Original Article

# Chemical Composition, Antibacterial and Antioxidant Activities of *Tagetes patula* L. Essential Oil Raised in Erbil, Iraq

**Ausama A. Safar[1,2], Anwar O. Ghafoor[2], Dara Dastan[3]**

*[1]Plant Production Department, Khabat Technical Institute, Erbil Polytechnic University, [2]Department of Biology, College of Education, Salahaddin University-Erbil, Erbil, Kurdistan Region, Iraq, [3]Department of Pharmacognosy, School of Pharmacy, Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*

## Abstract

**Background:** Over the years, management of human pathogenic microorganisms has primarily relied on the use of synthetic antibiotics. In the recent past, *Tagetes patula* essential oils (EOs) and their phytochemistry and bioactivities have received great attention in research. **Purpose and Methods:** In this study, the component, antimicrobial activity, and antioxidant capacity (ferric-reducing antioxidant power assay) of EOs from five plant parts (shoot at vegetative growth stage [TPSV], shoot at flowering growth stage [TPSF], flower [TPF], fruit [TPS], and root [TPR]) of *T. patula* were investigated. The antibacterial activity against five gram-negative bacterial isolates (including *Serratia fonticola, Klebsiella pneumoniae, Acinetobacter baumannii, Proteus mirabilis,* and *Escherichia coli*) and five gram-positive bacterial isolates (including *Staphylococcus aureus, S. epidermidis, S. saprophyticus, Streptococcus agalactiae,* and *Streptococcus oralis*) was studied using broth microdilution method. FRAP assay was also used to evaluate their antioxidant activity. **Results:** One hundred and twenty-five compounds of the total EOs were identified, constituting a mixture of oxygenated monoterpenes (33%), monoterpene hydrocarbons (25%), oxygenated sesquiterpenes (19%), sesquiterpene hydrocarbons (12%), and furanocoumarins (8%). In this paper, for the first time, more than 60 new compounds were isolated from *T. patula* such as bergapten, sylvestrene, (E)-β-farnesene, (E)-epoxy-ocimene, (Z)-jasmone, γ-gurjunene, and γ-himachalene. The EOs of *T. patula* showed potent antibacterial activity against the studied bacteria with the highest growth inhibition observed in *E. coli* after 24 h of incubation (MIC value 0.08 and MBC value 0.32 µL/mL). The TPS-EO had the highest mean value for ferric-reducing ability at the three test times, whereas TPR-EO had no activity. **Conclusion:** It was concluded that the potential biocidal activity of *T. patula* EOs could be substantially associated with their oxygenated constituents or the synergistic activity of their major and minor chemical components.

**Keywords:** *Tagetes patula, essential oil, gas chromatography–mass spectrometry, antibacterial, antioxidant*

## Introduction

*Tagetes* is an important genus belonging to the Asteraceae family, native to Mexico and Central America,[1] and widely cultivated in different parts of Kurdistan. However, the biological potency of the essential oil (EO) of this species has been broadly reported.[2-5] The most important EOs in *T. patula* with pharmaceutical efficacy are piperitone, piperitenone, trans-β-ocimene, terpinolene, dihydrotagetone, *cis*-tagetone, limonene, β-caryophyllene, and allo-ocimene, α-terpinolene, *trans*-caryophyllene, (Z)-ocimenone, dl-limonene, piperitenone, β–pinene, and car-3-en-2-one.[6,7] Although various antibacterial and antioxidant aspects of *T. patula* have been tested, the chemical constituents of different parts of its EO based on experimental models have still not been explored. In this study, we investigated the effect of EO constituents, hydrodistilled from several parts of *T. patula*, and its antibacterial and antioxidant activities. However, to the best of our knowledge, this is the first publication that provides the list of more than 60 new compounds, which will be discussed in the following sections.

## Materials and Methods

### Plant material

The five different parts of *T. patula* were sampled freshly early morning in different periods, as shoots at vegetative growth (TPSV) were collected during July, whereas shoots at flowering stage (TPSF), flowers (TPF), fruits (TPS), and root (TPR) parts were sampled during August. All samples were placed into polythene bags and collected from different



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***Address for correspondence:***
*Ausama A. Safar, Plant Production Department, Khabat Technical Institute, Erbil Polytechnic University, Kirkuk Road, Erbil, Kurdistan Region, Iraq.*
*E-mail: allelopathy.81@gmail.com; ausama.safar@epu.edu.iq*

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areas of Erbil Province (latitude: 36.2062; longitude: 44.0088 at 429 altitude). The collected parts were initially washed in tap water to remove soil and other contaminants and were cut into small pieces to be prepared for distillation. A voucher specimen was deposited by Dr. Abdullah Shakur Sardar at the herbarium of the College of Education, Salahaddin University-Hawler, Erbil, Iraq, under the voucher number (no. 7591).

### Isolation of essential oils

Six hundred grams of fresh plant parts were submitted to hydrodistillation using a Clevenger-type apparatus to produce oil for 3 h. The process was performed separately for each plant part by using 4750, 3650, 4150, 3560, and 3910 g from TPSV, TPSF, TPF, TPS, and TPR, respectively, until the required amount of oil was obtained. The oil was separated from the water by adding anhydrous sodium sulfate ($Na_2SO_4$) and stored in tightly closed dark vials at 4°C until analysis.[8]

### Gas chromatography and gas chromatography–mass spectrometry analysis

The gas chromatography (GC) analysis of the oil was conducted using a ThermoQuest (UK) gas chromatograph with a flame-ionization detector (FID). The analysis was carried out using fused silica capillary DB-5 column (60 m × 0.25 mm; film thickness, 0.25 µm). The injector and detector temperatures were 250°C and 300°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL/min; oven temperature was programmed from 60°C to 250°C at a rate of 5°C/min, and finally held isothermally for 10 min. GC–mass spectrometry (MS) analysis was performed using a ThermoQuest–Finnigan gas chromatograph equipped with the above mentioned column, used under the same conditions coupled to a TRACE mass spectrometer (UK). Helium was used as the carrier gas. Ionization voltage was kept at 70 eV. Ion source and interface temperatures were kept between 200°C and 250°C, respectively. Mass range was scanned from 43 to 456 *m*/*z*.[9,10]

### Identification of compounds

The constituents of the volatile oils in different stages were identified by the calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C6–C24) and the oil on a DB-5 column. Identification of individual compounds was made by comparing their mass spectra library (Wiley and Adams) or with authentic compounds. For confirmed compounds, their GC retention indices were compared with authentic compounds or with those reported earlier.[10] Each sample was then analyzed three times with GC–FID to obtain the percentage concentration of each constituent without performing any correction.

### Bacterial strains

The following microorganisms were obtained from Rizgari Hospital in Erbil: *Serratia fonticola*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Escherichia coli*, and five gram-positive bacterial isolates including *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus agalactiae*, and *Streptococcus oralis*. Each strain was grown in a test tube containing 45 mL of sterile nutrient broth (Lab M, Heywood, England) at 37°C for 24 h. The bacteria identification was confirmed by VITEK 2 Compact (bioMérieux, Nürtingen, Germany) instrument.

### Antibacterial susceptibility testing

To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EOs, the broth microdilution method was performed[11] with some modifications. Ninety-six well-cultured plates were used. Stock solutions of EOs were diluted in dimethyl sulfoxide (DMSO) to give serial twofold dilutions that were added to each medium, resulting in concentrations ranging from 5 to 0.005 µL/mL. Ciprofloxacin was used as a positive control (500 µg/mL). The volume of dispensing extract was 100 µL per well along with 100 µL of Mueller Hinton broth. Twenty µL of bacterial culture at a density of $5 \times 10^5$ CFU/mL was added to the wells.[11] Three control wells were maintained for each test batch: the positive control (antibiotic, Mueller Hinton broth and test organism), sterility control (Mueller Hinton broth and DMSO), and negative control (Mueller Hinton broth, test organism, and DMSO). The plates were incubated at 37°C for 24 h. The absorbance for each well was measured at 630 nm before incubating by enzyme-linked immunosorbent assay (ELISA) Reader (BioTech, Vermont, USA). Then, the plates were incubated under shaking conditions (100–120 rpm) at 37°C for 24 h. After the incubation time, the absorbance was remeasured to determine the final absorbance and was compared with the initial absorbance.[12]

The MIC and MBC were confirmed by subculturing bacterial cells on the solid nutrient agar. The plates were incubated at 37°C for 24 h overnight. The lowest concentration of the EO required to inhibit visible growth of the tested microorganism was designated as the MIC, whereas the plates that did not show growth were considered to be the MBC. Using the values of MIC in the micro broth dilution assay method, the MIC index values (MBC/MIC) for both the EO and the standard control drug were calculated against the test strains. The tests were performed in triplicate. However, the EOs and the compounds with MICs less than 500 µg/mL were considered to be of interest.[13]

### Ferric-reducing antioxidant power assay

The $Fe^{3+}$ reducing antioxidant power of the EOs was performed according to Pulido *et al.*,[14] with modification in terms of the time interval. The reaction was evaluated at three different times (5, 25, and 50 min). Briefly, ferric-reducing antioxidant power (FRAP) assay reagent was freshly prepared and contained in 25 mL of 300 mM sodium acetate buffer (pH, 3.6), 2.5 mL of 20 mM 2,4,6-tripyridyl-S-triazine (TPTZ) (Sigma-Aldrich, UK) in 40 mmol/L HCl, and 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 and warmed at 37°C. The FRAP reagents (2 mL) were mixed with 100 µL of each sample, then the mixture was incubated at 37°C. The absorbance was read at 593 nm after each 5, 25, and 50-min interval using a UV-Vis spectrophotometer (EMCLAB 11 UV, Duisburg, Germany). The FRAP value was determined by designing a standard curve produced by the addition of $FeSO_4 \cdot 7H_2O$ to the FRAP reagent. Antioxidant

**Table 1: Essential oil composition of different parts and stages of *Tagetes patula* L. from Erbil**

| No. | Compounds/classification | RI[a] | TPSV | | TPSF | | TPF | | TPS | | TPR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RT[b] | Area% | RT | Area% | RT | Area% | RT | Area% | RT | Area% |
| 1. | α-pinene | 932 | 4.11 | 0.54 | 4.11 | 0.47 | 4.1 | 0.1 | 4.11 | 0.25 | | |
| 2. | Camphene | 946 | 4.36 | 0.09 | 4.37 | 0.06 | | | | | | |
| 3. | Sabinene | 969 | 4.78 | 1.23 | 4.79 | 1.27 | 4.77 | 0.3 | 4.78 | 0.52 | | |
| 4. | β-pinene | 974 | 4.86 | 0.06 | 4.86 | 0.07 | | | | | | |
| 5. | Myrcene | 988 | 5.07 | 0.31 | 5.08 | 0.26 | 5.06 | 0.11 | 5.07 | 0.43 | | |
| 6. | α-phellandrene | 1002 | 5.37 | 0.39 | 5.38 | 0.4 | | | 5.37 | 0.11 | | |
| 7. | *p*-cymene | 1020 | 5.79 | 0.25 | | | | | | | | |
| 8.* | *o*-cymene | 1022 | | | 5.81 | 0.15 | 5.78 | 0.11 | 5.79 | 0.1 | | |
| 9.* | Sylvestrene | 1025 | 5.89 | 9.85 | 5.91 | 8.74 | 5.86 | 3.4 | 5.88 | 5.61 | 5.88 | 0.13 |
| 10. | (Z)-β–ocimene | 1032 | 6.05 | 10.02 | 6.07 | 9.09 | 6.03 | 8.36 | 6.06 | 10.21 | | |
| 11. | (E)-β–ocimene | 1044 | 6.27 | 1.04 | 6.27 | 1.06 | 6.25 | 0.7 | 6.26 | 1.95 | | |
| 12. | Dihydro-tagetone | 1046 | 6.36 | 2.61 | 6.36 | 0.84 | 6.34 | 0.63 | 6.35 | 0.72 | | |
| 13. | γ-terpinene | 1054 | 6.53 | 0.07 | 6.53 | 0.08 | | | 6.52 | 0.05 | | |
| 14. | Terpinolene | 1086 | 7.24 | 14.36 | 7.26 | 12.88 | 7.2 | 3.11 | 7.24 | 9.31 | | |
| 15.* | Dihydro-linalool | 1088 | | | 7.41 | 0.03 | | | | | | |
| 16. | *p*-cymenene | 1089 | 7.28 | 0.81 | | | | | | | | |
| 17. | Linalool | 1095 | 7.48 | 0.3 | 7.49 | 0.34 | | | 7.48 | 0.14 | | |
| 18.* | α-pinene oxide | 1099 | | | | | 7.38 | 0.13 | | | | |
| 19. | *cis*-thujone | 1101 | | | | | | | 7.57 | 0.11 | | |
| 20.* | *trans*-vertocitral C | 1105 | 7.58 | 0.19 | 7.58 | 0.14 | | | | | | |
| 21.* | (2E,4E)-octadienol | 1113 | 7.81 | 0.53 | 7.82 | 0.55 | 7.8 | 0.16 | 7.81 | 0.3 | | |
| 22. | (Z)-epoxy-ocimene | 1128 | 8.24 | 3.01 | 8.25 | 2.65 | 8.22 | 2.59 | 8.23 | 2.4 | | |
| 23. | Allo-ocimene | 1128 | 8.17 | 0.05 | 8.18 | 0.15 | | | 8.16 | 0.1 | | |
| 24. | *cis-p*-mentha-2,8-dien-1-ol | 1133 | 8.38 | 0.18 | | | | | 8.37 | 0.06 | | |
| 25.* | (E)-epoxy-ocimene | 1137 | 8.48 | 1.47 | | | | | 8.48 | 2.47 | | |
| 26. | *trans*-sabinol | 1137 | | | 8.38 | 0.15 | | | | | | |
| 27.* | Geijerene | 1138 | | | 8.49 | 1.48 | | | | | | |
| 28. | (E)-tagetone | 1139 | 8.58 | 3.44 | 8.59 | 3.1 | 8.77 | 1.27 | 8.57 | 2 | | |
| 29.* | (E)-myroxide | 1140 | | | | | 8.47 | 2.58 | | | | |
| 30. | Camphor | 1141 | 8.71 | 0.41 | 8.71 | 0.3 | 8.56 | 2.23 | | | | |
| 31. | (Z)-tagetone | 1148 | 8.78 | 4.47 | 8.8 | 4.51 | | | 8.78 | 1.78 | | |
| 32. | Isoborneol | 1155 | 9.04 | 0.1 | 9.05 | 0.14 | | | | | | |
| 33. | Terpinen-4-ol | 1174 | | | | | 9.42 | 0.61 | | | | |
| 34.* | (E)-isocitral | 1177 | 9.38 | 0.49 | 9.39 | 0.45 | | | 9.43 | 0.54 | | |
| 35. | *p*-cymene-8-ol | 1179 | 9.69 | 1.25 | | | | | 9.67 | 0.63 | | |
| 36. | *cis*-pinocarveol | 1182 | | | 9.44 | 0.53 | | | | | | |
| 37. | α-terpineol | 1186 | 9.8 | 0.52 | | | | | 9.79 | 0.08 | | |
| 38.* | *trans-p*-mentha-1(7),8-dien-2-ol | 1187 | | | 9.67 | 0.18 | | | | | | |
| 39.* | 2-allyl-phenol | 1189 | | | 9.69 | 0.75 | 9.68 | 2.35 | | | | |
| 40. | Myrtenol | 1194 | 9.97 | 0.06 | 9.8 | 0.19 | | | 9.97 | 0.1 | | |
| 41.* | Methyl chavicol | 1195 | | | | | 9.79 | 0.52 | | | | |
| 42.* | *trans*-4-caranone | 1196 | 10.07 | 0.28 | | | | | 10.05 | 0.31 | | |
| 43. | γ-terpineol | 1199 | 10.11 | 0.48 | | | | | | | | |
| 44.* | *cis*-4-caranone | 1200 | | | 9.98 | 0.28 | 9.96 | 2.3 | 10.24 | 0.16 | | |
| 45. | *trans*-carveol | 1215 | | | 10.27 | 0.08 | | | | | | |
| 46. | *cis*-sabinene hydrate acetate | 1219 | | | 10.51 | 0.05 | | | | | | |
| 47.* | Coahuilensol methyl ether | 1219 | 10.58 | 0.18 | 10.59 | 0.09 | | | 10.55 | 0.12 | | |
| 48. | (Z)-ocimenone | 1226 | 10.82 | 6.57 | 10.85 | 8.09 | | | 10.82 | 5.33 | | |
| 49. | (E)-ocimenone | 1235 | 11.04 | 6.61 | 11.07 | 8 | 10.82 | 12.08 | 11.03 | 3.77 | | |
| 50.* | Car-3-en-2-one | 1244 | | | | | 11.02 | 3.92 | | | | |
| 51. | Piperitone | 1249 | 11.43 | 6.53 | 11.45 | 5.78 | 11.39 | 2.52 | 11.4 | 1.79 | | |
| 52.* | Perilla aldehyde | 1269 | 11.8 | 0.14 | 11.8 | 0.09 | 11.91 | 0.66 | | | | |
| 53.* | Neo-3-thujanol acetate | 1273 | 11.98 | 0.07 | | | | | | | | |
| 54.* | *p*-menth-1-en-7-al | 1273 | | | 11.88 | 0.15 | | | | | | |
| 55. | Isobornyl acetate | 1283 | 12.15 | 0.44 | 12.16 | 0.34 | | | 12.14 | 0.21 | | |

**Table 1: Continued**

| No. | Compounds/classification | RI[a] | TPSV | | TPSF | | TPF | | TPS | | TPR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RT[b] | Area% | RT | Area% | RT | Area% | RT | Area% | RT | Area% |
| 56. | Bornyl acetate | 1287 | | | | | 12.15 | 0.5 | | | | |
| 57.* | *trans*-verbenyl acetate | 1291 | | | 12.33 | 0.11 | | | | | | |
| 58.* | Carvacrol | 1298 | 12.63 | 0.14 | 12.66 | 0.11 | | | | | | |
| 59.* | Neo-dihydro carveol acetate | 1299 | | | 12.78 | 0.16 | | | | | | |
| 60. | Terpinene-4-ol acetate | 1299 | 12.77 | 0.17 | | | | | | | | |
| 61.* | Silphiperfol-5-ene | 1326 | | | 13.18 | 0.05 | 13.17 | 0.42 | 13.17 | 0.12 | | |
| 62. | Piperitenone | 1340 | 13.67 | 6.37 | 13.7 | 6.68 | 13.66 | 6.34 | 13.64 | 1.78 | | |
| 63. | Piperitenone oxide | 1366 | 14.28 | 0.11 | 14.3 | 1.87 | 14.27 | 1.53 | 1427 | 0.84 | | |
| 64.* | Longicyclene | 1371 | | | | | | | 14.46 | 0.09 | 14.22 | 0.86 |
| 65.* | Silphiperfol-6-ene | 1377 | | | | | 14.46 | 0.33 | | | | |
| 66. | Geranyl acetate | 1379 | 14.62 | 0.13 | 14.62 | 0.1 | | | 14.62 | 0.06 | | |
| 67.* | α-isocomene | 1387 | | | | | 14.76 | 0.17 | | | | |
| 68. | β-elemene | 1389 | | | | | | | 14.87 | 0.2 | 14.89 | 0.97 |
| 69.* | 7-epi-sesquithujene | 1390 | 14.83 | 0.08 | 14.84 | 0.07 | | | | | | |
| 70.* | (Z)-Jasmone | 1392 | 15.04 | 0.16 | 15.04 | 0.22 | 15.04 | 0.64 | 15.07 | 0.2 | | |
| 71. | Cyperene | 1398 | | | | | | | | | 15.11 | 12.76 |
| 72.* | β-longipinene | 1400 | | | 15.19 | 0.23 | | | 15.18 | 0.16 | | |
| 73.* | α-funebrene | 1402 | 15.15 | 0.08 | | | | | | | | |
| 74.* | β-isocomene | 1407 | | | | | 15.26 | 0.19 | | | | |
| 75.* | Longifolene | 1407 | | | | | | | 15.27 | 0.12 | | |
| 76. | α-gurjunene | 1409 | | | | | | | | | 15.44 | 0.67 |
| 77. | (E)-caryophyllene | 1417 | 15.59 | 4.54 | 15.61 | 6.43 | 15.61 | 20.59 | 15.68 | 26.62 | 15.59 | 5.96 |
| 78.* | *cis*-carvyl propanoate | 1420 | 15.65 | 0.92 | | | | | | | | |
| 79.* | α-*trans*-bergamotane | 1432 | | | | | | | | | 15.93 | 0.21 |
| 80. | Aromadendrene | 1439 | | | | | | | | | 16.01 | 0.77 |
| 81.* | α-himachalene | 1449 | | | | | | | | | 16.29 | 0.41 |
| 82. | α-humulene | 1452 | 16.43 | 0.13 | | | | | 16.42 | 0.64 | | |
| 83. | Geranyl acetone | 1453 | | | | | | | 16.34 | 0.05 | | |
| 84. | (E)-β-farnesene | 1454 | | | 16.44 | 0.49 | 16.43 | 0.73 | | | 16.46 | 8.98 |
| 85.* | *trans*-carvyl propanoate | 1454 | 16.54 | 0.16 | | | | | | | | |
| 86.* | γ-decalactone | 1464 | | | | | 16.52 | 0.81 | | | | |
| 87. | γ-gurjunene | 1475 | | | | | | | | | 16.94 | 1.44 |
| 88.* | γ-himachalene | 1481 | | | | | | | | | 17 | 1.81 |
| 89. | Germacrene D | 1484 | 17.09 | 0.89 | 17.1 | 0.96 | 17.09 | 1.64 | 17.11 | 3.97 | 17.1 | 0.15 |
| 90.* | *cis*-eudesma-6,11,diene | 1489 | | | | | | | | | 17.16 | 0.37 |
| 91.* | α-zingiberene | 1493 | | | | | | | 17.36 | 0.12 | | |
| 92.* | α-selinene | 1498 | | | | | | | | | 17.44 | 0.12 |
| 93. | α-muurolene | 1500 | | | | | | | | | 17.54 | 0.52 |
| 94. | Bicyclogermacrene | 1500 | 17.47 | 0.38 | 17.47 | 0.9 | 17.47 | 0.69 | 17.47 | 1.23 | | |
| 95. | (E,E)-α-farnesene | 1505 | | | 17.69 | 0.31 | 17.69 | 0.28 | 17.69 | 0.56 | | |
| 96. | β-bisabolene | 1505 | 17.71 | 0.18 | | | | | | | 17.74 | 9.44 |
| 97. | δ-cadinene | 1522 | | | | | | | 18.09 | 0.1 | | |
| 98.* | Italicene epoxide | 1547 | 18.79 | 0.31 | 18.79 | 0.13 | | | 18.79 | 0.26 | 18.79 | 0.31 |
| 99. | (E)-nerolidol | 1561 | | | 19.02 | 0.19 | 19.03 | 0.18 | | | | |
| 100. | epi-longipinanol | 1562 | 18.94 | 0.11 | 18.94 | 0.03 | | | 18.93 | 0.04 | 18.94 | 0.42 |
| 101.* | Germacrene d-4-ol | 1574 | | | 19.35 | 0.06 | | | | | | |
| 102. | Spathulenol | 1577 | 19.43 | 0.3 | 19.43 | 0.33 | | | | | | |
| 103. | Caryophyllene oxide | 1582 | 19.52 | 0.78 | 19.53 | 0.57 | 19.53 | 6.51 | 19.54 | 4.89 | 19.52 | 2.54 |
| 104.* | Thujupsan-2α-ol | 1586 | | | | | | | 19.76 | 0.05 | | |
| 105.* | β-atlantol | 1608 | | | | | | | | | 20.1 | 0.81 |
| 106. | Humulene epoxide II | 1608 | | | | | | | 20.12 | 0.1 | | |
| 107.* | anti-syn-syn-helifolen-12-al[c] | 1619 | | | | | | | | | 20.22 | 0.27 |
| 108.* | 2-epi-α-cedren-3-one | 1626 | | | | | | | | | 20.36 | 0.31 |
| 109.* | Muurola-4,10(14)-dien-1-β-ol | 1630 | | | | | | | | | 20.52 | 0.16 |

**Table 1: Continued**

| No. | Compounds/classification | RI[a] | TPSV | | TPSF | | TPF | | TPS | | TPR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RT[b] | Area% | RT | Area% | RT | Area% | RT | Area% | RT | Area% |
| 110. | allo-aromadendrene epoxide | 1639 | 20.77 | 0.27 | 20.8 | 0.14 | | | 20.8 | 0.52 | 20.78 | 0.53 |
| 111.* | Selina-3,11-dien-6-α-ol | 1642 | | | | | 20.81 | 0.52 | | | | |
| 112.* | 14-hydroxy-(Z)-caryophyllene | 1666 | | | | | 21.18 | 0.13 | | | | |
| 113.* | Helifolenol B | 1679 | | | | | | | | | 21.63 | 0.1 |
| 114.* | Germacra-4(15),5,10(14)-trien-1α-ol | 1685 | | | | | | | 21.9 | 0.08 | | |
| 115.* | (Z)-α-*trans*-bergamotol | 1690 | | | | | | | | | 21.9 | 0.14 |
| 116. | 2-pentadecanone | 1697 | | | | | | | 22.02 | 0.1 | | |
| 117.* | 14-hydroxy-4,5-dihydro-caryophyllene | 1706 | | | 22.36 | 0.04 | | | | | | |
| 118.* | (Z)-α-atlantone | 1717 | | | | | | | | | 22.49 | 0.8 |
| 119.* | 6S,7R-bisabolone | 1748 | | | | | | | | | 23.15 | 0.27 |
| 120.* | (E)-α-atlantone | 1777 | | | | | | | | | 23.89 | 0.42 |
| 121. | *n*-nonadecane | 1900 | | | | | | | 26.22 | 0.22 | | |
| 122.* | Phytol | 1942 | | | | | 26.74 | 0.22 | | | | |
| 123.* | Bergapten | 2056 | | | | | | | | | 26.84 | 39.68 |
| 124.* | *n*-heneicosane | 2100 | | | | | | | 30.15 | 0.41 | | |
| 125.* | Oleic acid | 2141 | 30.46 | 0.61 | | | | | | | | |
| | Total No. & percentage (%) | | **59 -(96.22%)** | | **63 (94.14%)** | | **42 -(93.16%)** | | **58 -(95.19%)** | | **31 (92.33%)** | |
| | Monoterpene hydrocarbon | % | **41%** | | **39%** | | **18%** | | **30%** | | **0.5%** | |
| | Oxygenated monoterpene | % | **47%** | | **48%** | | **39%** | | **27%** | | **0%** | |
| | Sesquiterpene hydrocarbon | % | **2%** | | **3%** | | **4%** | | **8%** | | **40%** | |
| | Oxygenated sesquiterpene | % | **8%** | | **9%** | | **34%** | | **35%** | | **13%** | |
| | Oxygenated diterpene | % | **0%** | | **0%** | | **1%** | | **0%** | | **0%** | |
| | Furanocoumarins (FCs) | % | **0%** | | **0%** | | **0%** | | **0%** | | **43%** | |
| | Others | % | **2%** | | **2%** | | **5%** | | **1%** | | **4%** | |

[a]RI, Retention indices relative to C6–C24, *n*-alkanes on the DB-5 column
[b]RT, Retention time
*Compounds identified for the first time in *Tagetes patula*

capacity based on the ability to reduce ferric ions of samples was calculated from the linear calibration curve and expressed as μmol $Fe^{+2}$ per gram of fresh weight (μmol $Fe^{+2}$/g FW).

### Statistical analysis

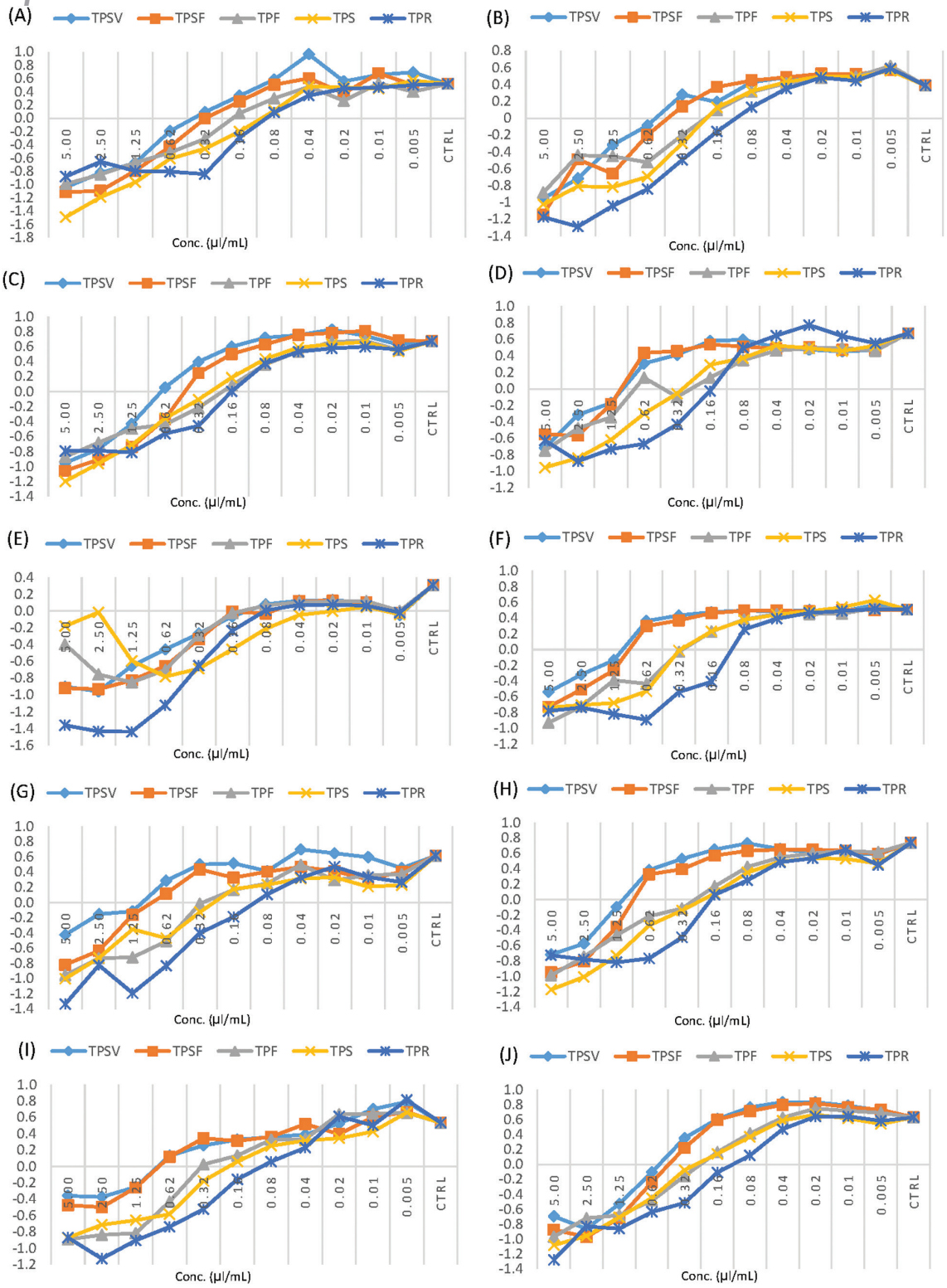
For analyzing antibacterial activity, the experiment was performed in a completely randomized design (CRD) with three replicates for each treatment, including a control to assess the efficiency of EOs against the tested strains. For analyzing antioxidant activity, statistical analysis was carried out using factorial CRD between the EOs and the time interval. All results were expressed as mean ± standard error (SE). These evaluations were performed by two-way analysis of variance, and statistical significance between mean values was carried out by the least significant difference (LSD) test using the Statistical Package for the Social Sciences software of SPSS, version 18, for Windows (SPSS, Chicago, Illinois). The values of $P < 0.01$ were considered statistically significant.

## Results and Discussion

### Chemical constituents of essential oils

The obtained volatile components from different parts of *T. patula* (TPSV, TPSF, TPF, TPS, and TPR) are shown in Table 1. The components are listed in order of their elution on the DB-5 column. All compounds and their percentages were identified by comparing and matching the mass spectra and GC retention indices of the unknown compounds with those of reference. The maximum oil yield was found in TPSV (0.057%) followed by TPS (0.042%), TPSF (0.037%), TPF (0.022%), and lowest in TPR (0.012%). In total, 125 chemical compounds were identified from the EO of studied species. The total amount of identified EOs at TPSV and TPSF were 59 and 63 compounds representing 96.22% and 94.14%, respectively, whereas 42, 58, and 31 compounds were isolated from the TPF, TPS, and TPR parts expressing 93.16%, 95.19%, and 92.33%, respectively [Table 1].

The chemical constituents of EOs as in Table 1 were revealed to be variable and similar among *T. patula* parts, and the percentage of each component also showed high variation. Monoterpene hydrocarbons characterized by terpinolene, (Z)-β-ocimene, sylvestrene, (E)-ocimenone, (Z)-ocimenone, piperitone, piperitenone, (Z)-tagetone, (E)-tagetone, and sesquiterpene (E)-caryophyllene were the most prolific components of TPSV and TPSF with quantitatively variation. Similar compositions are cited in studies with the EO constituents from the aerial part of *T. patula*.[4,15] In addition, the EO chemical profiles of TPF and TPS closely resembled




**(Figure 1)** Antibacterial activity of different concentrations of EOs from *T. patula* on: A) *S. fonticola*, B) *K. pneumoniae*, C) *A. baumannii*, D) *P. mirabilis*, E) *E.coli*, F) *S. aureus*, G) *S. epidermidis*, H) *S. saprophyticus*, I) *S. agalactiae*, J) *S. oralis*

**Figure 1: Antibacterial activity of different concentrations of essential oils from *Tagetes patula* on: (A) *Serratia fonticola*, (B) *Klebsiella pneumoniae*, (C) *Acinetobacter baumannii*, (D) *Proteus mirabilis*, (E) *Escherichia coli*, (F) *Staphylococcus aureus*, (G) *Staphylococcus epidermidis*, (H) *Staphylococcus saprophyticus*, (I) *Streptococcus agalactiae*, and (J) *Streptococcus oralis***

each other, with at least 20 components occurring in both parts. Among the components, (E)-caryophyllene, (E)-ocimenone, (Z)-β-ocimene, piperitenone, (Z)-ocimenone, caryophyllene oxide, sylvestrene, (E)-myroxide, car-3-en-2-one, germacrene D, terpinolene, and (E)-epoxy-ocimene were the highest constituted oil. Our results resemble the composition reported

by previous studies with some differences in the quantitative data.[1,7,16,17]

Conversely, the composition of the TPR oil was considerably different from the rest of the aerial parts. Bergapten (39.68%) proved to occur in very high concentrations followed by cyperene (12.76%), β-bisabolene (9.44%), (E)-β-farnesene (8.98%), and (E)-caryophyllene (5.96%). The consistency of the oil was also notably different from the oil from aerial parts due to it forming a solid crystalline substance. It is interesting to note that thiophenes, the main constituents isolated by several previous studies[18-20] are absent in our oil, similar to the finding in a study by Romagnoli *et al.*[9]

Correspondingly, more than 60 new compounds were isolated in this study for the first time in *T. patula*, which include bergapten, sylvestrene, (E)-β-farnesene, (E)-epoxy-ocimene, (Z)-jasmone, γ-gurjunene, γ-himachalene, β-bisabolene, (E)-myroxide, geijerene, and italicene epoxide. Consequently, the variations of oil components between the plant parts and their ratios might be affected by several factors including used plant part as well as the developing stage.[4,21]

### Antibacterial activity of essential oils of *Tagetes patula*

The antibacterial activity of different EOs of *T. patula* (TPSV, TPSF, TPF, TPS, and TPR) against all tested bacteria is shown in Figure 1. In general, the EOs showed high-to-mild antibacterial activity against all tested bacterial strains. The TPF and TPR EOs showed considerably more activity than the other parts. Minimum antibacterial activity was shown by both TPSV and TPSF EOs. In general, all the EOs were able to inhibit the tested bacteria with different ratios. The results of the determination of the antimicrobial activity of all five EOs of *T. patula* are presented as MIC and MBC values [Table 2]. The values of the MIC are those obtained after 24 h incubation at 37°C; MBC was determined by streaking out a sample on Mueller Hinton agar followed by incubation for a further 24 h at 37°C. The range of MIC and MBC of EOs recorded was between 0.16 and 0.64 µL/mL. In this investigation, the lowest MIC and MBC were recorded against *E. coli* by TPS EO with the values 0.08 and 0.32 µL/mL, respectively. According to the comparison between the effects of EOs and the positive control, ciprofloxacin antibiotic (CIP), the results showed a relatively significant difference between them. On the basis of this study, we can consider that the EOs of *T. patula* possessed strong-to-mild antibacterial activity against tested gram-positive and gram-negative bacteria strains. This observation is in agreement with other studies.[15,22]

EOs are natural plant products that contain a complex mixture of components; therefore, they have multiple antimicrobial properties. Most of these compounds are derived from oxygenated terpenoids, particularly phenolic terpenes, phenylpropanoids, and alcohols.[23,24] Our results support the notion that the strong biological activity of *T. patula* EO against the test pathogenic organisms is attributed to the presence of terpinolene, (Z)-β-ocimene, sylvestrene, (E)-ocimenone, (Z)-ocimenone, piperitone, piperitenone, (E)-caryophyllene, (Z)-tagetone, (E)-tagetone, caryophyllene oxide, car-3-en-2-one, germacrene D, (Z)-epoxy-ocimene, (E)-epoxy-ocimene, and (E)-myroxide in the aerial parts, and bergapten, cyperene, β-bisabolene, (E)-β-farnesene, (E)-caryophyllene, caryophyllene oxide, γ-himachalene, and γ-gurjunene in the root part. Likewise, major compounds such as piperitone, terpinolene, β-caryophyllene, piperitenone, dihydrotagetone, *cis*-tagetone, limonene, allo-ocimene, limonene, (Z)-β-ocimene, E-caryophyllene, piperitone, *P*-cymen-8-ol, (E)-β-ocimene, (Z)-tagetone, (Z)-tagetenone, (E)-tagetenone, (E)-tagetone, δ-elemene, linalool, and α-terthienyl obtained from other countries have been found to show significant biological activities including gram-positive and gram-negative bacteria strains.[5,6,9,15,25-27] Moreover, a number of EOs with low activities due to their high hydrocarbon content can also be used to interact with each other, acting as synergistic agents to increase their bioactivities.[23]

### Antioxidant activity of essential oil of *Tagetes patula*

The FRAP assay is a simple and reproducible method, which can be applied to the study of the antioxidant capacity of pure dietary antioxidants.[14] The antioxidant activity results of five *T. patula* EO parts (TPSV, TPSF, TPF, TPS, and TPR), which

**Table 2: Minimum inhibition concentration (µL/mL) and minimum bacterial concentration (µL/mL) of *Tagetes patula* essential oils against test bacterial strains**

| Test bacteria | EOs concentration (µL/mL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TPSV | | TPSF | | TPF | | TPS | | TPR | |
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| ***Serratia fonticola*** | 0.32 | 0.64 | 0.32 | 0.64 | 0.16 | 0.32 | 0.16 | 0.64 | 0.16 | 0.64 |
| ***Klebsiella pneumoniae*** | 0.64 | 1.25 | 0.64 | 1.25 | 0.32 | 0.64 | 0.32 | 1.25 | 0.16 | 2.5 |
| ***Acinetobacter baumannii*** | 1.25 | 2.5 | 0.64 | 1.25 | 0.32 | 1.25 | 0.32 | 1.25 | 0.32 | 2.5 |
| ***Proteus mirabilis*** | 1.25 | 2.5 | 1.25 | 2.5 | 0.32 | 0.64 | 0.32 | 2.5 | 0.32 | 1.25 |
| ***Escherichia coli*** | 0.16 | 0.64 | 0.16 | 0.32 | 0.16 | 0.64 | 0.08 | 0.32 | 0.16 | 0.64 |
| ***Staphylococcus aureus*** | 1.25 | 2.5 | 0.64 | 1.25 | 0.32 | 1.25 | 0.32 | 1.25 | 0.16 | 0.32 |
| ***Staphylococcus epidermidis*** | 0.32 | 1.25 | 1.25 | 2.5 | 0.32 | 1.25 | 1.25 | 2.5 | 0.16 | 0.64 |
| ***Staphylococcus saprophyticus*** | 0.32 | 2.5 | 1.25 | 5.0 | 0.32 | 2.5 | 1.25 | 5.0 | 0.32 | 2.5 |
| ***Streptococcus agalactiae*** | 0.64 | 1.25 | 0.64 | 1.25 | 0.32 | 1.25 | 0.32 | 1.25 | 0.16 | 1.25 |
| ***Streptococcus oralis*** | 0.32 | 0.64 | 0.64 | 1.25 | 0.32 | 1.25 | 0.64 | 1.25 | 0.16 | 0.64 |

**Table 3: Antioxidant capacity of essential oils from different parts of *Tagetes patula*, determined by the ferric-reducing antioxidant power assay at 5, 25, and 50 min of reaction**

| Plant part | *FRAP value (µmol/g)* | | |
|---|---|---|---|
| | After 5 min | After 25 min | After 50 min |
| TPSV | 81.56 ± 4.52 | 98.69 ± 5.47** | 100.64 ± 5.68** |
| TPSF | 57.38 ± 4.87 | 68.43 ± 5.90 | 71.20 ± 6.13 |
| TPF | 86.19 ± 1.41 | 103.28 ± 1.70** | 105.46 ± 1.77** |
| TPS | 222.88 ± 11.85** | 248.17 ± 13.04** | 259.69 ± 14.34** |
| TPR | 0.00 | 0.00 | 0.00 |
| Ascorbic acid | 76.33 ± 0.91 | 76.33 ± 0.91 | 76.33 ± 0.91 |
| LSD value | EO: 11.597 | Time: 6.35 | EO*Time: 20.09 |

**Significant at $P < 0.01$

FRAP assay expressed as µM of $FeSO_4.7H_2O$

Significantly different at $P < 0.01$, level of confidence based on LSD's multiple range test. Mean values denoted with a double star within the same column were observed to differ significantly (three replications ± standard error (SE)

were recorded at three different times to evaluate the reducing power of ferric tripyridyltriazine ($Fe^{+3}$-TPTZ) complex to its ferrous ($Fe^{+2}$-TPTZ) blue-colored form with an absorption maximum at 593nm[14] are shown in Table 3. FRAP value was significantly different depending on the plant parts and the time lapse. After 5-min reaction, the TPS-EO (222.88 ± 11.85) showed a significantly potent FRAP reduction, that is, three times greater than positive control ($P < 0.01$). Similar results were found after 25 and 50 min of reaction. In TPSV and TPF, at 5 min of reaction, results were markedly similar to the positive control (ascorbic acid), but they significantly showed FRAP reducing activity after 25 min (98.69 ± 5.47 and 103.28 ± 1.70, $P < 0.01$) and 50 min (100.64 ± 5.68 and 105.46 ± 1.77, $P < 0.01$), respectively. The TPR-EO of this species did not show antioxidant activity [Table 3].

Similar results have also been reported by other studies.[7,28] The strong antioxidant properties of the aerial part EOs of *T. patula* might be attributed to a comparatively high content of oxygenated terpenoids components or the synergistic activity between a variety of major and minor constituents. Furthermore, the differences in the antioxidant reactivity of these compounds affected the EOs ranking by the time of the reaction. Our results are in agreement with those findings that the antioxidants follow diverse kinetic behavior with time-dependent increase in their ferric-reducing ability.[14] Formerly, Henríquez *et al.*[29] emphasized that the time of reaction is one of the most important factors regarding the quantitation of the biological antioxidant potency of the plant extracts. However, our results showed that the antioxidant activity of the EOs depends on (1) the part of the plant and (2) the time of the reaction.

## Conclusion

This study showed that chemical characteristics, antimicrobial and antioxidant efficacy of EOs obtained from *T. patula* vary among the analyzed plant parts. The difference in chemical composition (qualitatively and quantitatively) of our investigation and the reported data was likely due to plant parts and environmental conditions. Among the recorded new compounds in the study, bergapten, sylvestrene, (E)-β-farnesene, (E)-epoxy-ocimene, (Z)-jasmone, γ-gurjunene, γ-himachalene, β-bisabolene, (E)-myroxide, geijerene, and italicene epoxide were the major chemical constituents. *E. coli* (MIC = 0.08 µL/mL) was the most affected microorganism by *T. patula* EOs. Also, among all EOs isolated from the tested plant, TPS had the highest antioxidant capacity at three different test times. One possible mechanism of the biological efficacy of these EOs appears to be related with the presence of either the high amount of oxygenated terpenoids (more than 50%) and fewer hydrocarbons in the aerial parts or with the synergistic activity of some major and minor components. Therefore, the plant could be a potential candidate source of natural antibacterial and antioxidant agents.

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## Conflicts of interest

There are no conflicts of interest.

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