



Chemical Composition and Antibacterial Activity of the Essential Oil from *Tagetes erecta* L. Grown in Northeastern Brazil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MMMM and ACNS designed, planned, and carried out the experiments and performed the statistical analysis. Authors SMDM and MIFG acquired funding, administered the project, and participated in review and editing of the manuscript. Authors SMDM and RODSF analyzed the data and contributed to the drafting and editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Faced with the increase in microbial infections linked to the phenomenon of drug resistance, research with natural products is promising. *Tagetes erecta* L., known as "cravo-de-defunto" in Brazil, is an ornamental plant used in folk medicine as an antimicrobial and to treat skin infections, fever, and digestive disorders. This study aimed to describe the chemical composition and report the antimicrobial activity of *T. erecta*.

Study Design: The medicinal plant was collected in the state of Ceará, Brazil, followed by extraction of the essential oil, using the hydrodistillation method. This study was analyzed by gas chromatography/mass spectroscopy to the essential oil. In addition, agar-well diffusion, and broth microdilution methods to the antimicrobial assays.

Place and Duration of Study: Ceara State University, Fortaleza, Brazil, between 2016 and 2018.

Methodology: *T. erecta* essential oil was obtained by hydro-distillation in a modified Clevenger type apparatus and analyzed by gas chromatography/mass spectroscopy. The antimicrobial activity was performed by agar-well diffusion and broth microdilution methods. The minimum inhibitory concentration (MIC) was determined against strains of Gram-positive and negative bacteria.

Results: Fourteen components, representing 87.99% of the oil, were identified. Monoterpenoid ketones represented the main fraction with piperitone (45.72 %) as the major constituent. The agar well-in method using EO exhibited high activity against *E. faecalis*, *S. aureus*, *S. epidermitis*, *S. pyogenes*, *E. coli* and *P. mirabilis*. EO from *T. erecta* was more active against Gram positive than Gram negative bacteria.

Conclusion: The essential oil of *T. erecta* showed strong antibacterial activity against important human pathogenic Gram positive and Gram-negative bacteria probably due to the antibacterial compound piperitone, which is present in high yield, nevertheless synergism could occur with other minor active constituents. In silico molecular docking studies are needed to investigate possible pharmacological mechanisms of action.

Keywords: *Tagetes erecta* L.; piperitone; antimicrobial effect; essential oil.

1. INTRODUCTION

"Bacterial resistance to antimicrobial agents has emerged over the past few decades and has been a major problem for the treatment of infectious diseases. There is a continuous need to develop novel antimicrobial agents to minimize the phenomenon of drug resistance" (Upadhayay et al., 2023). Recently, the acceptances of traditional medicine as an alternative form for health care have led researchers to investigate the antimicrobial activity of medicinal plants. Ethnobotanical and ethnopharmacological studies are essential in the prospection of bioactive substances with pharmacological potential. (Sobrinho et al., 2017). In particularly, the results of studies with essential oil (EO) have been especially promising. Antimicrobial properties of EO have formed the basis in diverse commercial products, such as dental root canal sealers, antiseptics, and feed supplements for lactating sows and weaned piglets (Calo et al., 2015). Emergence of drug resistant pathogenic strains motivated the use of therapeutic alternatives, including essential oils and other secondary plant metabolites (Keita et al., 2022).

Essential oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives, as well as other volatile compounds as phenylpropenes and specific sulphur- or nitrogen-containing substances (Miri, 2025), being considered as potential sources for the screening of antimicrobial agents (Barros et al., 2015). According to Chrysargyris et al. (2020), antimicrobial activity of essential oils depends on their chemical composition, determined by the genotype of each plant with influence of environmental and the agronomic conditions.

Tagetes erecta L. (Asteraceae) in Brazil, it is known as "cravo-de-defunto", is native of Mexico, its natural range extends from southwestern United States into Argentina, and it has been used as an ornamental plant and in traditional medicine. This species originated in Mesoamerican cultures, with multiple uses in herbal medicine, ritual plant, ornamental, and feed product (Estrada et al., 2025). Various folk traditions have used the herb for a variety of medicinal purposes, the leaves are used to prepare a bath to treat fever (Giovannini & Heinrich, 2009), stomach pain, bronchitis,

erysipelas and in wounds (Andrade-Cetto, 2009) and the flowers are used externally in the treatment of skin infections (Lopez *et al.*, 2001). The topical medicinal use of this species has been documented in India for earache and eye infections, in China for conjunctivitis and mumps and in Belize for fever, common cold, flu and diarrhea (Giovannini & Heinrich, 2009). A series of studies have demonstrated the potential medicinal effect of *T. erecta*, such as antiinflammatory (Khan, 1999), nematicide (Natarajan *et al.*, 2006), insecticidal (Sarin, 2004), larvicide (Pathak *et al.*, 2000), herbicidal (Laosinwattana *et al.*, 2018) and antimicrobial against strains of Gram-positive and negative bacteria and fungi strains by agar-well diffusion method (Grover & Rao, 1978).

The chemical composition of EO from *Tagetes erecta* L. growing in Northeastern Brazil has been reported (Machado *et al.*, 1994), but no study on the antibacterial potential, thus of the aim of this study was identify the antimicrobial potential and correlate the chemical composition with antibacterial activity of essential oil (Craveiro *et al.*, 1976).

2. MATERIALS AND METHODS

2.1 Plant Material

Tagetes erecta (L.) was collected in the Medicinal Plants Garden of the Ceará State University, Brazil. Taxonomic identification was confirmed by botanist Ligia Queiroz Matias of the Prisco Bezerra Herbarium (Ceará Federal University, Brazil), where a voucher sample was deposited with a reference number 35.649.

2.2 Essential Oil Extraction and Analysis Procedure

"The EO was extracted from the leaves and stems (2 Kg) of *T. erecta* by hydro-distillation for four hours in a modified Clevenger type apparatus" [16]. The oil was dried over anhydrous sodium sulphate and, after filtration, stored at 4°C until and analyzed. All the essential oils were kept in tightly stoppered bottles in a freezer until used for biological tests. "The EO was analyzed by gas chromatography-mass spectrometry (GC/MS) to identify their components. GC/MS was performed using a Hewlett-Packard 5971 instrument employing C; the following conditions: column (30 m x 0,25 mm); carrier gas: He (1 ml/min); injector temperature: 250°C; detector temperature:

200°C; column temperature: 35-180°C at 4°C/min then 180-250°C at 10°C/min; mass spectra: electron impact 70 eV. The identification of the constituents was performed by computer library search" (NIST), retention indices and visual interpretation of the mass spectra (Adams, 2012). The identified constituents are listed in their order of elution from a non-polar column, calculated by linear interpolation relative to retention times of a series of n-alkanes.

2.3 Microorganisms Tested

A total of nine bacterial strains (*Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus epidermidis*, *Streptococcus pyogenes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*) were grown in Brain Heart Infusion (BHI) broth at 37°C and maintained on slopes of Mueller Hinton agar (MHA) at 4°C. Antimicrobial assay was carried out according to M7-A9 guidelines of Clinical and Laboratory Standards Institute (CLSI, 2012).

2.4 Screening for Antibacterial Activity

2.4.1 Well-in agar method

The inoculum suspension, 1×10^8 colony forming unit (cfu/ml), was spread uniformly over the agar, plates using sterile glass rod spreader, to get uniform distribution of bacteria. Subsequently, using a sterile borer, a well of 0,6 cm diameter was made in the inoculated media. The EO were weighed and dissolved in dimethylsulphoxide (DMSO) followed by sterilization using a 0.45 µm membrane filter. Addition of 20 µl (100 mg/ml) of oil was aseptically filled into the well. "Later the plates were placed at room temperature for an hour to allow diffusion of EO into the agar. Then the plates were incubated for 24h at 37°C. The results were recorded by measuring the diameter of the inhibition zone at the end of 24h". All tests were performed in triplicate (Zhang *et al.*, 2016).

2.4.2 Disc diffusion method

Briefly, a suspension of the tested microorganism (10^8 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µl of the oil placed on the inoculated plates. These plates, after staying at 4°C for 2h, were incubated at 37°C for 24h. Amoxicillin (30 µg/disc) was used as positive control. The diameters of the inhibition zones were performed in triplicate (CLSI, 2012).

2.4.3 Microdilution assays

The minimal inhibitory concentration (MIC) of *T. erecta* oil against bacterial strains was determined based on a micro-well dilution method (20). The 96-well plates were prepared by dispensing 95 μ L of Müller Hinton broth (MHB) and 5 μ L of the inoculum into each well. A 100 μ L aliquot of the oil initially prepared at the concentration of 500 μ g/ml was added into the first wells. Then, 100 μ L from their serial dilutions was transferred into ten consecutive wells. The last well containing 195 μ L of nutrient without compound and 5 μ L of the inoculum on each strip was used as negative control. "Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 620 nm and confirmed by plating 5 μ L samples from clear wells on nutrient agar medium". "The MIC of each extract was taken as the lowest concentration that showed no growth" (CLSI, 2012; Sugumar et al., 2014).

2.4.4 Statistical analysis

The antibacterial activity was performed by linear correlation for individual analysis and the two-tailed Student's t-test ($p < 0.05$). All experiments were performed in triplicate.

3. RESULTS AND DISCUSSION

The hydrodistillation of the aerial parts of *T. erecta* yielded 0.06% oil, based on the dry weight of the plant parts. The chemical constituents of this oil are shown in Table 1. A total of 14 components were identified, representing 87.99% of the oil. The main constituents of the oil were the monoterpenoid ketones, piperitone (45.72%) and piperitenone (5.89%). Other monoterpenoids such as D-limonene (9.67%), p-cymen-8-ol (6.08%), and myrtenol (5.86%) were also identified. The low relatively amounts of sesquiterpene consisted mainly of β -caryophyllene (3.35%) and caryophyllene oxide (3.51%). Similar composition was observed between EO from leaves of *T. erecta* in Nigeria (Ogunwande & Olawore, 2006), India (Krishna et al., 2004), and Italy (Piccaglia et al., 1997). On the other hand, an early study identified monoterpene piperitone as the main constituent in *T. erecta* from the northeastern region of Brazil (Machado et al., 1994).

Piperitone, main constituent found in our study, is an oxygenated monoterpene with biologically active, being widely used in the cosmetics

industry and perfumery as fragrance (Abdelgaleil et al., 2008; Gulluce et al., 2004; Yaguchi et al., 2009). It is a main component of essential oil from *Eucalyptus dives* (Delaquis et al., 2002; Weber et al., 2006), *Micromeria fruticosa* (Gulluce et al., 2004) and *Mentha longifolia* (L.) L. (Ghazizadeh et al., 2025).

The antibacterial activity of *Tagetes erecta* EO measured by agar well-in agar and disc diffusion is summarized in Table 2. A repertory of nine bacterial strains (four Gram positive and five Gram negative) was used in this study. The agar well-in method using EO exhibited high activity against *E. faecalis*, *S. aureus*, *S. epidermitis*, *S. pyogenes*, *E. coli* and *P. mirabilis*. A low activity was observed against *E. aerogenes*, *K. pneumoniae* and *P. aeruginosa*. Nevertheless, when the disc diffusion method was used, we observed only a low activity against *S. aureus*, *E. aerogenes* and *K. pneumoniae*. According to the results, the well-in agar method was better suited for studying antimicrobial activity of EO than disc diffusion method and this finding is in accordance with the work presented by Natarajan et al. (2005). The data also showed that EO from *T. erecta* was more active against Gram positive than Gram negative bacteria. This can be due to the membrane structural of Gram-negative bacteria, where the outer membrane is rich in lipopolysaccharide molecules, almost impermeable to lipophilic compounds, presenting a barrier to the penetration of the antimicrobial substances contained in the EO, as well as the enzymes associated in the periplasmic space, which can break down the antimicrobial substances introduced from outside (Zgurskaya et al., 2018).

A previous study evaluated the antibacterial action of four different extracts of *T. erecta* against strains of *Staphylococcus aureus* and *Escherichia coli*. Among all the extracts evaluated, the dichloromethane extract was most effective in inhibiting bacterial growth (Burlec et al., 2019).

Gram-negative bacteria *P. aeruginosa* is known to have a high level of intrinsic resistance against many antimicrobials and antibiotics due to very restrict outer membrane barrier, being highly resistant even to synthetic drugs (Breidenstein et al., 2011). As shown in Table 2, *P. aeruginosa* has a modest sensitivity to the EO of *T. erecta* when analyzed using the well-in agar method, indicating that this compound can be used as an alternative supplement for treatment of highly resistant bacteria.

Table 1. Percentage composition of *Tagetes erecta* L. aerial parts essential oil

Constituents	RI*	% Yield
Monoterpenes		
Thujene	968	0.56
D- limonene	1029	9.67
(Z)- β -cis-Ocimene	1037	0.58
Terpinolene	1089	0.65
β -linalool	1097	0.52
p-cymen-8-ol	1183	6.08
Myrtenol	1196	5.86
Piperitone	1250	45.72
Piperitenone	1343	5.89
Sesquiterpenes		
β -Caryophyllene	1416	3.35
Trans-nerolidol	1563	1.53
Spathulenol	1574	1.31
Caryophyllene oxide	1580	3.51
Diterpene		
Phytol	1943	2.76
Total		87.99

*The retention indices (RI) of the compounds were estimated by linear regression using the Kovat index from the NIST library and the retention times of the main compounds

Table 2. Antibacterial activity of the essential oil of *Tagetes erecta*

Bacterial species	Source N°	Zone of inhibition			MIC values
		EO Disc diffusion	EO Well-in agar	Amoxicillin	EO
Gram-positive					
<i>Enterococcus faecalis</i>	ATCC 29212	-	18	35	125
<i>Staphylococcus aureus</i>	ATCC 9144	10	28	45	31.2
<i>Streptococcus epidermidis</i>	ATCC 12228	-	19	26	125
<i>Streptococcus pyogenes</i>	ATCC 19615	12	38	48	7.8
Gram-negative					
<i>Enterobacter aerogenes</i>	ATCC 13048	-	12	-	250
<i>Escherichia coli</i>	ATCC 35218	9	17	-	125
<i>Klebsiella pneumoniae</i>	ATCC 13883	7	13	-	250
<i>Proteus mirabilis</i>	ATCC 25933	-	16	27	125
<i>Pseudomonas aeruginosa</i>	ATCC 15442	-	9	-	>500

(-) inactive; (7-13 mm) moderately active; (>14 mm) highly active
 Zone of inhibition: essential oil (100mg/ml); amoxicillin (30 μ g/disc)
 MIC: minimum inhibitory concentration (μ g/ml)

The results of the minimum inhibitory concentration (MIC) shown in Table 2 confirm that EO of *T. erecta* were in the range of 7.8-500 μ g/ml, being *S. pyogenes* and *S. aureus* the most sensitive microorganisms.

The antimicrobial activity of EO of *T. erecta* reported in this study can be attributed to the

presence of a high concentration of piperitone. This argument might be supported by previous work where oils rich in this compound show a high antimicrobial activity (Mahboubi & Haghi, 2008; Gilles *et al.*, 2010). Shahverdi *et al.* (2004a) demonstrated that the antimicrobial activities of both furazolidone and nitrofurantoin, synthetic antibacterial drugs, were increased by

piperitone. Piperitone exhibited antibacterial action against Gram-positive than Gram-negative bacteria (Delaquis *et al.*, 2002), and antifungal activity against plant pathogenic filamentous fungi by broth microdilution method (Abdelgaleil *et al.*, 2008). Piperitone from *Mentha longifolia* produced antibacterial action against nitrofurantoin-resistant strains of Enterobacteriaceae bacteria to the disk diffusion method (Shahverdi *et al.*, 2004b). These results corroborate our findings, since piperitone is the major constituent of *Tagetes erecta* essential oil.

On the other hand, other components such as limonene, piperitenone, β -caryophyllene have been reported previously to have antibacterial activity (Dahham *et al.*, 2015; Umagiliyage *et al.*, 2017) and could be also responsible for this activity. Some studies have concluded that whole EO has a greater antibacterial activity than the major components isolated, suggesting that the minor components are essential for activity and may have a synergistic effect with the other compounds present in the essential oils (Dahham *et al.*, 2015).

4. CONCLUSION

The essential oil from *Tagetes erecta* showed high content of piperitone and an excellent antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus epidermitis*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus mirabilis*, important human pathogenic Gram-positive and Gram-negative bacteria, probably due to the important antibacterial compound piperitone, which is present in high yield, nevertheless synergism could occur with other minor active constituents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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