Chapter

Bioactive Compounds and Antioxidant Activity of Essential Oil of Species of the Genus Tagetes

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Abstract

This study investigated the bioactive compounds and antioxidant activity of the essential oil of two species of the genus Tagetes (*Tagetes minuta L*. and *Tagetes elliptica Sm*). The essential oil was obtained by steam distillation, and its extraction performance, relative density, refractive index, and solubility in ethanol (70% v/v) were determined. The chemical components were evaluated by gas chromatography coupled to mass spectrometry (GC-MS). Antioxidant activity was determined by the free radical 2,2-diphenyl-1-picrylhydrocyl (DPPH) method and the trapping capacity of the ABTS*+ radical cation. In the essential oils of the species Tagetes, it was possible to identify 26 chemical components for the species *Tagetes elliptica* Sm. and 16 for *Tagetes minuta* L., both species presented as main components monoterpenes (61%) and sesquiterpenes (44%). The compounds found were β-myrcene, trans-tagetone, β-trans-ocimene, and β-caryophyllene. Essential oils showed a variation in extraction yields and density. The refractive index was higher in the species *Tagetes elliptica* Sm., finding a high solubility in both species. A variation was found between 1.77 and 2.56 mg/mL of antioxidant activity by the DPPH method and 21.02–41.06 mg/mL for ABTS*+. The essential oils of the species *Tagetes elliptica* Sm.y and *Tagetes minuta* L. have bioactive components with antimicrobial and antioxidant potentialities for use for food preservatives.

Keywords: chromatography, density, monoterpenes, sesquiterpenes, solubility

1. Introduction

Peru is one of the 12 countries with the greatest biological diversity, with approximately 10% of the world's flora, estimated at 25,000 species, 30 of which are endemic [1]. There is a growing interest in bioactive compounds and the antioxidant properties of substances from natural sources that can potentially be used in food industries. Essential oils from aromatic and medicinal plants are known to possess biological activity [2, 3]. Essential oils are natural plant products that contain a complex mixture and therefore have multiple antimicrobial properties [4]. To be the constituents of the most important groups of raw materials for the food, pharmaceutical, perfumery, and related industries [5]. Most of these compounds are derived from oxygenated terpenoids, particularly phenolic terpenes, phenylpropanoids, and alcohols [6, 7]. Tagetes species were originally used as a source of essential oils that were extracted from leaves, stems, and flowers, being applied as flavorings in the food industry; in addition, their pigments have potential as a natural food colorant.

Tagetes is an important genus belonging to the family Asteraceae [8], aromatic, native to Central and South America with a cosmopolitan distribution due to anthropic activities [9]. *Tagetes minuta* L. is an aromatic plant with a broad spectrum of biological activity that has medicinal, antioxidant, and antimicrobial properties [10]. The great importance of Tagetes is due to the presence of essential oil in almost all parts of its plants, except in the stem [11]. It has biological activities such as antibacterial, antifungal, antiviral, antioxidant, anticancer, acaricide, nematicide, insecticidal, and allelopathic activities [12]. The growing interest in the food, flavor, and perfumery industries contributes to the investigation of environmental conditions affecting qualitative composition and yield [13].

Tagetes minuta L. is known by the common name of "huacatay" in Peru; in Mexico, it is known as "Mexican marigold" [14]. It is a species that accumulates a long world history of uses such as food, therapeutics, and aromatherapy that are inherent in the unique chemistry of the plant, its composition, and bioactivities. According to the research background review on bioactive metabolites and antioxidant activity of the aromatic species *Tagetes minuta* L. and *Tagetes elliptica* Sm., no publications are reported in our country; however, there are many reports on essential oils of these species in other countries [15].

Despite their importance as food species, research on the species *Tagetes minuta* L. and *Tagetes elliptica* Sm. in terms of chemical composition, genetic diversity, and biological properties is limited. Therefore, the objective was to determine the physical properties and identify the bioactive components and antioxidant activity of the essential oils of both species of the genus Tagetes that grow wild and are adapted to moderate-altitude ecosystems of the Andean region of Peru.

2. Materials and methods

2.1 Plant matter and botanical identification

The sheets of *Tagetes minuta* L. and *Tagetes elliptica* Sm were used. The samples were collected from the high Andean zone of the district of José María Arguedas (13°42 S.73°24 W at an altitude of 2935 m above sea level) belonging to the province of Andahuaylas, Apurimac region. With climate Cwd according to Koppens with average annual rainfall around 1000 mm/year, average relative humidity of 50% and temperature of −5 to 21°C, with moderate incidence of frost. The sample sheets were collected

during the months of February to March 2019. The plants were identified and authenticated by Dra. María del Carmen Delgado Laime and deposited in the botany laboratory of the Basic Sciences pavilion of the José María Arguedas National University.

2.2 Essential oil extraction

For the extraction of essential oils, fresh leaves of *Tagetes minuta* L. were selected and *Tagetes elliptica Sm.*; 2.5 kg of fresh leaves of each species were used and subjected to extraction by distillation by dragging water vapor at a pressure of 10 psi. Once distilled, the essential oils were separated by difference in densities using a graduated Florentine decanter. Then dried in anhydrous sodium sulfate and stored at 4°C until the time of analysis, extraction yields were evaluated according to (Eq. 1).

$$\%P = \frac{Masafinaldeaceiteesencial\left(g\right)}{Masainicialdemuestraofollaje\left(g\right)} * 100 \quad (1)$$

2.3 Determination of the physical properties of the essential oil

In the essential oils obtained from each species, the relative density at 20°C was determined according to the Peruvian technical standard: NTP 3129.081:1974; refractive index in the ABBE refractometer; optical rotation in polarimeter and solubility in ethanol. For the latter, a 70% solution was used taking 100 μL of essential oil.

2.4 Determination of chemical compounds by gas chromatography coupled to mass spectrometry (GC-MS)

The analysis of the chemical composition of essential oils was identified by gas chromatography coupled to mass spectrometry (GC-MS) at the natural products research center of the Universidad Peruana Cayetano Heredia.

For the analysis of each sample, 20μL of essential oil in 980 μL of dichloromethane was used, which was injected into the gas chromatograph coupled to a selective mass detector. The compounds were separated in a mixture by an apolar capillary column DB-5MS (60 m × 250 μm × 0.25 μm) (J and W Scientific of 5% phenyl-polymethylsiloxane).

The temperature of the injector was maintained at 250°C with an injection in Split mode (50:1), the programming of the furnace temperature was: initial temperature 50°C, maintained for 5 mins; then increasing to 10°C/min to reach 100°C and finally to 10°C/min to 270°C, maintaining the final temperature for 1 min. The execution time was 77.8 mins, helium was used as a drag gas at a constant flow of 1 ml/min. The compounds of Tagetes oils *minute* L. and *Tagetes elliptica* Sm. were identified using software provided by Agilent; MSD chemstation (verse EO2.00.493), by comparing the mass spectra of each peak with those of the mass spectra library of the flavor databases and the National Institute of Standards and Technology (NIST, 08).

2.5 Evaluation of the antioxidant activity of essen0tial oils

For the determination of the antioxidant activity of the essential oils of the species of the genus Tagetes, two methodologies were used:

2.5.1 DPPH radical method

Aqueous ethanol dilutions of hydroalcoholic extracts were prepared to obtain concentrations of 0.0–150.0 μg/mL. About 1.0 mL of each dilution was combined with 0.5 mL of a 0.3 mM solution of DPPH in ethanol and allowed to react at room temperature for 30 mins, then the absorbance of the mixtures at 517 nm was measured in the spectrophotometry equipment. The percentage of antioxidant activity of each sample was calculated according to the following (Eq. 2):

$$Actividad\ Antioxidante\,(\%) = \frac{MAC - AM - AB(g)}{AC} X\,100 \qquad (2)$$

AM: is the absorbance of the sample + DPPH,
AB: is the absorbance of the target (sample + ethanol),
AC: is the absorbance of the reactant target (DPPH + ethanol).

The concentration of the hydroalcoholic extract was neutralized at 50% of the DPPH radicals (EC_{50}, mean effective concentration) and was obtained directly by drawing the line between the percentage of antioxidant activity, compared with the concentration of the sample of essential oils mg/mL.

2.5.2 Radical method ABTS+*

The $ABTS^{+}$ free radical scavenging activity was determined by the method developed by Re et al. (1999), with some modifications.

About 3.5 mM of ABTS was reacted with 1.25 mM of potassium persulfate. The samples were incubated at temperatures of 2–8°C for 16–24 h in darkness. The formed ABTS*+ radical is diluted with ethanol to an absorbance of 0.7+ minus 0.05 to 734 nm. At a volume of 190 μL dilution of the ABTS*+ radical A, 10 μL of the AE sample was added and incubated at room temperature for 5 mins. After the time it took to determine by means of the spectrophotometer equipment at 734 nm in the Themoscientific microplate reader. For the positive control of the absorption of A radicals ABTS*+, ascorbic acid (4 μg/mL) was used.

2.6 Statistical analysis

The analyses were performed in triplicate, for the statistical evaluation, the completely randomized design (DCA) was used; The analysis of variance was worked with 0.05 significance; upon finding a significant difference, the Fisher's mean comparison test (LSD) was performed at a level of $\alpha = 0.05$. The data were processed with the help of the statistical programs Centurion XVII and the Microsoft Excel 2016 spreadsheet.

3. Results

3.1 Performance and physical properties of essential oils

The determination of the physicochemical properties allows us to know the quality control and purity in essential oils.

Table 1 shows the percentage of extraction yield and the physical properties of the essential oils of both species of the genus Tagetes. Where:, a is different from b.

3.2 Chemical composition of essential oils of two species of the genus Tagetes

The main components of the essential oils of both species of the genus Tagetes are shown in **Table 2**.

Retention time (TR) and relative abundance (%) of essential oils, Not detected (ND).

In the analysis of the chemical composition, a total of 26 chemical compounds were detected and quantified in the essential oil of *Tagetes elliptica* Sm., with main fraction in monoterpenes in (61.00%) and 16 chemical compounds for the essential oil of *Tagetes minuta* L. being found as the main fraction to the monoterpenes (50.0%); between both species, a standard deviation below 5% was obtained between the percentages of each analyte in both columns used. They were identified as bioactive compounds in essential oils in species of the genus Tagetes to β-trans-ocimene, trans-tagelone, cis-tagelone, β-myrcene, and β-caryophyllene.

Analysis	***Tagetes minuta* L.**	***Tagetes elliptica* Sm.**
Performance (%)	0.05 ± 0.002^{to}	0.048 ± 0.001^{to}
Density (g/mL) at 24°C	0.900 ± 0.0004^{to}	0.882 ± 0.0043^{B}
Refractive index at 24°C	1.93 ± 0.05^{to}	1.482 ± 0.04^{to}
EtOH solubility 70% (v/v)	Positive	Positive
Specific gravity at 20°C	$0.872^{to} \pm 0.01$	0.945 ± 0.034^{b}

Table 1.
Performance and physical properties of the essential oils of Tagetes minuta *L. and* Tagetes elliptica *Sm.*

Compound	**% relative abundance, (TR. %)**	
	***Tagetes minuta* L.**	***Tagetes elliptica* Sm.**
β-transocimeno	21.07 (25.03)	16.5 (11.45)
β-Myrcene	ND	15.01 (2.78)
β-Linalool	ND	18.56 (1.18)
Cis-Tagetone	25.6 (3.5)	20 (16.27)
M-tert-butyl-phenol	ND	22.6 (1.44)
Trans-Tagetona	25.94 (51.37)	20.22 (10.25)
β-caryophyllene	36.39 (0.48)	28.21 (1.17)
Guaiol	41.96 (1.25)	ND
Apiol	42.45 (3.28)	33.41 (0.43)
α-Bisabolol	44.29 (1.1)	ND

Table 2.
Main components detected in the essential oils of Tagetes minuta *L. and* Tagetes elliptica *Sm*.

Essential oil	Methods	
	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)
Tagetes minuta L.	1.77 ± 0.02	21.02 ± 0.14
Tagetes elliptica Sm.	2.56 ± 0.12	0.06 ± 41.23

Table 3.
Antioxidant activity by DPPH and ABTS methods.

3.3. Antioxidant activity of AE of *Tagetes minuta* L. and *Tagetes elliptica* Sm

The antioxidant activity of the essential oil, evaluated by the DPPH and ABTS methods, is shown in **Table 3**.

Significant differences were found in the antioxidant activity of both Tagetes samples as shown in **Table 3**. According to the DPPH methodology, CI_{50} varied from 1.77 to 2.56 mg/mL; however, the CI_{50} of ABTS*+ varied from 21.02 to 41.06 mg/mL, finding a higher antioxidant activity the value of CI $_{50}$ 41.06 mg/mL. *Tagetes* essential oil had a lower CI_{50} of 1.77 mg/mL, respectively, exhibited considerable DPPH radical scavenging activity compared with ABTS*+ method A.

4. Discussion

The essential oils of *Tagetes minuta* L. and T*agetes elliptica* Sm. did not show significant differences in the percentage of performance. The yield of the essential oil depends on the plant and the district where it is grown [16]. According to the results of the physical properties of the essential oil, the density showed a variation for both species of the genus Tagetes; however, the refractive index did not show a variation between both species. The presence of a lower refractive index and density value is related to an amount of phenols [17].

The refractive index of both species presented high values, which indicate the presence of high-molecular-weight compounds such as sesquiterpenes and diterpenes and eventually oleoresins in high concentrations [18], being also indicative of essential oils of higher quality and purity.

According to the results of specific gravity of the essential oils of both species, significant differences were found with a presence of higher quality (0.945 ± 0.034) in the essential oil of *Tagetes elliptica* Sm., finding similar values obtained according to previous studies [19].

The analysis of the chemical components in the essential oils of the species *Tagetes minuta* L. *and Tagetes elliptica* Sm. showed mainly the presence of the following compounds: Trans-Tagetone, β-trans-Ocimene, Cis-Tagetone, β-Caryophyllene, and Apiol.

The essential oils of the species *Tagetes spp*. are rich in monoterpene hydrates (Ocimenes, limonene, terpinene, myrcene, and acyclic monoterpene ketones (tagetone, dihydrotagetone, and tagetenone), which are the main odors in addition to smaller amounts of sesquiterpene hydrocarbons oxygenated compounds [20].

According to the results of the study in species *of Tagetes patula*, strong bioactivity was found in its essential oils against pathogenic test organisms, which is attributed to the presence of terpinolene, E-karyophene, Z-tagetone, E-tagetone, Caryophene oxide, and Germacrene D.

Regarding its bioactivities of the species family of the genus Tagetes, strong-to-mild antibacterial activity was found against strains of large-positive and large-negative bacteria tested in the study [4].

Regarding its applications of the essential oil according to the presence of metabolites, it was found that the metabolites synthesized by plants of the genus Tagetes show significant effects as antioxidants, enzyme inhibitors, precursors of toxic substances, and pigments. The activity of secondary metabolites in species of the genus Tagetes is thought to be related to their composition, concentration, and environmental conditions affecting their content.

Essential oils obtained from different parts of the plant may show different biological capabilities and can therefore be used in a variety of industries, such as cosmetics, pharmaceuticals, or food production [21].

According to [22], I report the antimicrobial activity of the essential oils of *Tagetes minuta* L. against phytopathogenic bacteria, *Pseudomonas savastanoi* pv, and *Phaseoli axonopodis* pv, which are responsible for different plant diseases.

The results indicated that *Tagetes spp*. plays a role of great importance for the preparation and preservation of food, considered as an excellent source of food spice. Even from a traditional point of view, the nature of *Tagetes spp*. and its composition affect the quantity and quality of extracts [23]. Despite the promising results obtained in vitro, more detailed studies of the mechanisms of action of the extracts and essential oils of *Tagetes spp*. would be beneficial to reach its potential in biotechnology. It was documented that the components of essential oils, especially terpenoids such as dihydrotagetones, tagetones, and ocymenones, were sufficient to explain the observed antimicrobial activity [24].

The difference in antioxidant activity between the two samples could be attributed to the presence of monoterpenes in their polyphenolic compounds, and oxygenated monoterpenes lead to increased antioxidant, antibacterial, and antifungal activities [25–27].

5. Conclusions

In this study, it was possible to determine the bioactive metabolites of the essential oils of the species of *Tagetes minuta* L. and *Tagetes elliptica* Sm., finding greater abundance of the bioactive metabolites: β-trans-ocimene, trans-tagelota, cis-tagelone, β-myrcene, and β-caryophyllene being the monoterpenic acyclicos, with significant effects as antioxidants, enzyme inhibitors, precursors of toxic substances, and pigments beneficial to reach their potential in biotechnology. The abundance in monoterpenes leads to antioxidant activities, being in the study greater presence of antioxidants in the species of *Tagetes elliptica* L. The physical properties of both species of the genus Tagetes were found in the quality ranges of essential oils.

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