BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902025e24072

Article

Chemical composition and larvicidal activity of the essential oil of *Tagetes minuta* Linnaeus from the southern Brazilian highlands

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Tagetes minuta L. is a naturalized species that is not endemic to the high-altitude fields of Santa Catarina in southern Brazil. In this study, we investigated the composition of the essential oil (EO) from the flowers of the plant and its larvicidal activity against *Aedes aegypti*. The EO was obtained by hydrodistillation using a Clevenger apparatus. The chemical composition was determined using gas chromatography coupled with mass spectrometry (GC-MS). The EO extraction yield was 4.9%, and the major compounds (Z)-tagetone, (Z)-β-ocimene, and dihydrotagetone were identified. The 24 h larvicidal activity was verified against that of L3 larvae of the Rockefeller strain of *Ae. Aegypti*. The EO showed larvicidal activity against *Ae. aegypti*, with LC_{50} = 17.28 µg/mL^{-1}. The results indicate that the bioinsecticidal potential of *T. minuta* EO is promising for the development of research in the field of natural products.

Keywords: *Tagetes minuta*. Essential oil. Larvicidal. Brazilian highlands. Tagetone.

INTRODUCTION

Aedes aegypti Linnaeus (Diptera, Culicidae) is the main vector for arboviruses that cause dengue, Zika, chikungunya, and yellow fever (Pierson, Diamond, 2020), which are diseases that have a great impact on global health.

Control strategies are based on vector control, by employing synthetic and biological chemicals integrated with environmental management programs that mainly use chemical insecticides (Silva *et al*., 2019). However, frequent use of chemical insecticides favors the development of resistant insect populations (Dusfour *et al*., 2019; Vivekanandhan *et al*., 2021). In the search for inputs of natural origin as alternatives for the control of the *Ae. aegypti* mosquito, essential oil (EO)-based bioinsecticides have been created (Valli *et al*., 2021; Vivekanandhan *et al*., 2018; Vivekanandhan *et al*., 2020; Vivekanandhan *et al*., 2021; Vivekanandhan *et al*., 2024). Promising bioinsecticide molecules can overcome the majority of the challenges associated with synthetic insecticides, and they are biodegradable and have moderate selectivity, minimizing the emergence of resistant insects (Isman, 2020).

Tagetes minuta L (family Asteraceae) is a herbaceous plant native to Central and South America that is found in grasslands and elevated regions of Argentina, Peru, Bolivia, Chile, and Paraguay (McVaugh, 1943). With the Spanish colonization of the Americas, *T. minuta* was introduced into different countries (Soulé, 1993) and is currently widely spread in Europe, Africa, Asia,

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and Oceania (Salehi *et al*., 2018). It is commonly known as wild marigold, wild clove, clove, chinchilho, rocket stick, or rocket butt. Is an aromatic, small-sized plant with small yellow flowers, hence the species name minuta. It is considered a source of economic interest mainly due to the broad spectrum of uses of its EO in folk medicine (Martinez *et al*., 2020). This plant is commercially cultivated in several countries to extract EO from leaves and flowers (Singh *et al*., 2015; Walia, Kumar, 2020).

T. minuta has biological properties of economic importance in the pharmaceutical, cosmetic and food industries, particularly in its EO (Salehi *et al*., 2018; Walia, Kumar, 2020). Studies have described the EO as a biocidal, herbicidal, nematicidal, larvicidal, anti-inflammatory, antibacterial, and antiviral agent (Singh *et al*., 2015; Wanzala *et al*., 2016; Salehi *et al*., 2018; Kumar, Pandey, Varshney, 2019). Its chemical composition is rich in secondary metabolites including monoterpenes, sesquiterpenes, flavonoids, and thiophenes (Walia *et al*., 2020).

T. minuta L. is an annual plant and is considered a weed. In Santa Catarina, it occurs spontaneously in fallow land or along roads and paths. The aim of this study was to investigate and characterize the composition of the EO produced by this species from the high-altitude fields in southern Brazil and evaluate its larvicidal activity against *Ae. aegypti.*

MATERIAL AND METHODS

Plant Material

Flowers of *T. minuta* L. were collected from the southern Brazilian highlands (28°24'57.3"S and 49°44'29.8"W) in the autumn (June), at an altitude of 1230 m above sea level, with temperature of 10 °C. The present study was authorized by the Genetic Heritage Management Council (CGEN), a legislative and deliberative body under the Ministry of the Environment of Brazil, under number 02001.001165 / 2013-47.

Extraction of EO

T. minuta L. was dried at room temperature for 20 days. Subsequently, the sample was pulverized using a cutting mill. The EO was extracted from 100 g of the flower by hydrodistillation for 6 h using a Clevenger apparatus. The EO was then stored in a sealed amber jar glass at -8 °C until subsequent analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

The EO from *T. minuta* was analyzed by gas chromatography-mass spectrometry using a Shimadzu GC-MS - QP 2010 Plus analyzer equipped with a capillary column Rtx -5MS (30 m × 0.25 mm × 0.25 µm.) with a splitless injector mode at 250 °C, and an ion source and interface at 300 °C. The mass window was analyzed from m/z 40 and m/z 350, using helium as the carrier gas. Ramp injection for analysis had the injector temperature set at 250 °C, pressure of 20 psi column, starting at 50 °C for 5 min and increasing to 200 °C at a rate of 5 °C/min. Identification of the oil components was undertaken by comparing their Kovats indices and mass spectra with the NIST library as well as comparing them with those reported in the literature (Adams, 2007).

Larvicidal activity against *Ae. aegypti*

The methodology applied was adapted from the WHO (2005) and Betim *et al.* (2019). The eggs of *Ae. aegypti* (eggs from the Rockefeller strain made available by Oswaldo Cruz Foundation - Fiocruz) were placed in dechlorinated water and incubated in a B.O.D incubator at a controlled temperature of 27 °C and relative humidity of 80%. The larval diet consisted of fish feed (Aldon basic, MEP 200 complex), from hatching until they reach the third developmental larval stage.

The EO was diluted in 0.5% of polysorbate 80 (obtained from Sigma Aldrich), and then dissolved in dechlorinated water to obtain the desired concentration. An aqueous solution of 0.5% of polysorbate 80 was used as a negative control and the insecticide temephos was used as a positive control. The positive control was used at a concentration of 6 µg/mL (twice the lethal concentration that causes 99% mortality of susceptible strains) according to the protocol recommended.

After hatching, 10 larvae in the 3rd stage were placed in contact with the negative and positive control and essential oil at concentrations (1000, 100 and 10 µg/mL) for 24h and then live and dead larvae were counted. Four repetitions were used for each treatment giving a total of 40 larvae to each sample dose.

Statistical analysis

The probit method (Finney, 1971) was used to determine the lethal concentration (LC_{50} and LC_{90}) values, as well as the corresponding 95% confidence intervals and chi square values for the assays with *Ae. aegypti*, using the SPSS Statistical Software Package version 23.0.

RESULTS AND DISCUSSION

Essential Oil Yield and Composition

Hydrodistillation of the flowers of *T. minuta* produced a characteristic yellow, fruit scented EO with a yield of 4.9%, with the highest volume obtained in the first 30 min.

T. minuta produces the highest EO yield among all cultivated species of the genus *Tagetes*. Oliveira *et al.* (2018) obtained a 2.09% EO yield in a sample from Brazil, while an extraction South Africa retrieved 0.33% (Igwaran *et al*., 2017), and Indian EO from the Himalayan region returned 0.37 to 0.79% (Walia *et al*., 2020). According to Kumar *et al*., (2014), EO yield can vary according to environmental factors, geographical location, collection time, cultivation method, and climatic conditions.

The present study was conducted in southeastern Santa Catarina, where the Atlantic Forest biome in southern Brazil has grassland formations called Campos de Altitude do Planalto das Araucárias and Campos de Cima da Serra. These areas predominate at higher altitudes, with elevations greater than 1000 m above sea level (Boldrini, 2009).

The climate is characterized, according to the Köppen-Geiger classification, as humid mesothermal (Cfb), with mild summers, cold winters, well-distributed rainfall during the year, and an average temperature of 14.4 ºC . The city is considered one of the coldest in Brazil, with records of frost and snowfall in the winter months. The maximum and minimum annual average temperatures are 26.0 ºC and – 9.2 ºC , respectively (Potter *et al*., 2004).

The high EO yield obtained in our study compared to those listed above may be related to post-flowering collection phase and the particular climatic and altitude conditions of the geographical region, corroborating the results of studies conducted in India (Kumar *et al*., 2014). In addition, the altitude difference appears to have affected the chemical composition of the EO (Rathore, Walia, Kumar, 2018).

GC/MS analysis detected and identified 11 terpene-derived compounds (mono- and sesquiterpenes) corresponding to 97.72% of the components present in the EO of *T. minuta* (Table I).

TABLE I – Chemical composition of EO from *T. minuta* flowers

RT	RIcal	RIlit	Compound	Classification	%
9.295	972	969	Sabinene	Monoterpene	0,06
11.590	1029	1024	Limonene	Monoterpene	0,80
11.825	1034	1032	(Z)- β-Ocimene	Monoterpene	21,34
12.310	1045	1044	(E)- β-Ocimene	Oxygenated monoterpene	0,22
12.500	1050	1046	Dihydrotagetone	Oxygenated monoterpene	5,61
16.030	1128	1128	Allo-Ocimene	Monoterpene	0,36
16.780	1145	1139	(E)-Tagetone	Oxygenated monoterpene	1,76
17.110	1152	1148	(Z)-Tagetone	Oxygenated monoterpene	62,69
20,795	1232	1226	(Z)-Ocimenone	Oxygenated monoterpene	1,05
21.145	1239	1235	(E)-Ocimenone	Oxygenated monoterpene	3,64
32.305	1496	1500	Bicyclogermacrene	Sesquiterpene	0,19
Monoterpene					22,56
Oxygenated monoterpene					74,97
Sesquiterpene					0,19
Total identified					97,72

Notes: RT = Retention time (min), RIcal = Kovats retention index experimentally calculated, RIlit = Kovats retention index (Adams 2007), % = Composition.

Of the 11 compounds, 10 (99.8%) were monoterpenes and 1 (0.2%) was a sesquiterpene. The predominance of monoterpenes is in agreement with the results obtained in most studies on *Tagetes* species (Salehi *et al*., 2018).

The main compounds found were monoterpenes (Z)-tagetone (62.69%), (Z)-β-ocimene (21.34%) and dihydrotagetone (5.61%).

Z-tagetone, dihydrotagetone, and ocimene are associated with antimicrobial, insecticidal, herbicidal, and nematicidal activity. Ocimene is used in the perfume and fragrance industries (Salehi *et al*., 2018). In addition, tagetone and ocimene derivatives have been reported to be chemical constituents of EOs with potential cytotoxic effects (Ali *et al*., 2014; Shirazi *et al*., 2014). The result obtained is consistent with the characterization of EOs from previous research; however, a quantitative difference was observed.

The genus *Tagetes* is known to contain species rich in aromatic compounds, which are generally high in monoterpene hydrocarbons (ocimenes, limonene, terpinene, and myrcene), and acyclic monoterpene ketones (tagetone, dihydrotagetone, and tagetenone), which are the primary odorants. Lower amounts of sesquiterpene hydrocarbons and oxygenated compounds are also present (Ibrahim *et al*., 2016). *T. lucida* and *T. filifolia* are the only species with marked differences in EO composition, dominated by phenylpropanoids such as methyleugenol, methylchavicol, and anethole (Salehi *et al*., 2018). According to Singh *et al*. (2015), the EOs from *T. minuta* can be grouped into two chemotypes: those dominated by (Z)-β-ocimene and monoterpenes, and those with mainly tagetone and dihydrotagetone derivatives.

The EOs of *T. minuta* from Argentina showed different chemical compositions, as dihydrotagetone (42.9%) was the main constituent present in leaves, whereas β-ocimene (45.4%) was found in flowers (Chamorro *et al*., 2008). In another study, there was a predominance of limonene (66.3%) (Gillij, Gleiser, Zygadlo, 2008), whereas in our research, we only identified traces of this compound (0.80%).

Verbenone was the most abundant compound obtained in Uganda (Kyarimpa *et al*., 2014) and Turkey (Bahadirli, 2020), with 15% and 32.68%, respectively. Verbenone was not identified in the present study.

In Brazil, we observed variations in the chemical composition of EO across different geographical regions (Table II).

TABLE II – Major EO components, reported as percentages, of *T. minuta* flower from Brazil

Major components	Central-western	Northeastern	Southern
Limonene	7,0	13,73	-
(Z) -β-Ocimene	5,1	-	-
Trans-Ocimene	-	-	16,23
Cis-Ocimenona	-	-	-
Trans-Ocimenona	-	-	-
Dihydrotagetone	54,2	-	67,64
Cis-Tagetone	-	-	-
Trans-Tagetone	-	-	10,14
(E) -Tagetone	6,7	-	-
(Z) -Tagetone	-	-	-
Piperitone	-	86,27	-
Verbenone	-	-	2,98%
1,3,6-octatrieno-3,7-dimetil-E	-	-	-

Garcia *et al*. (2012) found dihydrotagetone as the main compound (54.2%) in the EO from the central-western region, whereas the EO from the northeastern region was dominated by piperitone (86.3%) (Macedo *et al*., 2013). On the coast of the southern region near sea level, there was also a predominance of dihydrotagetone 67.64% (Chaaban *et al*., 2019).

Comparing our results with the data in Table II, it is clear that the composition of the EO of *T. minuta* grown in Brazil differs with respect to geographical region. The EO obtained from the central region showed higher tagetone content and traces of terpenes, while that from the northeastern region was dominated by piperitone (86.27%) and limonene (13.73%).

In the pioneer study by (Craveiro *et al*., 1988), the EO of *T. minuta* from the northeastern region of Brazil, Bahia, and Pernambuco, was observed to be rich in dihydrotagetone (reaching 99%), while in our study, the cold-climate plants from the altitude region of Santa Catarina produced an essential oil rich in (Z)-tagetone and β-ocimene, similar to the results obtained by Oliveira *et al*. (2018). The sample from Pernambuco contained thymol (7.4%), a compound that was not identified in our study.

Larvicidal activity against *Aedes aegypti*

The EO of *T. minuta* showed potential larvicidal activity for third-stage larvae of *Ae. aegypti*, with an LC_{50} of 17.28 $\mu g.mL^{-1}$ (concentration required to inhibit 50% of treated larvae), as shown in Table III.

TABLE III – Induction of mortality in *Ae. aegypti* larvae by *T. minuta* EO

Sample	Concentration ($\mu g/mL^{-1}$)	Mortality (%) ± SD	LC50 ($\mu g/mL^{-1}$) LCL - UCL	LC90 ($\mu g/mL^{-1}$) LCL – UCL	x^2	(df)
EO T. minuta	1000 100 10	100 75,00 ± 0,50 42,50 ± 0,43	17, 28 (7,57 – 30,42)	225,35 (112,96 – 802,29)	1,9	1,0

Notes: No mortality was observed in the negative controls; the positive control killed 100% of *Ae. aegypti* larvae; (LC_{50}) lethal concentration that killed 50% of the exposed organisms; (LC_{90}) lethal concentration that killed 90% of the exposed organisms; (UCL) 95% upper confidence limit; (LCL) 95% lower confidence limit; x^2, chi-square statistics; df, degrees of freedom.

The EO of *T. minuta* has insecticidal and repellent properties against a number of pests and insects (Walia, Kumar, 2020), including *Pediculus humanus capitis* (Cestari *et al*., 2004), *Brevicoryne brassicae*, and *Rhipicephalus appendiculatus* (Wanzala *et al*., 2020). The EO extracted from *T. minuta* seeds showed insecticidal activity against pests found in grain storage: *Tribolium castaneum*, *Rhyzopertha dominica*, and *Callosobruchus maculatus* (Jayaram *et al*., 2022).

Studies have been conducted to evaluate the repellent and insecticidal effects of EO on different mosquito species, such as *Anopheles arabiensis*, *A. stephensi,* and *Culex quinquefasciatus* (Walia, Kumar 2020).

The repellent activity of EO against *Ae. aegypti* has been described in Argentina (Gillij, Gleiser, Zygadlo, 2008) and Kenya (Wanzala, Ogoma, 2013), with limonene being the major compound in both cases. In Peru, *T. filifolia* obtained a higher larvicidal activity (LC_{50}=47.7 ppm) for *A. aegypti* when compared to that of *T. minuta* (LC_{50}=52.3 ppm), probably due to its high concentration of trans-anethol (88.2%) (Ruiz *et al*., 2011).

The result obtained in our work is similar to the study by Kyarimpa *et al*. (2014), in which an LC_{50} of 1.49 mg/L was obtained against *Anopheles gambiae*, with an EO mainly composed of terpenic derivatives. Although that study also noted the presence of verbenone, which is a compound known to be nematicidal. In contrast, Furtado *et al*. (2005) obtained an LC_{50} of 72.85 mg/mL, which is lower than our results.

The EO insecticidal activity of *T. minuta* against *Ae. aegypti* can be attributed to the presence of the main constituents, limonene, E-ocimenone, dihydrotagetone, β-ocimene, and (Z)-tagetone (Bakshi, Ghosh 2022).

Green *et al.* (1991) reported the larvicidal activity of the EO of three species of *Tagetes* grown under controlled laboratory conditions. They reported that the EO of the flowers of *T. minuta* had the greatest biocidal effect on the larvae of *Ae. aegypti*. However, the components of the EO responsible for this activity were not characterized. Notably, the plant materials were obtained through controlled cultivation, which can directly interfere with the chemical composition of the EO. Lima *et al.* (2009) evaluated the larvicidal activity of the EO (of an unknown composition) obtained from a commercially acquired *T. minuta* of Indian origin. The authors reported a LC_{50} of 0.24 $mL{\cdot}L^{-1}$, and suggested that the compounds derived from thiophene and ocimenone were responsible for the toxicity in *Ae. aegypti*.

In this study, *T. minuta* was collected in situ from different soils and climatic conditions to characterize the chemotype of the EOs and elucidate their primary composition. β-ocimene, (E)-ocimenone, dihydrotagetone, and (Z)-tagetone were the major compounds identified, and possibly the ones responsible for the larvicidal activity, however, EOs are complex mixtures of substances, and synergism may occur with the minority compounds and their combinations (Jayaram *et al*., 2022). These compounds are believed to act on the insect nervous system by altering GABAergic functions (Bloomquist *et al*., 2008) and inhibiting acetylcholinesterase (Abdelgaleil *et al*., 2015), causing toxicity.

CONCLUSION

The major compounds found in the EO of the flowers of *T. minuta* from the altitude fields of Santa Catarina, southern Brazil, were the monoterpenes (Z)-tagetone (62.69%), (Z)-β-ocimene (21.34%) dihydrotagetone (5.61%), and (E)-ocimenone (3.64%). The EO yield obtained by vapor drag hydrodistillation was 4.9%, favoring its use in the pharmaceutical, cosmetic (perfumes and fragrances), and food industries. The high yield obtained can be correlated to the edaphoclimatic characteristics of the Campos de Altitude in the Serra Catarinense. Planting of the altitude cultivar should be encouraged, favoring the production chain of the EO and generating a new source of income for the cultivation region. The EO showed larvicidal activity against *Ae. aegypti*, with an LC_{50} = 17.28 $\mu g.mL^{-1}$, which is promising for the generation of bioinsecticidal products for mosquito control. The results of this study demonstrate the need for further research with the Brazilian high-altitude chemotype of *T. minuta* EO, particularly in terms of the structural elucidation of its components, as well as its bioactivity and toxicity.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ACKNOWLEDGMENTS

The authors would like to thank Federal University of Paraná, the Araucaria Foundation, the Paraná State Secretariat of Science, Technology and Higher Education (SETI-PR) and NAPI-Genomics Consortium.

FUNDING

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Funding Code 001.

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Received for publication on 23rd January 2024
Accepted for publication on 12th June 2024
Associated Editor: Alexandra Sawaya