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A comprehensive analysis of *Tagetes minuta* L. essential oil from Brazil







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Foreword

Brazil holds an immense potential in its vast array of plant species that could serve as alternative crops and sources of income for family farming. Those that yield essential oils present particularly compelling opportunities, as they allow the establishment of a production chain and their utilization across diverse market segments, including botanical pesticides, veterinary products, cosmetics, medicine, among others.

Tagetes minuta L., commonly known in Portuguese as chinchilho, cravo-do-mato, or rabo-de-foguete, is prevalent in various regions of Brazil albeit it has been mostlystudied in the Southern region. The biological properties of its essential oil have been discussed in dissertations, theses, and scientific articles produced by Brazilian research institutions, positioning Brazil as a leading producer of scientific knowledge on this species. Despite the remarkable research output, commercial cultivation of the species remains absent in the country.

However, in a mistaken way Brazil has been identified in numerous scientific papers, reports and academic works as one of the world's leading producers of *T. minuta* L. essential oil. This peculiar misconception arises from the misinterpretation of a study published in 1988, an error that has been supported over the years.

This study aims to understand the origin of this misconception and provide comprehensive and up-to-date information on the cultivation and essential oil composition of *Tagetes minuta* L. grown in Brazil. In this context, the study also contributes to the achievement of the Sustainable Development Goals (SDGs) proposed by the UN Agenda 2030, particularly in alignment with the promotion of sustainable agriculture (SDG 2).

Waldyr Stumpf Junior Acting Head of Embrapa Temperate Agriculture

Sumário

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Introduction

Brazil is a country with continental dimensions and boasts a vast diversity of plant species with potential for essential oil extraction. In the Amazon region alone, more than 12 plant families and countless species are known, over 80% of which are native. Their chemical compositions, antioxidant properties, and biological activities have been welldocumented (Ferreira et al., 2022). However, Brazil stands out as the world's leading exporter of citrus essential oil by volume, particularly orange oil, a byproduct of the juice industry rich in limonene (Bizzo; Rezende, 2022).

Tagetes minuta L. is a native species from South America, but it is widely distributed throughout the continent, ranging from the southeastern United States to the northern Patagonian region in Argentina (Schiavinato et al., 2017). In Brazil, it is considered a naturalized species occuring in almost all regions and biomes (Carneiro, 2024), although it is listed as adventive in Africa, Asia, Europe, and Australia (Gutiérrez; Stampacchio, 2015). It is an erect annual herb that can reach 2 meters in height, highly branched with a taproot, and has oil glands in the leaf blade and flower heads (Carneiro; Ritter, 2018). Studies have demonstrated the bioactive properties of its oil, enabling applications in various areas, including pharmaceuticals, medicine, and agriculture, among others (Gakuubi et al., 2016; Bandana et al., 2018; Salehi et al., 2018; Joshi; Barbalho, 2022). Its habitat includes cultivated areas, such as crops or grasslands, and ruderal areas in abandoned fields and roadsides (Gutiérrez; Stampacchio, 2015; Carneiro; Ritter, 2018).

Therefore, some studies have been conducted in southern Brazil in order to understand the potential of extracts and essential oil of *T. minuta* in various uses (Lovatto et al., 2013; Hellwig, 2019; Oliveira et al., 2019; Santos et al., 2021; Cepeda et al., 2023; Schiedeck, 2023). Nevertheless, when consulting the available scientific literature on the species, we frequently find the claim that Brazil is one of the world's largest producers of *T. minuta* essential oil. Such assertion is unrealistic, as there is no record of large-scale production of *T. minuta* essential oil in the country at any historical period to support it. Many articles asserting this misunderstanding have in common the citation of the study by Craveiro et al. (1988), often used as a reference to characterize the essential oil of plants growing in Brazil. Furthermore, distorted and even non-existent information is also attributed to the authors in the original article.

Hence, the objective of this study is to elucidate the origin of this misconception, which places Brazil as a major world producer of *T. minuta* essential oil and to describe aspects of the national academic production on the species. It also aims to systematize data on the composition of the essential oil of the species, taken from studies that used plants collected or cultivated in different regions of Brazil. This could provide insights for future research and reviews with correct and up to date information.

Exploring the source of the misconception

Since 1991, Craveiro et al. (1988) have been cited in 62 works, including papers, theses and dissertations, book chapters, and other types of scientific publications. This survey was carried out at Google Scholar¹ and Scopus² databases. Craveiro's article has a consistent frequency of almost two citations per year (Figure 1).

⁽¹⁾ Available at: https://scholar.google.com.br/.

⁽²⁾ Available at: https://www.scopus.com/.

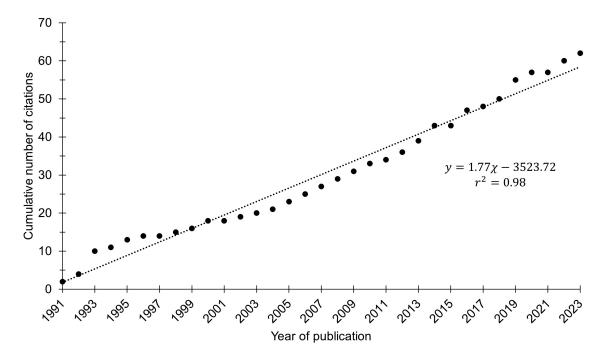
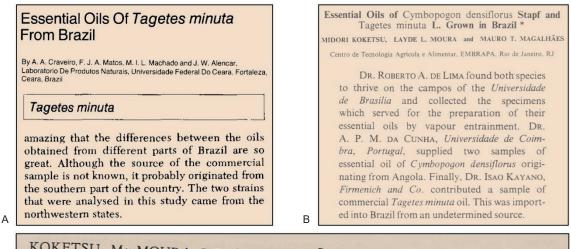


Figure 1. Number of citations to Craveiro et al. (1988), between 1991 and 2023.

The paper was published in a monthly magazine directed to the aroma, fragrance and flavor market, therefore targeting perfumers, chemists and industrial executives. The topics covered range from challenges on formulation, characterization of raw materials, technologies and trends to regulatory aspects and opinion articles, as well as commercial advertisements. Amazingly, it is noteworthy that Craveiro et al. (1988) remain consistently referred to contextualize the production of *T. minuta* essential oil in Brazil.

The one-and-a-half-page article explains that the interest in *T. minuta* essential oil is due to its aromatic and flavor properties and its potential use in controlling *Aedes aegypti* mosquito larvae. The authors cite Koketsu et al. (1976) as the only previous work comparing the chemical composition of *T. minuta* oil from Brazil to a commercial sample. Craveiro et al. (1988) then describe how they extracted the essential oil by steam distillation from flowers and inflorescences of two *T. minuta* samples, one from the municipality of Triunfo, State of Pernambuco, and the other from the municipality of Jacobina, State of Bahia (both in the Brazilian northeast region). There is no information about the site and harvest conditions in which these samples were collected, the date, or the time elapsed between collection and extraction. This information would have been vital since the distance between the two collection sites and the laboratory possibly used for analysis, in Fortaleza, State of Ceará, varies from 600 km to 1,000 km, respectively. From the chromatography data, the authors elaborated a table showing the composition of the samples from Triunfo and Jacobina, as well as the commercial sample published 12 years earlier by Koketsu et al. (1976). In regard to this commercial sample, Craveiro et al. (1988) explain that they do not have information about its origin, but they presume it to come from the southern region in Brazil.

It is presumable that the results of Koketsu et al. (1976) had already been presented at the 6th International Congress on Essential Oils, held in San Francisco, USA, two years earlier (Koketsu et al., 1974). In the complete paper dating from 1976, these authors analyzed the essential oil of *T. minuta* plants collected at the University of Brasilia campus, then compared it to a commercial sample clearly stating that it was an imported sample whose specific origin was impossible to determinate. Figure 2 presents excerpts from the three works mentioned above.



KOKETSU, M.; MOURA, L.L. & MAGALHÃES, M.T. Essential oils of Cymbopogon densiflorus and Tagetes minuta L. grown in Brazil. In: INTERNATIONAL CONGRESS OF ESSENTIAL OILS, 6., San Francisco, USA, 1974. Abstracts.

Aiming at an investigation of essential oils of exotic plants of possible economic use which have been introduced in Brazil, a comparative study was undertaken of oils of Cymbopogon densiflorus and Tagetes minuta grown in Brasilia (Brazil) and corresponding comercial samples from abroad. Physical constants, GLC chromatographic analysis, IR spectrometry and olfactory evaluation showed great similarity betheen the Brazilian and African products of C. densiflorus, whereas for the oils of T.minuta significant differences were found. Possible reasons for these findings are discussed.

Figure 2. Excerpt from Craveiro et al. (1988), assuming that the commercial sample comes from the southern region of Brazil (A); excerpt from Koketsu et al. (1976), reporting that the commercial sample of *T. minuta essential* oil was imported to Brazil with unknown origin (B); abstract of Koketsu et al. (1974) presented at the 6th International Congress of Essential Oils (C).

Koketsu et al. (1976) also fail to provide information about the site and harvest conditions, such like date, phenological stage of the plants, the parts used for oil extraction, or even the local of laboratory analysis. On their paper's conclusions, these authors emphasize that the commercial sample had stronger olfactory notes, possibly due to a higher degree of polymerization. The polymerization of essential oils is associated with significant changes in physical and chemical properties caused, among other factors, by exposure to light and heat (Turek; Stintzing, 2013).

С

Firmenich, the supplier of the commercial sample, is a Swiss company founded in 1895, in which makes the flavors and industrial fragrances, with offices in several countries around the world, including Brazil. In its current catalog, we can find *T. minuta* essential oil from South Africa, Zimbabwe, Madagascar and Azerbaijan (Firmenich, 2024).

In addition to the consideration of a sample with unknown origin as a standard for the phytochemical profile of *T. minuta* essential oil obtained in Brazil, another issue also emerged in subsequent studies. Although Craveiro et al. (1988) do not explicitly mention any commercial cultivation in Brazil, every year new scientific papers started citing it as a primary source to claim that the country is one of the largest producers of *T. minuta* essential oil in the world.

In summary, Koketsu et al. (1974) analyzed an imported commercial sample with unknown specific origin and, two years late, they published the results from their findings in a complete paper. Craveiro et al. (1988) ignored such information about the foreign origin of the commercial sample — clearly reported by Koketsu et al. (1976) — and assumed it to be from the southern of Brazil. Since then, inexplicably, new studies have taken for granted that there is a well-established and largescale production of *T. minuta* essential oil in Brazil, ranging the nation amongworld's largest producers.

Unusual distortions that multiply

Between 1991 and 2023, 16 studies were identifed containing the claim that Brazil is one of the world's largest producers of *T. minuta* essential oil or that the country has relevant commercial crops. Among them, 11 cite Craveiro et al. (1988) as the

primary source of this information. However, eight other publications also attribute assertions to these authors which are simply absent from the original work or heavily distorted or mistaken. Figure 3 illustrates the evolution of these errors over time.

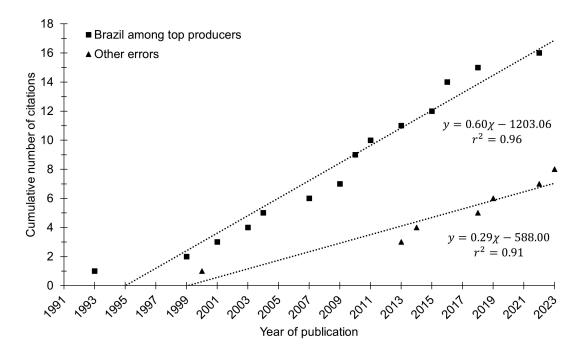


Figure 3. Evolution over the years of studies that cite Brazil as one of the world's largest producers of *T. minuta* essential oil and attribute non-existent, mistaken, or distorted information to Craveiro et al. (1988).

Soule (1993) is likely the first author to cite Craveiro et al. (1988) as the primary source to support the claim that Brazil is one of the world's largest producers of *T. minuta* essential oil. From 1993 to the present, this error, with some variations, has been repeated approximately every two years, the last one being found in Joshi and Barbalho (2022) (Table 1). On the other hand, publications that attribute to Craveiro et al. (1988) non-existent information or distort their work are less frequent, appearing every three or four years (Table 2).

Table 1. Authors who place Brazil among world's largest producers of *T. minuta* essential oil, excerpt from the publication, and factual discussion.

Author	Excerpt from the publication	Factual discussion
Soule (1993)	"Brazil is one major producer of <i>T. minuta</i> for Tagetes Oil (Craveiro et al., 1988)."	Craveiro et al. (1988) do not make this claim in their work.
Bansal et al. (1999)	"Commercial tagetes oil is produced from both wild populations and cultivated plants grown in several temperate and semi temperate parts of Asia, Africa, Europe, America and Australia (11-15) The main oil producing countries are Argentina, Brazil, Australia, Spain and France."	Craveiro et al. (1988) are reference #14, however the claim that Brazil is a major oil producer does not cite any source.

Table 1. Continued.

Author	Excerpt from the publication	Factual discussion
Singh (2001)	"South Africa, Brazil and Australia are the main producers of this oil in the world."	The sentence does not cite any source.
Gold (2003)	"In 1984, Brazil was the top producer in the world, while worldwide production was estimated at approximately 1.5 million tonnes (SOULE, 1993)."	Soule (1993) is quoted as a primary source, but this author uses Craveiro et al. (1988).
Cestari et al. (2004)	"Brazil is a major producer of <i>T. minuta</i> according to Craveiro et al. (1988)"	Craveiro et al. (1988) do not make this claim in their work.
Oliveira et al. (2007)	"According to Craveiro et al. (1988), Brazil is the largest producer of <i>T. minuta</i> , which characterizes it as a potential producer of species of this genus."	Craveiro et al. (1988) do not make this claim in their work.
Wanzala (2009)	"Brazil is one major producer of <i>T. minuta</i> for Tagetes Oil (Craveiro et al., 1988)"	Craveiro et al. (1988) do not make this claim in their work.
Shahzadi et al. (2010)	"In tropics and especially in Brazil and adjoining regions it is grown for essential oil production (Lawrence, 1985)"	The publication by Lawrence (1985) could not be located.
Meshkatalsadat et al. (2010)	"Brazil is one major producer of <i>T. minuta</i> for Tagetes Oil [12]"	Craveiro et al. (1988) are reference #12 and do not make this claim in their work.
Ghiasvand et al. (2011)	"Brazil is a major producer of <i>T. minuta</i> for Tagetes oil [4]."	The authors likely made an error in the citation numbering, as reference #4 is unrelated to the passage. Craveiro et al. (1988) are the source #46 and do not make this claim in their work.
Tankeu et al. (2013)	"Tagetes oil is commercially produced in many countries including South Africa, Brazil and Australia (Lawrence, 1993; Singh, 2001)."	Lawrence (1993) does not mention it. Singh (2001) makes this claim without reference to any source.
Ministério da Saúde (2015) [Brazil's Ministry of Health]	"Brazil is the largest producer of <i>T. minuta</i> for the extraction of its oil (34, 57, 76)."	Singh et al. (2003) are reference #34 and cite Craveiro et al. (1988), but in a different context of the discussion. Meshkatalsadat et al. (2010) are reference #57 and Ghiasvand et al. (2011) are #76; both studies use Craveiro et al. (1988) as a primary source, although they do not make this claim in their work.
Cornelius and Wycliffe (2016)	"Other countries contributing to the international production and marketing of the <i>T. minuta</i> essential oil include: Nepal, India, Brazil, Madagascar, Australia, Ukraine, Chile, Bolivia, Peru, Ecuador, Paraguay, Morocco, Kenya, China, United States of America, Argentina, and Madagascar (Craveiro et al., 1988)."	Craveiro et al. (1988) do not make this claim in their work.
Singh et al. (2016)	"The essential oil of <i>T. minuta</i> is commercially produced in Argentina, Australia, Brazil, France, Spain, Venezuela, Iran and other countries."	The sentence does not refer to any source.
Bandana et al. (2018)	"Brazil is one of the major producer of "Tagetes Oil" (Craveiro et al., 1988)."	Craveiro et al. (1988) do not make this claim in their work.
Joshi and Barbalho (2022)	"Brazil is one of the major producers of Tagetes Oil [139]."	Craveiro et al. (1988) are the reference #139 and do not make this claim in their work.

Authors	Excerpt from the publication	Factual discussion
Stojanova et al. (2000)	"In one publication (9) it has been recently reported that the oil of <i>T. minuta</i> grown in a N-deficient environment had been extremely rich in dihydrotagetone as compared to the same cultivar grown in a N-sufficient environment."	Craveiro et al. (1988) are reference #9 and do not provide any information in this regard.
Negahban et al. (2013)	"The EO is used in perfumes, and as a flavor component in most major food products, including cola beverages, alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins, puddings, condiments, and relishes (Craveiro et al., 1988)."	Craveiro et al. (1988) do not provide any information in this regard.
Lovatto et al. (2013)	"Despite the chemical composition and breadth of effects attributed to the plant, and even though Brazil is one of the countries with the highest occurrence of <i>T. minuta</i> (Craveiro et al., 1988)" ()	Craveiro et al. (1988) do not provide any information in this regard.
Andreotti et al. (2014)	"This species is the subject of studies that have shown promising results, with the species being effective against microbial agents, such as fungi [71], viruses [72] and bacteria [73]."	Craveiro et al. (1988) are reference #71 and do not provide any information in this regard.
Rathore et al. (2018)	"The essential oil is used in perfumes, and in flavor industry as a component in major food products, including alcoholic beverages, cola beverages, candy, frozen dairy desserts, baked goods, gelatins, condiments, relishes and puddings (6)."	Craveiro et al. (1988) are reference #6 and do not provide any information in this regard. This claim is similiar to the ones found in Negahban et al. (2013) and Monografia (2015).
Hellwig (2019)	"The literature commonly presents a wide range of references on the effects attributed to chinchilho against various pathogens (CRAVEIRO et al., 1988)" ()	Craveiro et al. (1988) do not provide any information in this regard.
Abdoul-Latif et al. (2022)	<i>"Tagetes minuta</i> L. is used by some healers to cure some illnesses such as flu or cough and to cure wounds and allergies [16–18]."	Craveiro et al. (1988) are reference #16 and do not provide any information in this regard.
Feskov et al. (2023)	The authors use the analysis of the commercial sample of unknown origin presented by Craveiro et al. (1988) to represent the essential oil of <i>T. minuta</i> obtained in Brazil.	The commercial sample described by Craveiro et al. (1988) was analyzed by Koketsu, Moura e Magalhães (1976) and, according to these authors, was imported to Brazil and has an uncertain origin.

Table 2. Authors who attribute mistaken or unrealistic claims to Craveiro et al. (1988), publication excerpt, and factual discussion.

The work of Singh (2001) is the only one that lacks any primary or secondary source for the claim that Brazil is a major producer of *T. minuta* essential oil. In turn, Gold (2003) attributes this claim to Soule (1993), whose primary source is Craveiro et al. (1988). Shahzadi et al. (2010) cite a work by Lawrence (1985) in *Perfumer & Flavorist* to inform that in the tropical region, especially in Brazil, *T. minuta* is cultivated for essential oil production. However, it was not possible to locate a copy of this article to verify if it was accuratedly cited. Tankeu et al. (2013) cite Singh (2001) and Lawrence (1993) as primary sources of the information. While the former makes the claim without indicating any source, the latter does not present any element allowing make inferences about the production of *T. minuta* essential oil in Brazil.

The fact is that there is not any known commercial production of *T. minuta* essential oil in Brazil, which could support the claim presented by these studies. Additionally, due to the lack of information regarding plants collection and processing, the chemical composition of the essential oil described by Craveiro et al. (1988) should be referred with caution, in order to avoid extrapolations in the context of a country as large as Brazil.

Regarding the other unrealistic information attributed to Craveiro et al. (1988), some of them seem absurd, such as the use of the plant by healers for the treatment of diseases and wounds (Abdoul-Latif et al., 2022) or the use of the essential oil in food, sweets, and beverage production (Negahban et al., 2013; Rathore et al., 2018). There is any kind of mention of such properties and uses in the original work. Such misunderstandings might originate from errors in the editing of the papers.

To sum up, 39% of the 62 citations to the work of Craveiro et al. (1988) are false or incorrect. It is also important to highlight that some of these citations are published in journals with high scientific impact, books, master's theses and doctoral dissertations.

Scientific production in Brazil

Brazil does not have commercial crops nor is one of the world's largest producers of *T. minuta* essential oil, however it is one of the countries that mostly publishes scientific articles about the species. Argentina and India dominate the scientific production about the species, each one representing nearly 16% of all publications. In fourth position, Brazil is responsible for 8.2% of *T. minuta* publications, and is ahead of countries such as the United States, the United Kingdom and the Netherlands (Figure 4).

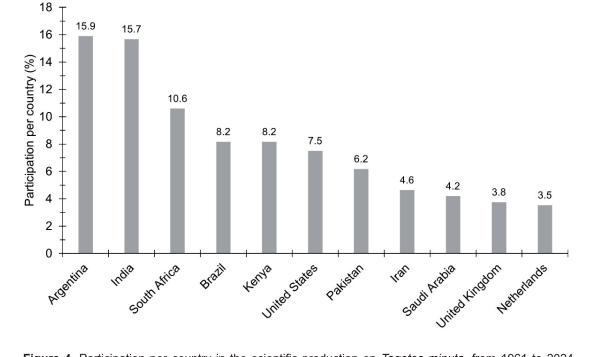


Figure 4. Participation per country in the scientific production on *Tagetes minuta*, from 1961 to 2024, according to the Scopus database.

In Argentina, there are 12 native species of the genus *Tagetes* (Gutiérrez; Stampacchio, 2015), including *T. minuta*, which may explain the interest of researchers. Recent studies on variety selection and essential oil composition have led to the registration of three varieties of *T. minuta* in this country (Massuh et al., 2017; Martinez et al., 2020).

In India, the species has become naturalized, well adapting to locations with altitudes between 1,000 and 2,500 m, where it occurs spontaneously on roadsides but also in cultivated areas during the early successional stages of plants (Cornelius;

Wycliffe, 2016. Bandana et al., 2018). Its potential for producing essential oil has raised the interest of different research institutes, and actually three varieties of *T. minuta* were released (Kumar et al., 1999; Singh et al., 2001; Kumar; Singh, 2020), with well-defined agricultural practices (Singh et al., 2001, 2003; Walia; Kumar, 2020).

In 2017, the Indian government created a program called CSIR-Aroma Mission, which involved planting 118 ha of *T. minuta* of the Himgold variety by 560 farmers. In total, around 3.5 tons of essential oil were extracted, representing a yield two to three

times higher than the one obtained with traditional crops such as wheat and rice (Council of Scientific and Industrial Research, 2019). The demand for the oil by the flavor and fragrance industry led to the expansion of cultivation and, between 2019 and 2020, an estimated area of 401 ha was cultivated in the western Himalayas (Council of Scientific and Industrial Research, 2020). In addition, farmers also

report an interest in the plant for its ability to attract pollinators (Kumar et al., 2022).

Within this context, over the past decade, the global scientific interest, expressed in publications on *T. minuta*, tended to increase in approximately 22 new papers each year. In Brazil, this number was almost 10 times lower, although the growth was also constant (Figure 5).

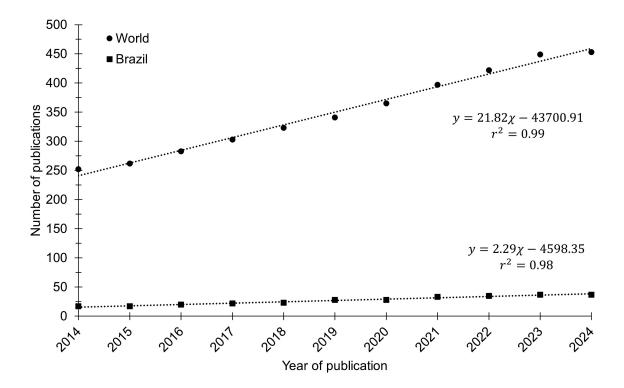


Figure 5. Scientific publications on *Tagetes minuta* worldwide and in Brazil, from 2014 to 2024, according to the Scopus database.

Overall, agricultural and biological sciences are the preferred areas for publishing research on the species, followed by biochemistry, chemistry, pharmacology and medicine. In Brazil, research prioritizes agricultural and biological sciences and pharmacology, but there is also a robust emphasis on medicine, microbiology and veterinary science (Table 3).

Table 3. Distribution (%) by knowledge areas of papers about *Tagetes minuta* in the world and in Brazil, published from 1961 to 2024, according to Scopus database.

Knowledge area	World	Brazil
Agricultural and Biological Sciences	35	20
Biochemistry, Genetics and Molecular Biology	12	8

Continued...

Table 3. Continued.

Knowledge area	World	Brazil
Chemistry	12	8
Pharmacology, Toxicology and Pharmaceutics	12	20
Medicine	10	12
Environmental Science	6	5
Immunology and Microbiology	4	12
Veterinary	2	11
Total	93	94

In Brazil, publications about *T. minuta* in veterinary medicine focuses on tick control (Andreotti et al., 2014; Cepeda et al., 2023) and bovine mastitis control (Schiavon et al., 2011). In medicine and pharmacology, *T. minuta* oil has been tested as

an antimicrobial in the development of endodontic sealants (Santos et al., 2021), as an antioxidant and antifungal against candidiasis and opportunistic pathogens (Cunha et al., 2016; Oliveira et al., 2019), and in the control of insects which are relevant in public health (Cestari et al., 2004; Furtado et al., 2005; Lima et al., 2009).

However, there is a considerable amount of work published as gray literature. In the Brazilian official thesis databases, there are records of 17 master's theses and 13 doctoral dissertations, published between 1998 and 2022 (Capes, 2024; Ibict, 2024). In Embrapa database, the largest public agricultural research company in Brazil, there are records of 39 publications as technical reports, abstracts and papers presented at conference proceedings, from 1974 to 2023 (Embrapa, 2024).

Essential oil from Brazil

Chemical composition

Over the years, there has been a considerable improvement in the sensitivity of the equipment used in the characterization of essential oils and in the reduction of the analysis costs. This has favored the increased number of publications and has qualified the information about the essential oil of *T. minuta* obtained in Brazil. Therefore, it is not reasonable to still cite the data from a work published more than three decades ago as a phytochemical standard, since there are more recent works with more precise data.

Hence, the information on the chemical composition of the essential oil of T. minuta obtained in the Brazilian territory was systematized from 18 papers published between 1976 and 2023, including theses and dissertations. These articles were obtained from the following databases: Google Scholar³, Scopus⁴, CAPES⁵, Ibict⁶ and Embrapa⁷. Among these papers, 16 examine spontaneous plants, one cultivated plants, and another one provides the composition of both spontaneous and cultivated plants. Many studies analyzed more than one sample, sometimes comparing spontaneous populations from different locations, other times analyzing different parts of the plants, such as leaves and flowers. A total of 123 compounds were identified, among which 75 were mentioned only once and 10 only twice. For the elaboration of Table 4, the compounds that had a frequency of occurrence equal to or greater than three analyses were considered.

 Table 4. Mean value (%) and standard error (SE) of major compounds of Tagetes minuta essential oil obtained in Brazil, number of samples (n) and references used.

Major compound	Mean (%)	SE	n	Reference
trans-Tagetone	6.22	1.04	53	Koketsu et al. (1976); Craveiro et al. (1988); Oliveira (2012); Oliveira (2013); Cunha et al. (2016); Gomes (2017); Albuquerque (2018); Fonseca (2018); Rostignoli (2019); Sperandio et al. (2019); Moreira (2021); Zimmermann et al. (2021); Cepeda et al. (2023)
Dihydrotagetone	24.54	3.37	51	Koketsu et al. (1976); Craveiro et al. (1988); Furtado et al. (2005); Garcia et al. (2012); Oliveira (2012); Oliveira (2013); Cunha et al. (2016); Gomes (2017); Fonseca (2018); Rostignoli (2019); Sperandio et al. (2019); Moreira (2021); Zimmermann et al. (2021)
cis-Tagetone	36.01	2.31	51	Koketsu et al. (1976); Oliveira (2012); Oliveira (2013); Cunha et al. (2016); Gomes (2017); Fonseca (2018); Sperandio et al. (2019); Moreira (2021); Zimmermann et al. (2021); Cepeda et al. (2023)
cis-beta-Ocimene	25.58	1.51	46	Oliveira (2013); Gomes (2017); Albuquerque (2018); Fonseca (2018); Rostignoli (2019); Sperandio et al. (2019); Zimmermann et al. (2021); Cepeda et al. (2023)

Continued...

⁽³⁾ Available at: https://scholar.google.com.br/.

⁽⁴⁾ Available at: https://www.scopus.com/.

⁽⁵⁾ Available at: https://catalogodeteses.capes.gov.br/.

⁽⁶⁾ Available at: https://bdtd.ibict.br/vufind/.

⁽⁷⁾ Available at: https://www.bdpa.cnptia.embrapa.br/.

Table 4. Continued.

Major compound	Mean (%)	SE	n	Reference
Caryophyllene	0.51	0.12	41	Cunha et al. (2016); Gomes (2017); Albuquerque (2018); Fonseca (2018); Sperandio et al. (2019); Moreira (2021); Santos et al. (2021); Zimmermann et al. (2021)
Humulene	0.28	0.05	39	Cunha et al. (2016); Gomes (2017); Fonseca (2018); Sperandio et al. (2019); Santos et al. (2021); Cepeda et al. (2023)
Sabinene	0.47	0.06	36	Gomes (2017); Albuquerque (2018); Fonseca (2018); Moreira (2021)
Bicyclogermac- rene	0.40	0.07	34	Gomes (2017); Albuquerque (2018); Fonseca (2018); Moreira (2021)
Limonene	6.22	1.40	25	Siqueira et al. (1982); Garcia et al. (2012); Macedo et al. (2013); Oliveira (2013); Cunha et al. (2016); Gomes (2017); Fonseca (2018); Moreira (2021); Rostignoli (2019);
D-Limonene	3.13	0.52	20	Albuquerque (2018); Fonseca (2018); Santos et al. (2021)
Caryophyllene oxide	0.51	0.29	18	Oliveira (2012); Albuquerque (2018); Fonseca (2018); Cepeda et al. (2023)
7-epi-Silphiperfol- -5-ene	0.24	0.06	15	Gomes (2017); Fonseca (2018)
trans-beta- Ocimene	4.51	2.81	14	Koketsu et al. (1976); Oliveira (2013); Cunha et al. (2016); Gomes (2017); Albuquerque (2018); Sperandio et al. (2019); Moreira (2021); Cepeda et al. (2023)
cis-Ocimenone	1.83	1.12	14	Koketsu et al. (1976); Fonseca (2018); Sperandio et al. (2019); Zimmermann et al. (2021)
Alloocimeno	0.52	0.05	13	Fonseca (2018); Cepeda et al. (2023)
Linalool	0.15	0.05	12	Albuquerque (2018); Fonseca (2018); Sperandio et al. (2019)
delta-Elemene	0.15	0.02	12	Albuquerque (2018); Fonseca (2018)
Myrcene	0.10	0.01	12	Albuquerque (2018); Fonseca (2018)
cis-Limonene oxide	0.81	0.04	11	Fonseca (2018)
cis-beta-Ocimene epoxide	0.14	0.03	11	Fonseca (2018)
2-Carene	0.36	0.02	10	Fonseca (2018)
trans-Ocimenone	3.10	1.71	10	Koketsu et al. (1976); Gomes (2017); Sperandio et al. (2019); Zimmermann et al. (2021)
3-Caren-2-one	0.27	0.04	9	Fonseca (2018)
Octanal	0.31	0.08	8	Cunha et al. (2016); Gomes (2017)
alpha-Phelland- rene	0.28	0.05	7	Gomes (2017); Albuquerque (2018)
Ethyl 2-methylbu- tyrate	0.05	0.02	6	Gomes (2017)
o-Cymene	35.71	5.72	5	Cepeda et al. (2023)
Isocaryophyllene	1.19	0.28	5	Cepeda et al. (2023)
Spathulenol	0.44	0.13	5	Cepeda et al. (2023)
Verbenone	5.07	0.86	5	Cepeda et al. (2023)

Major compound	Mean (%)	SE	n	Reference
Eucaliptol	0.41	0.09	5	Cepeda et al. (2023)
alpha-Pinene	0.98	0.48	4	Siqueira et al. (1982); Cunha et al. (2016); Albuquerque (2018)
Terpinolene	4.98	3.16	4	Oliveira (2012); Albuquerque (2018); Rostignoli (2019)
beta-Ocimene	10.29	4.44	3	Garcia et al. (2012); Oliveira (2012)
beta-Pinene	0.26	0.05	3	Cepeda et al. (2023); Zimmermann et al. (2021)
alpha-Cadinene	0.24	0.13	3	Cepeda et al. (2023)
beta-lonone	0.18	0.08	3	Cepeda et al. (2023)
Eugenol	0.16	0.06	3	Cepeda et al. (2023)

Table 4. Continued.

The existence of chemotypes of *T. minuta* is documented in the literature (Senatore et al., 2004; Tankeu et al., 2013) and may also occur in Brazil. Fonseca (2018) analyzed the phytochemical profile of 13 spontaneous populations of *T. minuta* from the State of Rio Grande do Sul and found a sample rich in cis-Ocimenone (11.35%) and car-3-en-2-one (10.16%), besides cis-beta-Ocimene (35.81%), Dihydrotegetone (25%) and cis-Tagetone (10.77%). In the other samples, the mean content of cis-Ocimenone and car-3-en-2-one was 0.12% and 0.27%, respectively. The composition of this sample is similar to the one from a chemotype described in Argentina by Gil et al. (2000).

It is also necessary to consider that variations in the composition of essential oils can occur due to different causes and at different moments, from the field to the laboratory. Among these factors are cultivation practices (Kumar et al., 2012; Rathore et al., 2018; Sood et al., 2020; Walia; Kumar, 2021a, 2021b), environmental conditions (Khare et al., 2020; Kumar et al., 2022), the ontogeny of the species (Tiwari et al., 2016; Kumar et al., 2020), post-harvest drying (Walia; Bhatt; Kumar, 2020), and extraction methods (Kant; Kumar, 2022). Additionally, differences among studies can also be attributed to the adopted procedures, analysis techniques, and identification of compounds (Rubiolo et al., 2010; Sadgrove et al., 2022; Syafri et al., 2022).

Nevertheless, taking into consideration the mean content of the major compounds from the essential oil of *T. minuta* obtained in Brazil, it is possible to notice the seasonal variation of the phytochemical profile in the main harvest months, when the plants are generally in full bloom or with mature fruits (Figure 6).

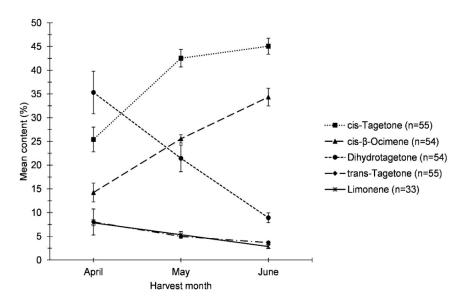


Figure 6. Seasonal variation of the major compounds of the essential oil of *T. minuta* harvested in Brazil. Mean of 18 studies; n = samples number. Vertical bars indicate a mean standard error.

The mean of the five major compounds represents 91%, 99%, and 95% of the essential oil composition in April, May, and June, respectively. With the development of flowering and the fruit maturation, the contents of cis-Tagetone and cis- β -Ocimene tend to increase, while those of Dihydrotagetone, trans-Tagetone, and Limonene tend to decrease. This behavior is similar to the one observed by Kumar et al. (2020) studying the relationship between the floral biology of *T. minuta* and the composition of the essential oil.

In principle, when the plant changes from the vegetative to the reproductive stage, the oil content of the leaves decreases and that of the flowers increases (Chalchat et al., 1995), to the point of containing more oil in the flowers than in the leaves (Oliveira, 2013; Mlala et al., 2018). The predominant compounds in the leaves are Dihydrotagetone and trans-Tagetone, while cis-Tagetone and cis- β -Ocimene prevail in the flowers (Chamorro et al., 2008; Oliveira, 2013). Thus, the composition of the essential oil of *T. minuta* changes throughout its development, mainly when the proportion of flowers increases in relation to the leaves.

Yield and productivity

In terms of essential oil yield, there are few references in Brazilian publications and, often, there is not enough information to understand the presented values. Data such as the characterization of the collection site, harvest date, cultivation practices (fertilization, spacing, etc.), the phenological stage of the plants, post-harvest processing (drying, storage, fractionation, etc.), the processed plant parts, the distillation method and even the moisture basis for estimating the yield (fresh or dry), are of extreme importance for comparative studies. Only four studies gathered the basic information to compose Table 5.

Table 5. Essential oil yield of *T. minuta* in Brazil, according to type and month of harvest, plant part, extraction method and moisture basis.

Author	Harvest type	Month	Plant part	Extraction method	Moisture basis	Sample number	Mean yield (%) (SE) ⁽¹⁾	Higher yield (%)
	Spontaneous	August	Flowers	Hydrodistillation	Dry	1	0.57	
Cepeda et al. (2023)	Spontaneous	March- April, August	Flowers	Hydrodistillation	Dry	4	1.04 (0.29)	1.87
Schiedeck (2023)	Spontaneous	April-May	Flowers, leaves and stems	Steam distillation	Fresh	9	0.63 (0.04)	0.81
Fonseca	Cultivated	May	Flowers and leaves	Hydrodistillation	Dry	14	3.31 (0.22)	4.46
(2018)	Cultivated	June	Flowers and leaves	Hydrodistillation	Dry	4	3.84 (0.33)	4.80
Oliveira	Spontaneous	April-May	Flowers	Steam distillation	Dry	1	2.09	
(2013)	Spontaneous	April-May	Leaves	Steam distillation	Dry	1	0.33	

(1) Standard error.

(...) Information not available.

The variation in essential oil yield also occurs in studies conducted in other countries. Walia et al. (2020) cultivated the Him Gold variety in 16 locations with different altitudes in India and the fresh weight yield ranged between 0.37% and 0.79%. In South Africa, the dry weight yield of essential oil from flowers and leaves of wild plants collected from 20 different locations ranged from 0.38% to 1.52% (Tankeu et al., 2013). In Turkey, different authors found dry weight yields of essential oil from flowers and leaves of wild *T. minuta* plants of 1.80% (Baser; Malyer, 1996 ; Bahdirli, 2020).

In general, the values obtained by Fonseca (2018) stand out from the others because they are much above the average. The composition of the

distilled sample may explain these results. The plants were harvested, and the leaves and flowers proportion was calculated. Then, 300 g of fresh mass were placed in the distillation flask, but maintaining the proportion observed in the plants. Neither stems nor stalks were used.

The study by Fonseca (2018) was also the only one that evaluated agronomic variables of *T. minuta* cultivation in Brazil. The plants harvested in May resulted a mean of 211 kg of essential oil per hectare. The comparison with other works is quite difficult due to the lack of standardization in the presentation of the results, however Table 6 provides information that can help to understand the productivity obtained in Brazil.

Table 6. Essential oil productivity of *T. minuta* obtained in Brazil by Fonseca (2018) compared to studies performed in other countries, considering the plant densities, the distilled parts and the moisture basis. All studies used plants in full bloom and extracted the oil by hydrodistillation.

		Spacing					Essen	tial oil	
Author	Country	between plants and rows (cm)	Plants per hectare	Plant part	Moisture basis	Biomass (ton ha ⁻¹)	(%)	(kg ha⁻¹)	Note
Fonseca (2018)	Brazil	20 x 25	200.000	Leaves and flowers	Dry	6.40	3.310	190.66 ⁽¹⁾	Average of 14 samples; oil volume adjus- ted for mass by factor 0.9
Singh et al. (2008)		30 x 30	111.111	Leaves and flowers	Dry	8.15	0.870	61.50	_
	al. India	30 x 45	74.074	Leaves and flowers	Dry	6.45	0.900	51.10	_
		30 x 60	55.556	Leaves and flowers	Dry	4.82	0.980	41.90	_
Kumar et al.	India	30 x 30	111.111	Leaves and flowers	Fresh	8.20	1.584	110.00(1)	_
(2012)	muia	30 x 30	111.111	Leaves and flowers	Fresh	18.48	1.194	180.00(1)	-
		30 x 45	74.074	Leaves, flowers and stems	Fresh	9.54	0.676	45.96	_
Kumar et al. (2014)	India	45 x 45	49.383	Leaves, flowers and stems	Fresh	6.43	0.740	38.38	_
		45 x 60	37.037	Leaves, flowers and stems	Fresh	4.86	0.686	27.44	_

		Spacing		Plant nart			Essen	tial oil	
Author	Country	between plants and rows (cm)	Plants per hectare		Biomass (ton ha⁻¹)	(%)	(kg ha⁻¹)	Note	
Pal et al. (2023)	India	8.3 x 60 ⁽¹⁾	200.706	Fresh plants	Fresh	28.13	0.330	83.55(1)	Oil volume ad- justed for mass by factor 0.9
Marotti et al. (2004)	Italy	50 x 50	40.000	Leaves	Fresh	26.00	0.660(1)	154.44(1)	Oil volume ad- justed for mass by factor 0.9
Sood et al. (2020)	India	30 x 45	74.074	Leaves and flowers	Fresh ⁽¹⁾	8.87	0.378(1)	30.18 ⁽¹⁾	Oil volume ad- justed for mass by factor 0.9

Table 6. Continued.

⁽¹⁾ Information obtained by inferences or estimates from the details available in the texts and graphs of the respective studies.

(-) Not applicable.

Although Fonseca (2018) did not use fertilizers, the study was conducted in an area where corn and beans were usually cultivated with organic fertilization, and the soil organic matter content was close to 2%. The effect of organic and chemical fertilizers on the increasing of the essential oil yield of *T. minuta* is documented in several studies (Singh; Rao, 2005; Pandey et al., 2015; Walia; Kumar, 2021a). Moreover, the density adopted was 1.8, which means 5.4 times greater than the recommendation in other countries. According to some studies, increases in plant densities provide greater plant biomass production and higher essential oil yield (Singh et al., 2008; Kumar et al., 2014; Pal et al., 2023).

The high plant population in the Brazilian study and the manual weeding performed after transplanting the seedlings may have reduced the competition with weeds during the development cycle of *T. minuta*, contributing to the high essential oil yield. Walia and Kumar (2021b) found that weed control practices can provide essential oil yields 3.4 times higher than those obtained without any control.

Another explanation may lie in the climatic conditions under which the work of Fonseca (2018) was carried out. The experimental area of Embrapa Temperate Agriculture, in Pelotas, state of Rio Grande do Sul, is located at an altitude of 180 m. The seedlings were transplanted on January 18th and had a mean cycle of 119 days, with harvest taking place between May 4th and 31st, when the plants had a mean leaf area index of 3.20. During this period, the average, maximum and minimum air temperatures were 20.4°C, 26.7°C and 16.1°C, respectively. The accumulated rainfall during the period was 522 mm, distributed on average every 3.4 days and 22

mm per week. Daily photossyntetic active radiation ranged from 825 μ mol s⁻¹ m⁻² to 290 μ mol s⁻¹ m⁻², with sunshine duration in average of 6h48. In Palampur, India, where *T. minuta* is cultivated, the accumulated rainfall between June and September can reach over 2,600 mm, with up to 220 mm per week, reducing sunshine hours to less than 3 hours per month on average (Walia; Kumar, 2021b, 2021a).

Final remarks

Brazil has never been one of the world's largest producers of *T. minuta* essential oil. This information is incorrect and stems from a mistake made by Craveiro et al. (1988) when citing the origin of a commercial sample from a previous work. This error led subsequent authors to believe that there were commercial *T. minuta* crops in Brazil. The lack of fact checking has allowed the perpetuation of this misconception over time. Nevertheless, contradictorily, the country is one of the largest producers of scientific knowledge about the plant and the potential uses of its essential oil.

The chemical composition of *T. minuta* essential oil obtained in Brazil is well documented in articles, and other types of publications, and is characterized by high levels of cis-Tagetone, cis- β -Ocimene and Dihydrotagetone. The proportion between the major compounds varies according to the harvest time.

Despite the limited number of studies focusing on the cultivation of the species in Brazil, the essential oil yield and productivity suggest that the country has the potential to produce volumes comparable to those obtained in India and other countries, possibly due to the high planting density used and the favorable environmental conditions.

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