FROM LEAVES OF *Eucalyptus robusta*

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Received 12 December 2023; accepted 30 May 2024

ABSTRACT

Essential oil (EO) from leaves of *Eucalyptus robusta* was extracted by hydrodistillation, and analyzed the chemical composition using gas chromatography- Flame ionization detector (GC-FID), and gas chromatography/mass spectrometry (GC/MS). Thirty constituents from leaf EO were identified accounting for 97.48% of the total composition of EO. Monoterpene hydrocarbons were the major chemical classes (83.34%) in which 1,8-cineole (29.23%), *α*-pinene (18.58%), *α*-phellandrene (14.05%) and *β*-pinene (6.40%) were the main components. The larvicidal activity test against *Culex quinquefasciatus* showed that the essential oil from the leaves of *E. robusta* strongly inhibited this southern house mosquito with LC_{50} values at 24 h and 48h to be 30.34 µg/mL and 28.77 µg/mL, respectively.

Keywords: *Eucalyptus robusta*, *Culex quinquefasciatus*, larvicidal, 1,8-cineole, α-pinene.

Citation: Nguyen Thi Hong Van, Tran Thi Tuyen, Pham Cao Bach, Dang Thu Quynh, Dinh Thi Thu Thuy, Vo Thanh Thuong, Ngo Gia Huy, Nguyen Quoc Thong, Nguyen Huy Hung, Do Huu Nghi, 2024. Chemical composition and larvicidal activity of essential oil from leaves of *Eucalyptus robusta*. *Academia Journal of Biology*, 46(2): 39–45. https://doi.org/10.15625/2615-9023/19644

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INTRODUCTION

The genus *Eucalyptus* is mainly native to Australia, has over 800 species, and belongs to the Myrtaceae family (Rivera, 2005; Elaissi et al., 2012). More than 300 species of this genus have been shown to contain volatile oil in their leaves. Less than 20 species, within these, have a high content of 1,8-cineole to produce essential oils used in the pharmaceutical and cosmetic industries (Elaissi et al., 2012; Pino et al., 2001). The use of natural products in medicine is growing in the treatment of diseases. In particular, multidrug resistance is becoming increasingly common and serious (Cermelli et al., 2007). In the folk medicine of Tunisie, *Eucalyptus* essential oil is used to treat respiratory disorders such as pharyngitis, bronchitis, and sinusitis (Boukef, 1986). Some studies showed that *Eucalyptus* essential oil inhibited *Haemophilus influenzae* and *Stenotrophomonas maltophilla* (Cermelli et al., 2007; Fabio et al., 2001). Besides, plant extracts and essential oils of some *Eucalyptus* species exhibited antibacterial and antifungal activities against, e.g., bacteria: *Pseudomonas aeruginosa, Pseudomonas fluorescens, Escherichia coli, Staphylococcus aureus*; fungi: *Penicillium digitatum*, *Aspergillus flavus, Aspergillus niger*, *Mucor* spp., *Rhizopus nigricans*, *Fusarium oxysporum*; and yeast: *Candida albicans, Sacchromyces cerevisiae* (Sartorelli et al., 2007; Tyagi & Malik, 2011). In Vietnam, about twenty *Eucalyptus* species have been recorded (Hang, 1995; Loi, 2011), in which *Eucalyptus robusta* is one of the *Eucalyptus* species commonly grown. Previous chemical compositions of essential oil of this plant showed that there are different major phytochemicals of essential oil from leaves of *E. robusta:* 1,8-cineole (50.0%), *α*-pinene (22.2%), *trans*-pinocarveol (13.0%), globulol (5.7%), and pinocarvone (5.4%) were the major components of EO from leaves of *E. robusta* in Algeria (Benayache et al., 2001), while *α*-pinene (30.18%), 1,8-cineole (26.08%), and spathulenol (5.31%) were the major components of EO from leaves of *E. robusta* in China (Liu et al., 2014). Moreover, *trans*-pinocarveol (26.6%), *α*-pinene (13.0%), pinocarveol (6.4%) were the main constituents of EO from *E. robusta* leaves in Australia (Bignell et al., 1997), while the essential oil from leaves of *E. robusta* collected from Mali mainly contained *α*-pinene (23.9%), *ρ*-cymene (23.2%), 1,8-cineole (14.5%), *α*-phellandrene (12.0%) and *β*-pinene (8.6%) as the major components (Traore et al., 2010). However, there are not many studies on the larvicidal activity of *E. robusta* essential oil. This study reported the chemical composition and *Culex quinquefasciatus* larvicidal activity of essential oil from leaves of *E. robusta.*

MATERIALS AND METHODS

The fresh leaves of *E. robusta* were collected in secondary forests mixed with planted forests located in Dong Luong commune, Cam Khe district, Phu Tho province in May 2023. The plant material was identified by Dr. Nguyen Quoc Binh, Vietnam Museum of Nature, Vietnam Academy of Science and Technology (VAST). The voucher specimen (EU-PT) was deposited at the Institute of Natural Products Chemistry (INPC), VAST.

Extraction of essential oils

The fresh leaves of *E. robusta* (1.0 kg) were cut into pieces and were hydrodistilled using a Clevenger-type apparatus (JSOW, India) for 3 hours. The essential oil was dried over anhydrous sodium sulfate and preserved at 4 ^{o}C in a refrigerator before analysis.

Physico-Chemical Properties of EOs

The refractive index (n_D^{20}) was determined according to ISO 280:1998.

The refractive density (d_{20}^{20}) was determined according to ISO 279:1998.

The optical rotation ($[\alpha]^{20}_D$) was determined according to ISO 592:1998.

GC-MS procedure

The gas chromatography (GC) method was used to analysis essential oils of *E. robusta* leaves with Mass Spectrometry (MS) and Flame Ionization Detector (GC-FID). The GC-MS analysis system was performed by a

GC Agilent Technologies 7890A connected with a mass spectrum detector (MSD) Agilent Technologies 5975C and an HP-5 MS column (60 m × 0.25 mm, film thickness 0.25 µm). The chromatographic conditions were set as: the temperature of the injector was at 250 ^{o}C; the temperature program began at 60 ^{o}C, then increased up to 240 ^{o}C, at 4 ^{o}C/min; carrier gas helium 1 mL/min constant speed; split ratio 100:1 and the volume of sample (diluted 3% EOs in methanol) was injected at 1 µL. The electron impact ionization voltage is 70 eV, the emission current is 40 mA, acquisitions scan mass range is 35–450 amu. The same chromatographic conditions were applied for GC-FID.

The constituents of EOs were identified by comparing the obtained retention indices (RI) and mass spectra (MS) data with HPCH1607, W09N08 libraries, NIST standard database, and description by Adams (Adams, 2007). The relative percentage of substances was calculated based on the GC-FID peak areas without any correction factors.

Larvicidal assay

Egg rafts of *C. quinquefasciatus* were collected in Da Nang, then hatched in tap water overnight. The larvae were fed on a mixture of dog food and yeast (3:1, w/w). The 3rd instar and early 4th instar larvae were used to evaluate the larvicidal activity of the EO according to the method previously described (Ngoc Anh et al., 2023). Ethanol (Sigma-Aldrich) was used to prepare a stock solution (1%) (WHO, 2005) of essential oil. Twenty-five larvae were transferred into 250 mL beakers containing 150 mL of test solutions of essential oil at concentrations of 100, 50, 25, 12.5 and 6.25 µg/mL. Each concentration was repeated 4 times, ethanol used to dissolve the essential oil was used as the negative control, permethrin (Sigma-Aldrich) was used as the positive control. The number of dead larvae when exposed to the test solutions was determined at 24 and 48 h later. Laboratory conditions (25 ^{o}C, 75% relative humidity, 12 h light/12 h dark cycle) were maintained throughout larval rearing and larvicidal activity testing.

Data analysis

Lethality data were subjected to log-probit analysis (Finney, 2009) to obtain LC_{50} values, LC_{90} values and 95% confidence limits using Minitab® version 19.2020.1 (Minitab, LLC, State College, PA, USA).

RESULTS AND DISCUSSION

Chemical composition of essential oil from the leaves of *Eucalyptus robusta*

Hydrodistillation of the leaves of *E. robusta* produced an EO yield of 3.7 % (w/w) with yellowish color and lighter than water. Thirty compounds were identified, accounting for 97.50% of the total composition of EO. The physico-chemical properties and chemical composition of EO are provided in Table 1 and Table 2.

The data in Table 2 showed that monoterpenes were the major chemical classes (83.34%) of the EO from leaves of *E. robusta*, with the main components: 1,8-cineole (29.23%), *α*-pinene (18.58%), *α*-phellandrene (14.05%), and *α*-terpinyl acetate (5.21%). The presence of 1,8-cineole and *α*-pinene may affect the biological activities of EO as the active elements (Atmani-Merabet et al., 2018; Liu et al., 2014). Compared with the EO of *E. robusta* in Brazil, its *α*-pinene was the main component (73%) while 1,8-cineole was not detected and very low content (2.3%) of phellandrene (Sartorelli et al., 2007). Besides, the main components of EO from leaves of *E. robusta* in Argentina were *α*-pinene (41.69%), *p*-cymene (8.50%) while 1,8-cineole was little (0.64%) (Alejandro et al., 2012). However, the main components of leaf EO of *E. robusta* in Congo were *p*-cymene (27.30%), myrtenal (12.8%), *β*-pinene (6.3%), terpineol (6.3%), and 1,8-cineol (4.3%) (Cimanga et al., 2002). The upper results showed the influential difference of phytochemicals of *E. robusta* essential oils from leaves among the areas or populations in *E. robusta*.

Table 1. Physico-chemical properties of essential oil from the leaves of *Eucalyptus robusta*

No.	Physico-Chemical Properties	Standard	At	Value
1	Specific Gravity	ISO 279:1998	20 ^oC	0.8788
2	Refractive Index	ISO 280:1998	20 ^oC	1.4628
3	Optical Rotation	ISO 592:1998	20 ^oC	-26.46

Table 2. Chemical compositions of essential oils from the leaves of *Eucalyptus robusta*

No.	^{a}Rt	^{b}RI$_E$	^{c}RI$_L$	Constituents	Classification	Content (%)
1	9.56	930	930	*α*-Thujene	Monoterpene	0.42
2	9.84	939	939	*α*-Pinene	Monoterpene	18.58
3	10.32	955	954	Camphene	Monoterpene	0.17
4	11.19	984	979	*β*-Pinene	Monoterpene	6.40
5	11.44	992	991	Myrcene	Monoterpene	0.90
6	12.04	1010	1003	*α*-Phellandrene	Monoterpene	14.05
7	12.42	1021	1017	α-Terpinene	Monoterpene	0.41
8	12.69	1029	1026	*o*-Cymene	Monoterpene	4.05
9	12.84	1034	1029	Limonene	Monoterpene	4.96
10	12.89	1035	1030	*β*-Phellandrene	Monoterpene	2.33
11	12.99	1038	1031	1,8-Cineole	Monoterpene	29.23
12	13.35	1049	1037	*E*-*β*-Ocimene	Monoterpene	0.27
13	13.83	1063	1060	*γ*-Terpinene	Monoterpene	0.92
14	14.88	1093	1089	Terpinolene	Monoterpene	0.65
15	15.86	1121	1117	endo-Fenchol	Monoterpenoid	0.26
16	16.10	1128	1121	Dehydrosabinaketone	Monoterpenoid	0.14
17	17.75	1175	1169	Borneol	Monoterpenoid	0.34
18	18.11	1185	1177	Terpinen-4-ol	Monoterpenoid	1.13
19	18.56	1198	1189	*α*-Terpineol	Monoterpenoid	1.85
20	20.82	1264	1253	Piperitone	Monoterpenoid	0.12
21	23.93	1356	1349	*α*-Terpinyl acetate	Monoterpenoid	5.21
22	26.50	1435	1419	*E*-Caryophyllene	Sesquiterpene	1.90
23	27.58	1470	1455	*α*-Humulene	Sesquiterpene	0.28
24	28.80	1509	1494	*trans*-Muurola-4(14),5-diene	Sesquiterpene	0.21
25	28.90	1512	1500	Bicyclogermacrene	Sesquiterpene	0.70
26	29.59	1535	1523	*δ*-Cadinene	Sesquiterpene	0.50
27	30.60	1569	1563	*E*-Nerolidol	Sesquiterpenoid	0.61
28	31.86	1612	1601	Guaiol	Sesquiterpenoid	0.22
29	32.81	1645	1629	1-*epi*-Cubenol	Sesquiterpenoid	0.45
30	33.23	1660	1654	*epi*-α-Cadinol	Sesquiterpenoid	0.22
				Total		97.50
				Monoterpenes		83.34
				Monoterpenoids		9.05
				Sesquiterpenes		3.59
				Sesquiterpenoids		1.5

Larvicidal activity of essential oil from the leaves of *Eucalyptus robusta*

The larvicidal activity of EO from the leaves of *E. robusta* (Table 3 & Fig. 1) demonstrated a strong activity against *C. quinquefasciatus* with LC_{50} values after 24 h and 48 h to be 30.34 µg/mL and 28.77 µg/mL, respectively. 1,8-Cineole showed relatively weak activity against larvae of *Culex* and *Aedes* mosquito species with LC_{50} values (24 h) > 100 and > 50.0 µg/mL, respectively (Ngoc Anh et al., 2023). In this study, α-pinene demonstrated strong larvicidal activity against *C. quinquefasciatus* with an LC_{50} value of 12.85 µg/mL. In the previous study, α-phellandrene showed strong larvicidal activity against *Aedes* mosquito species (Ngoc Anh et al., 2023). α-Terpinyl acetate was effective against *Culex pipiens* larvae with an LC_{50} value (24h) of 23.03 µg/mL (Kimbaris et al., 2012). Therefore, the compounds *α*-pinene, α-phellandrene, and α-terpinyl acetate may have been mainly responsible for the larvicidal activity of *E. robusta* EO. Essential oils with 24-h LC_{50} values between 10 µg/mL and 50 µg/mL (Hung et al., 2022) or with 24-h LC_{90} values less than 50 ppm (Pavela, 2015) against *C. quinquefasciatus* larvae were considered "very active". Our findings in the current study suggest that *E. robusta* essential oil can be considered as a source of biopesticides for mosquito larvae control.

Table 3. Larvicidal efficacy of essential oils from the leaves of *Eucalyptus robusta* against *Culex quinquefasciatus* (ppm)

Samples	LC_{50} (95% limits)	LC_{90} (95% limits)	χ^2	*p*
		24 h		
EO	30.34 (28.49–32.90)	40.06 (36.25–47.08)	0.108	0.999
α-Pinene	12.85 (12.13–13.67)	17.86 (16.25–21.00)	3.222	0.666
		48 h		
EO	28.77 (26.98–30.93)	41.19 (37.24–47.98)	4.185	0.382
α-Pinene	9.05 (8.38–9.75)	13.58 (12.37–15.38)	0.957	0.966

Figure 1. Testing of mosquito larvicides of *Eucalyptus robusta* essential oil against *Culex quinquefasciatus*

CONCLUSIONS

The essential oil from the leaves of *E. robusta* was obtained by hydrodistillation, and the chemical composition was analyzed by gas chromatography. EO yield was determined as 3.7 % (w/w, fresh weight). A total of thirty compounds were identified, accounting for 97.50%. Monoterpene was the primary chemical class with the highest amounts of 83.34%, of which 1,8-cineole (29.23%), α-pinene (18.58%), and α-phellandrene (14.05%) were significant components. The larvicidal activity of essential oil from the leaves of *E. robusta* exhibited very strong inhibition against *C. quinquefasciatus* (LC_{50} values of 30.34 to 28.77 µg/mL at 24 hours and 48 hours, respectively). These findings suggest that essential oils can become raw materials for producing bio-pesticides to control disease-transmitting mosquitoes.

Acknowledgements: This work is supported by a grant from the Vietnam Academy of Science and Technology (VAST02.02/23–24, PI: Do Huu Nghi).

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