Antioxidant and antibacterial activities of *Matricaria chamomilla* and *Teucrium polium* essential oils: Possible use in food preservation

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Abstract

The current work is devoted to using essential oils isolated from two wild-growing plants of *Matricaria chamomilla* and *Teucrium polium* in resistance to food spoilage, considering their antioxidant and antibacterial activities. Hydro-distillation extraction (HD) method was used to obtain essential oils and gas chromatography–flame ionization detection and gas chromatography–mass spectrometry were used to identify oils. Thus, the antioxidant activity of the oils was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging method. In all, 45 constituents, such as 1,8-cineole, germacrene-D, and artemisia ketone, were found in *M chamomilla* oil, contributing 89.06% of the total composition. *T. polium* L. oil contained 37 compounds, contributing 93.84% of the total composition; the two major compounds being beta-pinene at 74.81% and alpha-pinene at a proportion of 14.62%. The significant antioxidant and antibacterial properties of the above essential oils may affect the food sector to serve as natural and safe food preservatives. Thus, the study demonstrated that the identified essential oils of *M. chamomilla* L. and *T. polium* L. could be used as natural bioactive materials to form useful and prospective food products with enriched healthy properties.

Keywords: antibiotics; antioxidation; essential oils; food protection; GC-FID**;** GC-M**;** *Matricaria chamomilla* L.; natural conservation; *Teucrium polium* L.

Introduction

For thousands of years, fine herbs and aromatic plants have been used in treating illnesses, acting as natural remedies for conditions ranging from digestive issues to chronic diseases. Their therapeutic properties are harnessed in various fields, including culinary arts, cosmetics and pharmaceuticals because of their potent bioactive compounds

(Bruneton, 1999; Goetz and Ghedira, 2012; Singh *et al.*, 2011). Notably, these plants are revered for their essential oils, phenolic compounds, and flavonoids, which contribute to their antimicrobial and antioxidant properties. Although herbs are often consumed in small amounts, their regular incorporation into diets provides substantial health benefits, such as combating oxidative stress and enhancing immunity (Jiang, 2019; Yanishlieva *et al.*, 2006). The scientific exploration of plant extracts, mainly their active ingredients, has expanded significantly. While many compounds are well-documented, further research is necessary to understand the full spectrum of bioactivities and therapeutic potentials offered by these plants (PF Guiné and J Gonçalves, 2016; Sawicka *et al.*, 2022; Žlabur *et al.*, 2020). This study aimed to build on this foundation by examining *Matricaria chamomilla* L. and *Teucrium polium*, commonly known in Algerian traditional medicines, focusing on their chemical and pharmacological properties, including their essential oil composition and potential health benefits for their antioxidant and antibacterial properties. *M. chamomilla* L., an annual herb from the Asteraceae family, is widely recognized for its aromatic properties and historical medicinal uses. Known as chamomile, this plant is traditionally used to treat gastrointestinal disorders, inflammation, and insomnia. Its name originates from the Greek word *khamaimêlon*, meaning 'creeping apple', reflecting its low growth and apple-like fragrance (Hassanpour *et al.*, 2020; Kherraz *et al.*, 2023). Chamomile's medicinal reputation is largely attributed to its diverse secondary metabolites, including coumarins, flavonoids, terpenes, and sesquiterpenes. These compounds have demonstrated significant biological activities, such as antispasmodic and anxiolytic effects, making chamomile a staple in traditional medicine and modern pharmacology (Segura Campos, 2018). In addition to its soothing properties, chamomile is noted for its antibacterial and anti-diabetic potential, providing a natural alternative for managing metabolic disorders (Lardry and Haberkorn, 2007). However, despite its widespread use, there remains a need for comprehensive studies on its specific *in vitro* activities. In recent years, scientific interest in *M. chamomilla* L. has increased due to the diverse therapeutic properties of its essential oils. Essential oils extracted from chamomile have shown promising antiseptic properties, which can be crucial for developing new antimicrobial compounds (Marković *et al.*, 2020; Osman *et al.*, 2016). These oils are rich in bioactive compounds, such as 1,8-cineole, germacrene-D, and sabinene, contributing to their potent antibacterial and antioxidant properties. These properties are particularly significant in the context of rising antibiotic resistance and the global burden of chronic diseases driven by oxidative stress (Goetz and Ghedira, 2012; Singh *et al.*, 2011).

Teucrium polium L. is among the numerous species of the genus Teucrium (Labiatae) represented in the Algerian flora (Sharifi-Rad *et al.*, 2022). *T. polium* L., known as *Jaa'deh* in Algeria, is a small, hairy, scented shrub with dense clusters of white flowers and oval leaves with enrolled edges. Edible *T. polium* is commonly utilized in traditional and folk medicine. The aerial portions are infused to treat kidney stones, vermifuge, depurative, antispasmodic, headache, and abdominal colic. The plant has a variety of biological activities, as do its many extracts (Benchikha *et al.*, 2022).

Despite the extensive traditional and modern use of *M. chamomilla* and *T. polium*, particularly in Algerian traditional medicine, research on its mineral composition and certain pharmacological activities remains limited. Understanding these properties is crucial, given the importance of bioactive compounds in preventing and managing health issues (Bouasla and Bouasla, 2017; Gurib-Fakim, 2006). Our research is part of an ongoing project exploring natural products relevant to human health and nutrition. By highlighting the pharmacological potential of *M. Chamomilla* and *T. polium*, this work seeks to contribute to the growing database of medicinal herbs, offering valuable insights into their applications in pharmaceutical and nutritional contexts, particularly within Algeria's rich biodiversity.

Experimental

Reagents and materials

This study utilized various reagents and chemicals to analyze the properties of essential oils of *M. chamomilla* L. and *T. Polium.* Essential reagents, such as Folin–Ciocalteu reagent (FCR); sodium carbonate (Na_2CO_3); 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS); α-tocopherol (vitamin E); 2,6-ditert-butyl-4-methylphenol (BHT); gallic acid, quercetin, and $AlCl_3$, were purchased from Sigma (Sigma-Aldrich, Germany). Standards, such as α-tocopherol (vitamin E, $C_{29}H_{50}O_2$); 2,6-di-tert-butyl-4-methylphenol (BHT, $C_{15}H_{24}O$); and quercetin ($C_{15}H_{10}O_7$), were used for reference. Anhydrous sodium sulfate (Na_2SO_4) was employed to dry essential oils, and analytical-grade reagents and solvents were used (Sigma-Aldrich) to ensure purity and reliable experimental outcomes. The antimicrobial activity of this study was evaluated using the following laboratory reference strains (American Type Culture Collection [ATCC] for bacteria and *Candida albicans*, National Museum of Natural History [NMHN] for filamentous fungi) obtained from the Institute of Pastor Algeria: Gram-positive bacteria: *Staphylococcus aureus ATCC 6538; Bacillus subtilis ATCC 6633*; Gram-negative bacteria: *Pseudomonas aeruginosa ATCC 9027; Escherichia coli ATCC 8739*; and yeast: *Candida albicans ATCC 10231*.

Sampling and sample preparation

The aerial parts of *M. Chamomilla* and *T. polium* plants were collected in June 2020 from the El-Guetfa region, M'sila, located in a semi-arid area of Algeria (35°44'26"N and 3°23'05"E). Plants were identified at the Department of Chemistry, University of El Oued, Algeria. Upon collection, these plants were immediately transported to the laboratory in clean and airtight containers to minimize degradation and contamination. Once in the laboratory, the samples were thoroughly washed for more than three times with deionized water to remove any dirt or impurity. After washing, the samples were spread out in a single layer on clean trays and allowed to dry at room temperature (approximately 25°C) for 2 weeks, ensuring they were kept away from direct sunlight to prevent photodegradation of sensitive compounds. The dried plant materials were then crunched to a fine powder to a particle size of less than 200 µm using an agate mortar and pestle to ensure homogeneity. The powdered samples were stored in airtight containers in a cool and dry place until further processing for essential oils extract and phenolic extract.

Hydro-distillation and essential oil collection

Using a Clevenger apparatus, the powdered plant material was used to extract essential oils (EO). Approximately 100 g of each plant material was separately placed in a 2-L round-bottom flask with 1.5 L of distilled water. The hydrodistillation process was conducted for 3 h at a steady boil, ensuring that the water level in the apparatus remained constant by adding distilled water as necessary. The vapor mixture of water and essential oil was condensed and collected in the Clevenger-type apparatus, where the essential oil was separated by decantation. The collected essential oil was then dried over anhydrous sodium sulfate (Na_2SO_4) to remove any residual water. The dried essential oil was carefully transferred into amber glass vials with airtight caps and stored at 4°C until analysis. The yield of essential oil was calculated as the weight of oil obtained per 100 g of dried plant material.

Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of essential oil was analyzed using gas chromatography–flame ionization detection (GC-FID) with an Rtx-5MS fused silica capillary column (30-m length × 0.25-mm inner diameter; 0.25-µm film thickness). Nitrogen gas was the carrier gas at a 1 mL/min flow rate. The temperature program began at 60°C for 5 min, then increased by 3°C per minute to 250°C, where it was held for 10 min. The injector and detector temperatures were set at 300°C. The injection volume was 0.1 µL, using a split ratio 1:50. Electronic integration calculated the relative proportion of each component by comparing peak areas in the chromatogram.

For a more detailed analysis of essential oil's constituents, GC-MS was performed using a Shimadzu GCMS-QP2010 (Tokyo, Japan), equipped with an Rtx-5MS-fused bonded column (30 m × 0.25 mm i.d. × 0.25-µm film thickness) (Restek, USA). Helium served as the carrier gas at a 1.5 mL/min flow rate. The GC-MS temperature program started at 45°C, held for 2 min, and then increased by 5°C per minute to 300°C, which was maintained for 5 min. Mass spectra were obtained with a filament emission current (FEC) of 60 mA, ion source temperature (IS) of 200°C, and ionization voltage (IV) of 70 eV. Samples were diluted to 1% v/v before analysis. Components of essential oil were identified by comparing mass spectra with the National Institute of Standards and Technology (NIST) and WILEY mass spectral libraries and by calculating the retention index (RI) relative to n-alkanes (C8-C20) under the same conditions. The experimental RIs were compared with literature values to confirm compound identities, referring to Adams (2007) for standard retention indices.

Antioxidant activity *in vitro*

The antioxidant activity of essential oil was evaluated using DPPH and ABTS assays. In contrast, the antioxidant potential of raw extracts was assessed using DPPH, β-carotene, and the Galvinoxyl (GOR) radical scavenging assays. For each test, 0.5 mL of the sample (either essential oil or raw extract) was mixed with 1 mL of respective reagent: DPPH (0.2 mM) or ABTS (0.2 mM). The mixtures are incubated in the dark at room temperature for approximately 30 min. The absorbance of the samples was measured at 517 nm using a UV-Vis spectrophotometer for DPPH and ABTS assays, and at 450 nm for β-carotene and GOR47-1 (GOR) assays. The percentage of inhibition (I) was calculated using the following formula:

$$I\ (\%) = ((A_0 - A_1) \div A_0) \times 100, \quad (1)$$

Where A_0 is the absorbance of the control (DPPH, GOR, β-carotene, or ABTS solution) and A_1 is the absorbance of the sample. The results are expressed as IC_{50} values, indicating the concentration required to inhibit 50% of free radicals.

Antibacterial activity *in vitro*

The paper disc diffusion method was employed to evaluate the following antibacterial activity of essential

oil or methanol extracts: two Gram-positive strains: *Staphylococcus aureus ATCC 6538* and *Bacillus subtilis ATCC 6633*; two Gram-negative bacterial strains: *Pseudomonas aeruginosa ATCC 9027* and *Escherichia coli ATCC 8739*; and one fungal strain: *Candida albicans ATCC 10231*. The strains were obtained from RDC-SAIDAL, El-Harrah, Algeria. Paper discs with a diameter of 6 mm were impregnated with 35 µg of each extract solution.

These discs were then placed on agar plates inoculated with microorganisms. Reference standards, such as fosfomycin, carbenicillin, erythromycin, and cephalexin (35 µg per disc), were used to determine the sensitivity of Gram-positive strains. In contrast, fosfomycin was used for the sensitivity of Gram-negative strains. The plates were incubated at 37°C for 2 h to allow diffusion. After incubation, the antibacterial activity was assessed by measuring the diameter of the inhibition zones around each disc (Kot *et al.*, 2019).

Statistical analysis

Results were expressed as mean values ± SD of three measurements. The half-maximal inhibitory concentration (IC_{50}) values were calculated by linear regression analysis, and variance analyses were performed by ANOVA using XLSTAT. Tukey's test determined significant differences between mean values, and $p < 0.05$ was considered statistically significant.

Results and Discussion

Chemical composition of essential oils

Essential oils of *M. chamomilla* L. and *T. polium* L. had an average yield of 0.023% and 0.048% (mL/g, wet weight). Given the outcomes, it was observed that *T. polium* produced the highest yield. This was a regular occurrence since essential oil yields varied depending on different circumstances, including the species' geographic location, extraction method, etc. Numerous scientific investigations demonstrated that the yield of essential oils extracted varied according to the plant's place of origin (Aprotosoaie *et al.*, 2010; Marzouki *et al.*, 2010). The findings indicated that these two plants had a comparatively low yield of essential oils. First, GC-FID was used to identify all constituents of the essential oils of the two plants under study. The second phase involved using GC-MS to examine each essential oil and to identify the chemical components of *Hydro distillation Extraction* (HE) from both plants, retention indices (RI) on polar and apolar columns, mass spectra of constituent compounds, and comparison of these data with those of bibliographic databases and electronic databases, WILEY and NIST, were used (Adams, 2007).

Table 1 provides a detailed analysis of the chemical composition of the essential oil extracted from the aerial parts of *M. chamomilla*. The essential oil exhibited a complex mixture of compounds, with significant variations in their concentrations and potential effects. Among the major components, alpha-pinene (5.20%) was prominent for its pine-like aroma and was widely recognized for its anti-inflammatory and respiratory benefits. 1.8-Cineole (13.63%), known as eucalyptol, contributed a fresh, menthol-like scent beneficial for its expectorant and decongestant properties. Artemisia ketone (8.38%) added a unique aromatic profile associated with antioxidant and antimicrobial activities. Additionally, germacrene-D (12.40%) excelled for its earthy and spicy notes, which may offer therapeutic benefits, such as anti-inflammatory and antimicrobial effects.

The essential oil also contained several minor and trace compounds that contributed to its overall chemical profile. Sabinene (7.28%) and beta-pinene (3.25%) were key terpenes influencing the oil's scent and therapeutic properties. 4-Terpineol (2.74%) and alpha-muurolene (2.84%) were notable for their additional fragrance notes and potential bioactivities, including antimicrobial and anti-inflammatory properties. Camphene (0.29%) and citronellol (0.33%) in trace amounts added further complexity to the oil's aroma and therapeutic potential.

The essential oil composition revealed a diverse mixture of monoterpenes and sesquiterpenes, crucial for their functional and aromatic properties. The identified compounds constitute 89.06% of the total composition, yielding 0.023% (v/w). The essential oil included 20.06% hydrocarbon monoterpenes, 35.11% oxygenated monoterpenes, 26.94% hydrocarbon sesquiterpenes, 2.89% oxygenated sesquiterpenes, and 4.06% other compounds. This distribution highlights the oil's potential applications in aromatherapy, medicinal treatments, and fragrances. A high percentage of oxygenated monoterpenes suggests significant therapeutic potential, including antimicrobial and anti-inflammatory effects, which are of particular interest for further research and application.

Table 2 presents the composition of essential oil of *T. polium*, which was extracted by hydrodistillation of the aerial parts of the plants. Different compounds are listed according to their retention time (RT). In all, 37 compounds were identified; the predominant group was hydrocarbon monoterpenes, which accounted for 71.46% of the total essential oil composition. Hydrocarbon sesquiterpenes (16.32%) was the second prominent group. The proportion of oxygenated monoterpenes and sesquiterpenes in the total content was 4.64% and 0.60%,

Table 1. Chemical composition of essential oil obtained from *Matricaria chamomilla* (aerial parts).

No.	Compound	Retention index (KI)	Retention time (RT)	Hydrodistillation (HD) method (%)
1.	Santolina triene	908	11.831	0.39
2.	Alpha-pinene	939	13.576	5.20
3.	Camphene	953	14.427	0.29
4.	Sabinene	976	16.561	7.28
5.	Beta-pinene	980	16.694	3.25
6.	Myrcene	991	17.854	1.50
7.	Yomogi alcohol	998	18.663	0.77
8.	Alpha-terpinene	1018	19.538	0.73
9.	1.8-Cineole	1033	20.869	13.63
10.	Gamma-terpinene	1063	22.798	1.10
11.	Artemisia ketone	1071	23.414	8.38
12.	Artemisia alcohol	1083	24.795	0.26
13.	Erpinolene	1088	24.968	0.32
14.	Alpha-thujone	1102	26.295	1.30
15.	Chrysanthenone	1123	27.697	0.70
16.	Trans-pinocarveol	1139	28.607	0.58
17.	Camphor	1143	28.969	0.43
18.	Pinocarvone	1162	30.373	0.83
19.	Pinocamphone	1160	31.229	1.70
20.	4-Terpineol	1177	31.698	2.74
21.	Alpha-Terpineol	1189	32.656	0.90
22.	Myrtenal	1193	32.918	1.60
23.	Myrtenol	1194	33.049	0.74
24.	Citronellol	1228	35.457	0.33
25.	Trans-chrysanthenyl acetate	1235	35.837	2.27
26.	Geraniol	1255	37.170	0.22
27.	Chrysanthenyl acetate cis	1262	37.526	0.40
28.	Bornyl acetate	1285	39.151	0.52
29.	Delta-elemene	1339	42.676	2.55
30.	Neryl acetate	1365	44.548	0.55
31.	Cyclosativene	1368	44.417	0.60
32.	Alpha-copaene	1376	45.045	0.83
33.	Geranyl acetate	1383	45.811	0.32
34.	Beta-elemene	1391	46.165	0.99
35.	Trans-caryophyllene	1418	47.859	2.67
36.	Alpha-humulene	1454	49.840	0.52
37.	(E)-Beta-farnesene	1458	50.451	0.51
38.	Germacrene-D	1480	51.952	12.40
39.	Bicyclogermacrene	1494	52.661	1.70
40.	Alpha-muurolene	1499	52.964	2.84
41.	Delta-cadinene	1524	54.266	1.33
42.	Elemol	1549	55.728	0.26
43.	Spathulenol	1576	57.373	0.78
44.	Caryophyllene oxide	1581	57.648	1.22
45.	Alpha-cadinol	1653	61.695	0.63
	Total identified	–	–	89.060%
	Yield of EO (v/w) %	–	–	0.023%

(*continues*)

Table 1. Continued.

No.	Compound	Retention index (KI)	Retention time (RT)	Hydrodistillation (HD) method (%)
	Hydrocarbon monoterpenes	–	–	20.06%
	Oxygenated monoterpenes	–	–	35.11%
	Hydrocarbon sesquiterpenes	–	–	26.94%
	Oxygenated sesquiterpenes	–	–	2.89%
	Other compounds	–	–	4.06%

respectively. *T. polium* essential oil is distinguished for its high concentration of β-pinene (36.68%), which is followed by α-pinene (14.62%), D-germacrene (9.97%), limonene (9.78%), and myrcene (7.98%). The remaining compounds found were less than 1.5% in content.

Table 3 provides an overview of the physicochemical properties of the essential oils extracted from *M. chamomilla* and *T. polium*. The pale yellow color of *M. chamomilla* essential oil is indicative of its low level of pigments and suggests a relatively clean and refined product, free from excessive impurities or oxidation. The color also reflects the presence of specific compounds, such as chamazulene or flavonoids, known to impart a lighter hue. The essential oil density at 35°C was measured as 933.9 mg/mL, slightly higher than the typical range for most essential oils, often between 900 mg/mL and 930 mg/mL. This higher density could be due to the specific composition of chamomile oil, which contained higher proportions of heavier constituents, such as sesquiterpenes or waxes, which would increase oil's overall density. The refractive index of 1.4727 was within the expected range for essential oils, which generally varies between 1.45 and 1.55. This measurement is crucial for determining the purity and quality of essential oil. A consistent refractive index suggests that the oil will likely be highly quality, with minimal adulteration or contamination. It also reflects oil's chemical composition and could be used as a comparative measure in quality control processes.

Medicinal plants (MP), such as *M. chamomilla* L. and *T. polium* L., are an important source of natural substances used in therapeutics globally and mainly in Algeria. According to the literature, nearly half of the drugs used today are of plant origin, and a quarter of these drugs contain plant extracts or active molecules derived directly from plants. According to the World Health Organization (WHO), 21,000 plants have medicinal values out of the 300,000 species available worldwide (Pandita *et al.*, 2021). However, despite the importance of these natural substances, it is noted that barely 3000 plants have been the subject of scientific, chemical, biological, or pharmacological studies (Palici, 2016).

The essential oil of *M. Chamomilla* L. is the subject of this discussion. Its chemical originates from different locations (see Table 4), including Bosnia, Iran, Nepal, Egypt, and Ethiopia (Ayoughi *et al.*, 2011; EL-Hefny *et al.*, 2019; Mekonnen *et al.*, 2016; Stanojevic *et al.*, 2016). These studies exhibit a great deal of diversity, qualitatively (composition of various components) and quantitatively (proportions of specific components). Changes in climate, geography, and other conditions, as well as changes in fertilization practices, could be responsible for these variations. Additionally, the chemical composition of essential oil varies greatly depending on the exaction techniques used.

A comparison of the main constituents of the essential oil of *T. polium* L. is shown in Table 5. When these results are compared to those found in the literature, it becomes evident that hydrocarbon-based monoterpenes predominate among constituents. However, this does not rule out the occurrence of some differences, such as those between α-pinène and β-pinène, specifically in the case of *T. polium* L. from Jordan (Aburjai *et al.*, 2006), Croatia (Bezić *et al.*, 2011), and Tunisia (Boulila *et al.*, 2008). It is possible to attribute this variability to both intrinsic and extrinsic factors. Indeed, according to Sofowora (1996), geographical location, plant part, time, harvest time, and storage conditions affect a plant's composition of secondary metabolites. Several authors claim that these secondary metabolites give plants their bioactive qualities.

Antioxidant activity

The antioxidant activity of the essential oils of *M. chamomilla* L. and *T. polium* L. was evaluated using DPPH and ABTS assays (see Table 6). These assays helped to determine extracts' ability to scavenge free radicals and inhibit oxidation, which are crucial indicators of potential health benefits. Comparison with known standards, such as BHT, butylated hydroxyanisole (BHA), and vitamin E (α-tocopherol), highlights the antioxidant potency of oil extracts.

The DPPH assay measures the ability of antioxidants to donate hydrogen and neutralize DPPH radicals, a stable free radical. In this study, essential oils of *M. chamomilla* L. and *T. polium* L. demonstrated a weak antioxidant capacity with an IC_{50} value of 25091.5 ± 12 µg/mL

Table 2. Chemical composition of essential oil of *Teucrium polium* L. (aerial parts).

No.	Compounds	Retention index (KI)	Retention time (RT)	Hydrodistillation (HD) %
1.	2-Hexenal	854	8.462	0.13
2.	α-Thujene	931	13.073	0.15
3.	α-Pinene	939	13.661	14.62
4.	Camphene	953	14.454	0.3
5.	β- Pinene	980	16.948	36.68
6.	Myrcene	991	18.033	7.98
7.	Limonene	1031	20.642	9.78
8.	Cis-ocimene	1040	21.348	0.260
9.	Trans-ocimene	1050	22.126	1.30
1.0.	Gamma-terpinene	1062	22.738	0.1
11.	Terpinolene	1088	24.916	0.29
12.	Linalool	1098	25.96	0.68
13.	Nonanal	1098	26.272	0.13
14.	Nopinone	1137	28.387	0.20
15.	Trans-pinocarveol	1139	8.569	0.86
16.	Camphor	1143	28.912	0.19
17.	Trans-verbenol	1144	29.066	0.27
18.	Pinocarvone	1162	30.313	0.64
19.	Borneol	1165	30.539	0.14
20.	4-Terpineol	1177	31.417	0.18
21.	Alpha. Terpineol	1189	32.453	0.15
22.	Myrtenal	1194	32.779	1.26
23.	Nerol	1228	35.197	0.14
24.	Bornyl acetate	1285	39.097	0.27
25.	α-Cubebene	1351	43.346	0.10
26.	α-copaene	1376	44.977	0.12
27.	β-bourbonene	1384	45.565	1.06
28.	β-elemene	1391	46.072	0.16
29.	Germacrene-D	1480	51.799	9.97
30.	β-Selinene	1485	51.986	1.08
31.	Bicyclogermacrene	1494	52.603	2.56
32.	α-Guaiene	1500	48.972	0.18
33.	δ-Cadinene	1524	54.192	0.93
34.	Germacrene B	1556	56.009	0.16
35.	Spathulenol	1576	57.224	0.36
36.	β-Eudesmol	1649	61.294	0.18
37.	α-Cadinol	1653	61.564	0.28
	Total identified			93.84
	Yield of essential oil (v/w) %			0.048
	Monoterpene hydrocarbons			71.46
	Oxygenated monoterpenes			4.64
	Sesquiterpene hydrocarbons			16.32
	Oxygenated sesquiterpene			0.82
	Other compounds			0.60

and 5491.4 ± 3 µg/mL, respectively. Although this is notably higher than the standards such as BHT (12.99 ± 0.41 µg/mL) and BHA (15.21 ± 0.41 µg/mL), The results indicate that while the essential oils extract may not match synthetic antioxidants in potency, it still offers a natural alternative with substantial efficacy and its potential as a natural antioxidant source. At the same time, the ABTS assay data for the essential oils of *M. chamomilla* L. and *T. polium* L. demonstrated an IC_{50} equal to 1034.6 ± 2 and 373.45 ± 17 µg/mL, respectively, suggesting a lower antioxidant activity, compared to the standards. BHA was significantly more effective with an IC_{50} of 15.21 ± 0.77 µg/mL. The ABTS assay is particularly useful for evaluating both hydrophilic and lipophilic antioxidants, implying that the essential oil might lack certain compounds that could effectively neutralize ABTS radicals.

Generally, these results suggest that while essential oils contain compounds that can delay oxidation, they may not be as potent as synthetic antioxidants for this specific type of oxidative process. This observation aligns with the notion that natural extracts often provide a broader, *albeit* less intense, spectrum of antioxidant activity. Also, the weaker performance of these plants' extracts of essential oils could be due to the specific interaction between the extract's phytochemicals and the lipid radicals involved in the beta-carotene degradation process, which may require more targeted antioxidant compounds found in synthetic standards.

Table 3. Physicochemical properties of the essential oils of *Matricaria chamomilla* L. and *Teucrium polium* L.

Properties of essential oils	*Matricaria chamomilla* L.	*Teucrium polium*
Color	Pale yellow	Light green
Density at 35°C (mg/mL)	933.9	870.2
Refractive index	1.4727	1.4727

In vitro antimicrobial activity

The antimicrobial activity of *M. chamomilla* and *T. polium* essential oils was investigated using a combination of disk diffusion and minimal inhibitory concentration (MIC) methods. These assays were applied to a range of microbial strains, including Gram-positive and Gram-negative bacteria and fungi, to provide a thorough understanding

Table 4. Comparison of our data of main chemicals of essential oil of *M. chamomilla* L with that of the literature.

Major compounds	Country & content (%) of compounds					
	Experimental data	Bosnia (Stanojevic *et al.*, 2016)	Iran (Ayoughi *et al.*, 2011)	Nepal (Satyal *et al.*, 2015)	Egypt (EL-Hefny *et al.*, 2019)	Ethiopia (Mekonnen *et al.*, 2016)
α-pinene	5.20	1.9	0.32	0	0.22	6.953
Sabinene	7.28	0.6	0.14	0.2	0.29	ND
1.8-Cineole	13.63	0.1	0.13	ND	ND	4.139
Artemisia cetone	8.38	0.7	1.05	ND	ND	ND
Germacrene-D	12.40	6.2	3.82	9.1	3.71	ND

ND: not determined.

Table 5. Comparison of our data of main chemicals of essential oil of *Teucrium polium* L. with that of the literature.

Country	Content of major compounds present in the Hydro-distillation extraction (HD) method of *Teucrium polium* L. (%)					
	α-pinene	β-pinene	Myrcene	Limonene	Germacrene-D	Bicyclogermacrene
	14.62	36.68	7.98	9.78	9.97	2.56
Iran (Farahbakhsh *et al.*, 2021)	6.97	12.97	2.19	3.45	ND	1
Algeria Bouira (Bendjabeur *et al.*, 2018)	3.30	11.3	1.7	4	25	10.4
Jordan (Aburjai *et al.*, 2006)	0.74	1.48	0.48	0.66	2.38	ND
Croatia (Bezić *et al.*, 2011)	ND	0.3	0.1	5.9	8.7	ND
Tunisia (Boulila *et al.*, 2008)	6.6	5.8	15.5	2.2	9	ND
Morocco (Benali *et al.*, 2021)	6.76	19.82	2.9	5.71	18.33	3.21

ND: not determined.

Table 6. ***In vitro*** **antioxidant properties of essential oils of** ***Matricaria chamomilla*** **L. and** ***Teucrium polium*** **L.**

Samples	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Essential oil of *M. chamomilla*	25091.5 ± 12[c]	1034.6 ± 2[c]
Essential oil of *T. polium*	5491.4 ± 3[d]	373.45 ± 17[d]
Butylated hydroxytoluene (BHT)*	12.99 ± 0.41	NDC
Butylated hydroxyanisole (BHA)*	15.21 ± 0.41[a]	15.21 ± 0.77[a]
Vitamin E (α-tocopherol)*	15.81 ± 0.54[b]	37.79 ± 0.28[b]

*Standard compounds. IC_{50} is the concentration of 50% inhibition percentage. IC_{50} was calculated by linear regression analysis and expressed as mean ± SD (n = 3) of three parallel measurements. ND: not determined. The values with superscripts[(a,b,c,d)] in the same columns are significantly different ($p < 0.05$).

of extracts' antimicrobial efficacy. The *in vitro* antimicrobial activity of *M. chamomilla* highlights its potential to be a natural antimicrobial agent against various bacterial and fungal strains (see Tables 7 and 8).

First, antibacterial activity tests using the solid medium diffusion method using Mueller–Hinton medium for bacteria and Sabouraud medium for yeasts were performed on four Gram-positive and Gram-negative bacterial strains. The extracts were prepared from three examples. The results of the evaluation of the antibacterial potential of extracts are shown in Table 7. The antibacterial activity is determined as the diameter of the zone of inhibition (in mm) produced around the discs after incubation under conditions suitable for developing the test germ. This activity was evaluated on four bacterial reference strains (*Staphylococcus aureus*, *Bacillus subtilus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and a fungal strain (*Candida albicans*).

In the disk diffusion method, essential oil of *M. chamomilla*, compared to T. polium L. essential oil and the references used, demonstrated notable antibacterial activity, particularly against Gram-positive bacteria. For instance, against Staphylococcus aureus, the essential oil produced a significant inhibition zone of 24.66 ± 1.32 mm, indicating a strong antibacterial effect, although still less than the standard antibiotic fosfomycin, which showed an inhibition zone of 44.0 ± 0.5 mm. This suggests that *M. chamomilla* essential oil is effective and matches conventional antibiotics potency. Essential oil of *T. polium* L. also showed a good inhibitory effect against *Bacillus subtilis* with a zone size of 16.66 ± 01.32 mm, *albeit* slightly less than the *M. chamomilla* essential oil with a zone size of 21.5 ± 0.5 mm and the zone size of the standard antibiotic fosfomycin being 32.5 ± 0.3 mm. However, when tested against Gram-negative bacteria, such as Escherichia coli, the essential oil showed a moderate inhibition zone of 23.5 ± 0.5 mm. In contrast, the *T. polium* L. essential oil showed a lower inhibition zone at 12 ± 0.75 mm. This reduced effectiveness against Gram-negative bacteria could be attributed to their more complex cell wall structures, which could act as a barrier to the penetration of antimicrobial compounds. In addition to antibacterial activity, the study also evaluated the antifungal potential of essential oil extracts. Essential oil of *M. chamomilla* showed a particularly strong antifungal effect against *Candida albicans*, with an inhibition zone of 12.5 ± 0.5 mm, notably larger than the inhibition zone produced by *T. polium* L. essential oil at 9 ± 0.5 mm. This finding suggests that the essential oil of *M. chamomilla* might contain specific antifungal compounds that could be more effective than those found in *T. polium* L. essential oil. The higher efficacy of *M. chamomilla* essential oil against *Candida albicans* is significant, as fungal infections are often challenging to treat due to increasing resistance to conventional antifungal agents. The ability

Table 7. ***In vitro*** **antibacterial activity of** ***Matricaria chamomilla*** **and** ***Teucrium polium*** **essential oils against bacteria and yeast strains using the disk diffusion method.**

	Concentration (µL/disc) Groups		Our Data			
			Essential oil of *M. chamomilla* L.	Essential oil of *T. polium* L.	References used	
Gram-positive bacteria	*Staphylococcus aureus ATCC 6538*	Diam mean ± SD (mm)	24.66 ± 1.32	11.66 ± 1.32	Fosfomycin 44 ± 0.5	Carbenicilli 37.5 ± 0.4
	Bacillus subtilis ATCC 6633	Diam mean ± SD (mm)	21.5 ± 0.5	16.66 ± 1.32	Erythromycin 32.5 ± 0.3	Cephalexin 31 ± 0.3
Gram-negative bacteri	*Pseudomonas aeruginosa ATCC 9027*	Mean ± SD (mm)	12.0 ± 0.5	12 ± 0.5	Fosfomycin 31.2 ± 0.21	–
	Escherichia coli ATCC 8739	Diam mean ± SD (mm)	23.5 ± 0.5	12 ± 0.75	Fosfomycin 33 ± 0.31	–
Yeast	*Candida albicans ATCC 10231*	Mean ± SD (mm)	12.5 ± 0.5	9 ± 0.75	–	–

Table 8. Results of minimal inhibitory concentration values (µL/mL) using the it is mean Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods.

	Micro-organisms tested	MIC/MBC (µL/mL	Antibiotic/antifungal	*T. polium* L.	*M. chamomilla* L.
Gram-positive	**Reference antibiotic: Levofloxacin**				
	Staphylococcus aureus ATCC 6538	MIC	0.062	2.5	0.5
		MBC	0.062	5	2
	Bacillus subtilis ATCC 6633	MIC	0.125	20	2
		MBC	0.125	20	2
	Listeria monocytogenes CIP82110	MIC	0.125	6.125	ND
		MBC	0.125	6.125	ND
Gram-negative	*Pseudomonas aeruginosa* ATCC 9027	MIC	0.125	6.125	ND
		MBC	0.125	6.125	ND
	Escherichia coli ATCC 8739	MIC	0.062	<0.76	5
		MBC	0.062	<0.76	5
	Klebsiella pneumoniae CIP 8291	MIC	1	6.125	<0.76
		MBC	1	6.125	<0.76
	Pseudomonas aeruginosa ATCC 9027	MIC	1	<0.76	<0.76
		MBC	1	<0.76	<0.76
Champignons	**Antifungal: Nystatine**				
	Mucor ramanianus	MIC	0.94	3.06	6.125
	Aspergilus flavus	MIC	0.94	1.53	6.125
	Penicillium expansum	MIC	0.23	<0.76	1.53
	Fusarium culmorum	MIC	0.007	<0.76	<0.76

ND: not determined, MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration.

of the essential oil extract of *M. chamomilla* to inhibit *Candida albicans* effectively highlights its potential of being a viable alternative or complementary treatment in managing fungal infections.

Second, the minimal inhibitory concentration method provided further insights into the antimicrobial potency of *M. chamomilla* L. and *T. polium* L. essential oils (see Table 8).

The essential oil of *M. chamomilla* exhibited a low MIC value of 0.5 µL/mL against *Staphylococcus aureus*, indicating high antibacterial activity. However, it was less effective than the standard antibiotic levofloxacin, which had an MIC of 0.062 µL/mL, suggesting that while the raw extract had significant potential, it might require optimization or combination with other treatments for maximum efficacy. Similarly, *T. polium* L. essential oil showed a MIC of 2.5 µL/mL against *Escherichia coli*, demonstrating moderate antibacterial activity against this Gram-negative bacterium. Notably, *M. chamomilla* essential oil also displayed important antifungal activity with low MIC values against fungal strains, such as *Penicillium expansum* and *Fusarium culmorum* at 1.53 µL/mL and <0.76 µL/mL, respectively, indicating a potent antifungal effect.

These results collectively highlight the promising role of *M. chamomilla* and *T. polium* L. as natural antimicrobial agents, particularly when confronted with the rising antimicrobial resistance.

Conclusions

In this work, we investigated the composition of essential oil constituents of *T. polium* L. and *M. chamomilla* L., in addition to some biological properties, such as *in vitro* antioxidant and antimicrobial activity. The results showed that 26 compounds were identified, and beta-pinene was the major constituent of essential oil (36.68%), followed by alpha-pinene (14.62%), germacrene-D (9.97%), limonene (9.78%), and myrcene (7.98%). This study demonstrates that M. chamomilla and T. polium L. essential oils exhibit significant bioactive properties. The *in vitro* antioxidant tests revealed that the essential oil of *T. polium* L. had important antioxidant activity, particularly noted by the low IC50 value of 373.45 µg/mL in ABTS. However,

the antioxidant activity of the essential oil of *M. chamomilla* was less than that of the essential oil of *T. polium* L. and standards. Regarding antimicrobial activity, the essential oil of *M. chamomilla* showed an important activity. The results obtained underscored the potential of *M. chamomilla* as a source of valuable bioactive compounds with significant antioxidant and antimicrobial activities.

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Author Contributions

Abderrezak Ferhat: Conceptualization, methodology, software, validation; Amar Djemoui: Conceptualization, methodology, software, validation, formal analysis; Mohammed Messaoudi: Conceptualization, methodology, software, validation, formal analysis; Mohamed Amine Ferhat: Conceptualization, methodology, software, validation; Naima Benchikha: investigation, formal analysis; Hamza Ouakouak: investigation, formal analysis; Siham Boubekeur: investigation, formal analysis; Sabry M. Attia: validation, formal analysis, resources, funding acquisition; Sheikh F. Ahmad: validation, formal analysis, resources, funding acquisition; Maria Atanassova: investigation & Wafa Zahnit: Conceptualization, methodology, software, validation.

Conflicts of Interest

The authors declare no conflicts of interest.

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