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# Comparative analysis of chemical composition and antioxidant activity of essential oil isolated from orange and red marigold (*Tagetes patula* L.) flower petals

*Original Article*

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*Abstract:*

The present study aimed to determine and compare chemical composition and antioxidant activity of essential oils (EOs) isolated from marigolds (*Tagetes patula* L.) cultivated in the garden in southeast Serbia. The EOs were isolated from dry orange and red flower petals by Clevenger type hydrodistillation during 2 h by using 1:15 m/V hydromodule. Their qualitative composition was determined by GC/MS and quantitative by GC/FID method. The antioxidant activity was determined by using the DPPH assay. The most abundant components in the essential oil isolated from orange flower petals were geranyl acetate (36.7%) and (*E*)-caryophyllene (31.6%) while the one isolated from the red flower petals contained (*E*)-caryophyllene (69.4%) in the highest percentage. Since phototoxic thiophenes were identified in both EOs, they should not be used as components in cosmetic products for applications on areas of skin exposed to sunshine. Essential oils showed similar antioxidant activity, with orange flower EO being somewhat better.

*Key words:*
marigold, *Tagetes patula* L. flower petals, thiophenes, GC/MS, antioxidant activity

*Apstrakt:*

**Uporedna analiza hemijskog sastava i antioksidativne aktivnosti etarskog ulja izolovanog iz narandžastih i crvenih cvetnih latica baštenske kadife (*Tagetes patula* L.)**

Cilj ovog rada bio je određivanje i poređenje hemijskog sastava i antioksidativne aktivnosti etarskih ulja izolovanih iz baštenske kadife (*Tagetes patula* L.) gajene u jugostočnoj Srbiji. Etarska ulja su izolovana iz suvih narandžastih i crvenih cvetnih latica Clevenger hidrodestilacijom u toku 2 h pri hidromodulu 1:15 m/V. Kvalitativni sastav izolovanih etarskih ulja određen je GC/MS metodom a kvantitivni sastav GC/FID metodom. Antioksidativna aktivnost određena je DPPH testom. Najzastupljeniji sastojci u etarskom ulju izolovanom iz narandžastih latica bili su geranil acetat (36.7%) i (*E*)-kariofilen (31.6%) dok je u etarskom ulju izolovanom iz crvenih latica (*E*)-kariofilen bio zastupljen u najvećem procentu. S obzirom na to da su fototoksični tiofeni identifikovani u oba etarska ulja, ova etarska ulja ne bi trebalo koristiti kao komponente u kozmetičkim proizvodima namenjenim nezi delova tela izloženih sunčevim zracima. Etarska ulja su pokazala slična antioksidativna svojstva, pri čemu je etarsko ulje izolovano iz narandžastih cvetnih latica nešto bolje.

*Ključne reči:*
baštenska kadifa, *Tagetes patula* L. cvetne latice, tiofeni, GC/MS, antioksidativna aktivnost

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## Introduction

The life of people and animals on the Earth, as members of delicate world ecosystem, is possible due to the most important link of the chain with which they live in symbiosis – plants. People use plants more than 10,000 years. They cultivate them, consume (as the main source of vitamin C), and use as animal feed, as well as for fuel production (Parisi et al., 2010). The "dark side" of ethnobotany is reflected in phytodermatosis (dermatitis caused by plants) occurrence. It could be caused by the direct contact with plants or by association with sunlight. Skin reactions caused by contact with plants could

be divided as follows: dermatitis by physical trauma, dermatitis by pharmacological action, IgE-mediated dermatitis, dermatitis by irritation, sunlight-induced dermatitis and dermatitis by sensitization. The letter occurs when sensitizing substances are present. Among other plant families, these substances are present in ornamental plants such as chrysantemum, daisies, and marigolds from sunflower (Asteraceae) family (dos Reis, 2010).

The genus *Tagetes* consists of annual and perennial herbaceous plants, commonly called marigolds. It is native to the area from south-western America to Argentina, with maximum variations occurring in Mexico, but also naturalized all over the globe (Singh et al., 2015). Phytochemical studies of different plant parts revealed the presence of phenylpropanoids, carotenoids (such as lutein, zeaxanthin, neoxanthin plus violaxanthin, β-carotene, lycopene, α-cryptoxanthin, phytoene and phytofluene), flavonoids, thiophenes, and triterpeniods (Priyanka et al., 2013; Salehi et al., 2018; Singh et al., 2020). There are at least fifty-six *Tagetes* species (Salehi et al., 2018) and the most widely known are *T. erecta* (also known as Mexican marigold), *T. patula* (French marigold, dwarf marigold) and *T. minuta* (black mint or stinking roger). They are mainly studied in the field of agriculture, because of fungicidal, bactericidal, and insecticidal activities (Singh et al., 2015; Salehi et al., 2018). *T. minuta* is mainly cultivated for its essential oil, while *T. patula* and *T. erecta* have floricultural use. The flowers of African marigolds are yellow to orange and do not include red coloured marigolds, while French marigolds have red, orange and yellow as well as red and orange bicolour patterns flowers (**Fig. 1**).

The marigold species are traditionally used as analgesics, antiseptics, carminatives, diuretics, stimulants *etc*. in Mexico; in food seasoning and for insect repellence in USA; and in ceremonial purposes in India, Mexico and Guatemala. Recently, they have been recognised as commercial resources of essential oils and biologically active compounds for potential use as agrochemicals, colorants and nutritional supplements as well as in cosmetics (Singh et al., 2015).

The aerial parts of marigolds are rich sources of strongly aromatic EO (*Tagetes* oil), mainly used for perfumes production (Singh et al., 2020). Generally, *Tagetes* essential oils are rich in monoterpene hydrocarbons (such as ocimenes, limonene, *etc*.) and in acyclic monoterpene ketones (such as tagetone, dihydrotagetone, and tagetenone) which are the primary odorants – **Fig. 2**. There are also sesquiterpene hydrocarbons and oxygenated compounds but in much lower amounts. However, the chemical diversity is quite high (Tomova et al., 2005; Singh et al., 2015).

Marigolds are very popular as garden plants in southeastern Serbia. However, there is scarce information on the chemical composition and antioxidant properties of their essential oil. Therefore, the aim of the present study was to determine the chemical composition of essential oils isolated from red and orange *Tagetes patula* L. flower petals grown in southeastern Serbia in order to detect potentially present phototoxic compounds as well as to determine and compare their antioxidant activities.

## Materials and Methods

### *Plant material*

The seeds of *Tagetes patula* L. (Tagetes Patula Mix 5436) were purchased in local agricultural pharmacy (producer: Semenarna Ljubljana, Slovenia). Plant material was cultivated in the garden in village Manastirište (municipality of Vlasotince, southeastern Serbia; 42.9612° N, 22.1590° E). The orange and red flowers in their full flowering state (July and August 2022) were collected and dried in a shadowy place, at room temperature. Before


**Fig. 1.** The aerial parts of *Tagetes patula* L. (**a**, **c**) with dry orange (**b**) and red (**d**) flower petals used in this study

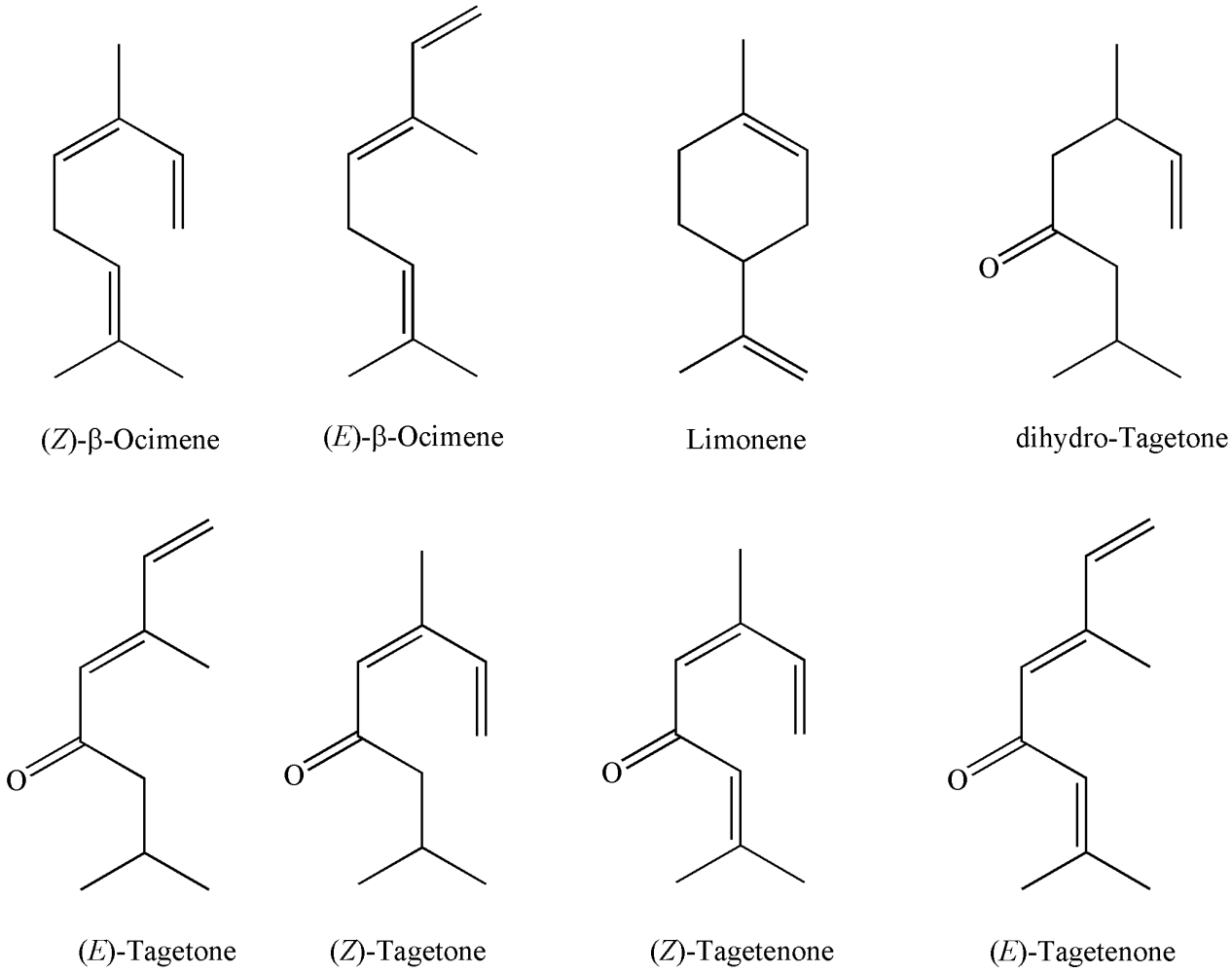



**Fig. 2.** Chemical structures of the primary odorants present in *Tagetes* EOs

the analysis, the dried flower petals were separated from the capitula and ground in an electric grinder (Moulinex Multi moulinette 3 in 1, 500 W).

### *Chemicals and reagents*

The following chemicals and reagents were used in this study: ethanol, 96% (Centrochem, Zemun, Serbia); 1,1-diphenyl-2-picrylhydrazyl (DPPH radical), butylated hydroxytoluene (BHT); alkane standard solution $C_8$-$C_{20}$ (~40 mg/l each, in hexane), alkane standard solution $C_{21}$-$C_{40}$; (*E*)-caryophyllene (≥98.0%, sum of enantiomers); β-pinene (≥99%); γ-terpinene (97%); linalool (97%) (Sigma Chemical Company, St. Louis, MO, USA); terpinen-4-ol (97%, J&K Scientific Ltd., Beijing, China); HPLC grade hexane (≥95%, Fisher Scientific, UK), and redistilled water.

### *Isolation of essential oil*

The essential oils (EOs) were isolated by Clevenger type hydrodistillation from dry flower petals during 2 h by using 1:15 m/V hydromodule. The volatile compounds were collected in hexane (1 ml) added in a graduated tube of the apparatus. The hexane solution was separated from the water phase and evaporated at room temperature. The residual essential oil was measured gravimetrically and stored at 4 °C until analysed. The yield of the essential oils was determined in g per 100 grams of the extracted plant material (g/100 g p.m.).

### *Gas chromatography-mass spectrometry (GC/MS) and gas chromatography-flame ionization detection (GC/FID) analyses*

GC/MS analysis was performed on Agilent Technologies 7890B gas chromatograph, equipped with nonpolar, silica capillary column, HP-5MS (5% diphenyl- and 95% dimethyl-polysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA) and coupled with inert, selective 5977A mass detector of the same company. The essential oils were dissolved in diethyl ether in concentrations of ~19 mg/ml (OFEO) and ~33 mg/ml (RFEO). One μl of the solution prepared was injected to the GC column through a split/splitless inlet set at 220 °C in 20:1

split mode. Helium was used as the carrier gas, at a constant flow rate of 1 ml/min. The oven temperature increased from 60 °C to 246 °C at the rate of 3 °C/min. Temperatures of the MSD transfer line, ion source and quadrupole mass analyzer were set at 300 °C, 230 °C and 150 °C, respectively. The ionization voltage was 70 eV and mass range *m/z* 41-415.

GC/FID analysis was carried out under identical experimental conditions as GC/MS. The flows of the carrier gas (He), make up gas ($N_2$), fuel gas ($H_2$), and oxidizing gas (Air) were 1, 25, 30, and 400 ml/min, respectively. The temperature of the flame-ionization detector (FID) was set at 300 °C.

Data processing was performed using MSD ChemStation, MassHunter Qualitative Analysis and AMDIS_32 softwares (Agilent Technologies, USA). Retention indices of the components from the analyzed samples were experimentally determined using a homologous series of n-alkanes from $C_8$-$C_{20}$ and $C_{21}$-$C_{40}$ as standards. Essential oil constituents identification was based on the comparison of their retention indices ($RI^{exp}$) with those available in literature (Adams, 2007) ($RI^{lit}$); their mass spectra with those of authentic standard as well as with those from Willey 6, NIST2011 and RTLPEST3 libraries (MS) and wherever possible, by co-injection with an authentic standard (Co-I).

Quantification was done by an external standard method according to the procedure described by Sparkman et al. (2011). The standards used were in the concentration ranges as follows: β-pinene (0.125-2 mg/ml), linalool (1.67-15 mg/ml), γ-terpinene (0.75-5 mg/ml), terpinen-4-ol (0.0625-1 mg/ml) and (*E*)-caryophyllene (0.0625-1 mg/ml).

The response factor (RF) for each standard used was calculated as follows:

$$\mathbf{RF=Area_{std}/S_{sd}}$$

where $Area_{std}$ is the peak area of the analyte standard and $C_{std}$ is the concentration of the standard used. The values of the mean response factors for the 5-points calibration curves of each standard used were: $8.39\times10^6$; $2.45\times10^7$; $3.02\times10^7$; $8.36\times10^6$; and $6.83\times10^6$ for β-pinene, γ-terpinene, linalool, terpinen-4-ol, and (*E*)-caryophyllene, respectively.

Then, the mean value of response factors ($RF_{mean}=1.56\times10^7$ for RFEO and $1.51\times10^7$ for OFEO) was calculated and used for quantification according to the following formula:

$$\mathbf{C_x=Area_x/RF_{mean}}$$

where $C_x$ is the concentration of an analyte in the sample (in mg/ml of EO), $Area_x$ is the peak area of the analyte in the sample and $RF_{mean}$ is the mean response factor of the standards used (Sparkman et al., 2011). The calculated concentrations of the β-pinene, and γ-terpinene in RFEO were: 0.02 mg/ml and 0.03 mg/ml respectively, while the concentrations of linalool, terpinen-4-ol, and (*E*)-caryophyllene were: 0.16 mg/ml; 0.15 mg/ml; and 45.79 mg/ml in RFEO and 0.20 mg/ml; 0.11 mg/ml, and 12.12 mg/ml in OFEO, respectively.

Finally, the content of each component in the sample expressed in % was normalized to get percents according to the formula:

$$\mathbf{C\ (\%)=(C_x/\Sigma C_x)x100}$$

where $C_x$ is the concentration of each component in the sample and $\Sigma C_x$ is the total concentration of all components in the sample.

### *DPPH assay*

The ability of the essential oils to scavenge free DPPH radicals was determined using the DPPH assay. Essential oils were dissolved in ethanol and a series of different concentrations (0.4-13.5 mg/ml and 0.3-10.3 mg/ml for EO isolated from red (RFEO) and orange flower petals (OFEO, respectively)) were prepared. Ethanol solution of DPPH radical (0.3 ml, 300 μmol solution ($3\times10^{-4}$ mol/l)) was added to 0.75 ml of the prepared essential oil solutions („sample") and the absorbance was measured at 517 nm after 20 min, 40 min, 60 min, 90 min, and 120 minutes incubation with radical ($A_S$). Absorbance at 517 nm was determined for ethanolic solution of DPPH radical („control" - $A_C$), diluted in the aforementioned ratio (0.3 ml of the DPPH radical of the given concentration with 0.75 ml ethanol added) as well as for the ethanolic solution of the essential oil which is not treated with DPPH radical solution ("blank" - $A_B$). Ethanol was used as a blank. Free radical scavenging activity was calculated according to the formula:

$$\mathbf{DPPH\ radical\ scavenging\ capacity\ (\%)=100-[(A_S-A_B)x(100/A_C)]}$$

All absorbances were measured on UV-VIS VARIAN-Cary 100 Conc. Spectrophotometer. BHT (dissolved in ethanol; concentration range 0.04-1.3 mg/ml) was used as a positive control.

## Results and discussion

### *Qualitative and quantitative composition of essential oils*

The yields of pale yellow coloured essential oils were 0.03 g/100 g p.m and 0.01 g/100 g p.m for RFEO and OFEO, respectively. Total Ion Chromatograms (TICs) of EOs studied are given in **Fig. 3**, while their qualitative and quantitative composition is given in **Tab. 1**. The chemical structures of the most abundant compounds are shown in **Fig. 4**.

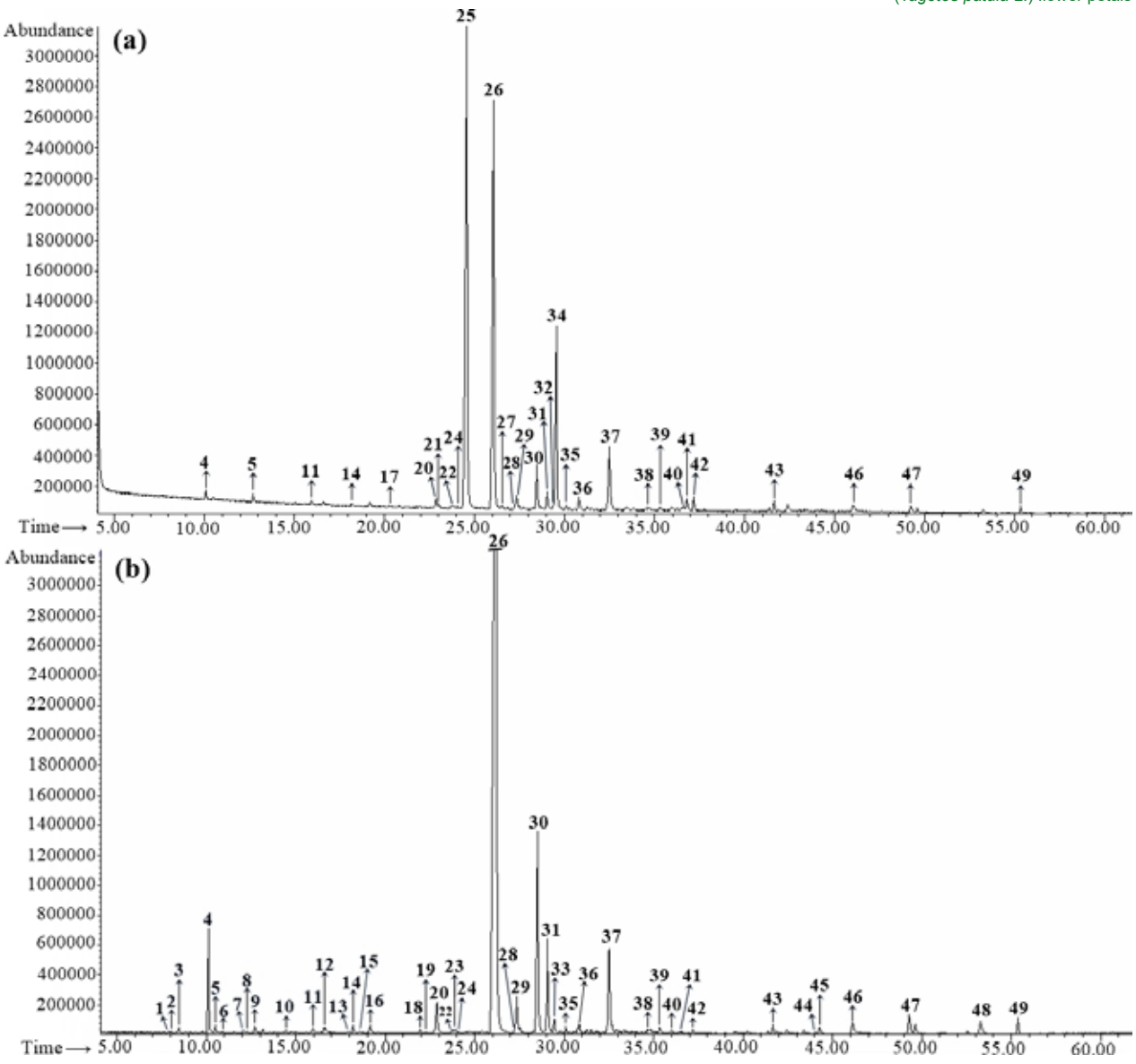
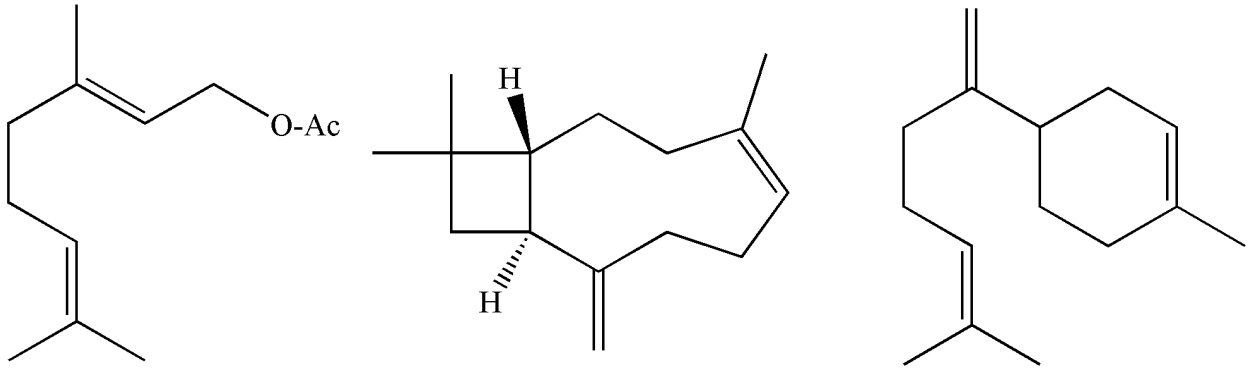



**Fig. 3.** TIC chromatogram of (**a**) OFEO, and (**b**) RFEO




Geranyl acetate (*E*)-Caryophyllene β-Bisabolene

**Fig. 4.** Chemical structures of the most abundant compounds present in isolated EOs

**Table 1.** Chemical composition of essential oils isolated from orange and red flower petals of *T. patula* L.

| No. | $t_{ret}$, min | Compound | $RI^{exp}$ | $RI^{lit}$ | Method of identification | Content (%) | | c (mg/ml of EO) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OFEO | RFEO | OFEO | RFEO |
| **1** | 7.92 | Sabinene | 965 | 969[a] | RI, MS | - | tr | - | tr |
| **2** | 8.03 | β-Pinene | 968 | 974[a] | RI, MS, Co-I | - | tr | - | tr |
| **3** | 8.46 | 2-Pentyl furan | 983 | 984[a] | RI, MS | - | tr | - | tr |
| **4** | 10.11 | (*Z*)-β-Ocimene | 1,030 | 1,032[a] | RI, MS | 0.4 | 2.7 | 0.1 | 0.9 |
| **5** | 10.49 | (*E*)-β-Ocimene | 1,040 | 1,044[a] | RI, MS | - | 0.2 | - | 0.1 |
| **6** | 10.92 | γ-Terpinene | 1,051 | 1,054[a] | RI, MS, Co-I | - | tr | - | tr |
| **7** | 12.06 | Terpinolene | 1,081 | 1,086[a] | RI, MS | - | tr | - | tr |
| **8** | 12.25 | *p*-Cymenene | 1,086 | 1,089[a] | RI, MS | - | tr | - | tr |
| **9** | 12.72 | Linalool | 1,098 | 1,095[a] | RI, MS, Co-I | 0.4 | tr | 0.1 | tr |
| **10** | 14.44 | (*E*)-Tagetone | 1,132 | 1,139[a] | RI, MS | - | tr | - | tr |
| **11** | 15.98 | Terpinen-4-ol | 1,169 | 1,174[a] | RI, MS, Co-I | tr | tr | tr | tr |
| **12** | 16.58 | *p*-Cymen-8-ol | 1,184 | 1,179[a] | RI, MS | - | tr | - | tr |
| **13** | 17.95 | Coahuilensol, methyl ether | 1,216 | 1,219[a] | RI, MS | - | tr | - | tr |
| **14** | 18.19 | (*Z*)-Ocimenone | 1,222 | 1,226[a] | RI, MS | tr | 0.3 | tr | 0.1 |
| **15** | 18.60 | (*E*)-Ocimenone | 1,232 | 1,235[a] | RI, MS | - | tr | - | tr |
| **16** | 19.15 | Piperitone | 1,245 | 1,249[a] | RI, MS | - | 0.3 | - | 0.1 |
| **17** | 20.30 | Isobornyl acetate | 1,276 | 1,283[a] | RI, MS | tr | - | tr | - |
| **18** | 21.97 | Silphiperfol-5-ene | 1,324 | 1,326[a] | RI, MS | - | tr | - | tr |
| **19** | 22.25 | Presilphiperfol-7-ene | 1,330 | 1,334[a] | RI, MS | - | tr | - | tr |
| **20** | 22.89 | Piperitenone | 1,337 | 1,340[a] | RI, MS | 0.5 | 1.5 | 0.1 | 0.5 |
| **21** | 23.04 | α-Terpinyl acetate | 1,340 | 1,346[a] | RI, MS | tr | - | tr | - |
| **22** | 23.73 | Eugenol | 1,357 | 1,356[a] | RI, MS, Co-I | tr | tr | tr | tr |
| **23** | 23.93 | Piperitenone oxide | 1,361 | 1,366[a] | RI, MS | - | tr | - | tr |
| **24** | 24.09 | α-Copaene | 1,365 | 1,374[a] | RI, MS | tr | tr | tr | tr |
| **25** | 24.59 | Geranyl acetate | 1,377 | 1,379[a] | RI, MS | 37.6 | - | 7.2 | - |
| **26** | 26.21 | (*E*)-Caryophyllene | 1,416 | 1,417[a] | RI, MS, Co-I | 31.6 | 69.4 | 6.0 | 22.8 |
| **27** | 26.51 | α-*trans*-Bergamotene | 1,423 | 1,432[a] | RI, MS | tr | - | tr | - |
| **28** | 27.30 | Geranyl acetone | 1,443 | 1,453[a] | RI, MS | tr | tr | tr | tr |
| **29** | 27.38 | α-Humulene | 1,444 | 1,452[a] | RI, MS | 1.0 | 1.7 | 0.2 | 0.6 |
| **30** | 28.53 | Germacrene D | 1,488 | 1,484[a] | RI, MS | 2.8 | 8.2 | 0.5 | 2.7 |
| **31** | 29.08 | Bicyclogermacrene | 1,490 | 1,500[a] | RI, MS | 1.1 | 3.6 | 0.2 | 1.2 |
| **32** | 29.39 | (*E*)-Methyl isoeugenol | 1,493 | 1,491[a] | RI, MS | tr | - | tr | - |
| **33** | 29.45 | (*E*,*E*)-α-Farnesene | 1,495 | 1,505[a] | RI, MS | - | 0.6 | - | 0.2 |
| **34** | 29.56 | β-Bisabolene | 1,498 | 1,505[a] | RI, MS | 12.5 | - | 2.4 | - |
| **35** | 30.10 | γ-Cadinene | 1,511 | 1,513[a] | RI, MS | tr | tr | tr | tr |
| **36** | 30.78 | (*E*)-γ-Bisabolene | 1,529 | 1,529[a] | RI, MS | 0.9 | 0.4 | 0.2 | 0.1 |
| **37** | 32.51 | Caryophyllene oxide | 1,574 | 1,582[a] | RI, MS | 5.7 | 4.5 | 1.1 | 1.5 |
| **38** | 34.65 | Caryophylla-4(12),8(13)-dien-5α-ol | 1,631 | 1,639[a] | RI, MS | tr | tr | tr | tr |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **39** | 35.32 | α-Cadinol | 1,648 | 1,652[a] | RI, MS | tr | tr | tr | tr |
| **40** | 36.63 | Shyobunol | 1,684 | 1,688[a] | RI, MS | tr | tr | tr | tr |
| **41** | 36.81 | Eudesm-7(11)-en-4-ol | 1,688 | 1,698[a] | RI, MS | 0.9 | tr | 0.2 | tr |
| **42** | 37.18 | (2*E*)-Tridecenol acetate | 1,698 | 1,703[a] | RI, MS | 0.8 | tr | 0.2 | tr |
| **43** | 41.65 | Hexahydrofarnesyl acetone | 1,850 | 1,846[b] | RI, MS | 0.6 | 0.3 | 0.1 | 0.1 |
| **44** | 44.05 | (5*Z*,9*E*)-Farnesyl acetone | 1,897 | 1,889[a] | RI, MS | - | tr | - | tr |
| **45** | 44.27 | 5-(3-buten-1-ynyl)-2,2'-bithiophene (BBT) | 1,902 | 1,892[c] | RI, MS | - | 0.4 | - | 0.1 |
| **46** | 46.12 | Palmitic acid | 1,960 | 1,959[b] | RI, MS | 1.2 | 1.9 | 0.2 | 0.6 |
| **47** | 49.29 | 5-(3-penten-1-ynyl)-2,2-bithiophene (PBT) | 2,053 | 2,043[c] | RI, MS | 0.7 | 1.5 | 0.1 | 0.5 |
| **48** | 53.28 | 2,2':5',2''-Terthiophene (α-Terthiophene) | 2,181 | 2,171[c] | RI, MS | - | 0.9 | - | 0.3 |
| **49** | 55.35 | Tricosane | 2,296 | 2,300[a] | RI, MS, Co-I | 0.5 | 0.7 | 0.1 | 0.2 |
| | Total identified | | | | | 99.2 | 99.1 | 19.0 | 32.6 |
| | Grouped components | | | | | (%) | | (mg/ml of EO) | |
| | Monoterpene hydrocarbons (1, 2, 4-8) | | | | | 0.4 | 2.9 | 0.1 | 1.0 |
| | Oxygenated monoterpenes (9-21, 23, 25, 28) | | | | | 38.5 | 2.1 | 7.4 | 0.7 |
| | Sesquiterpene hydrocarbons (24, 26, 27, 29-31, 33-36) | | | | | 49.9 | 83.9 | 9.5 | 27.6 |
| | Oxygenated sesquiterpenes (37-41, 43, 44) | | | | | 7.2 | 4.8 | 1.4 | 1.6 |
| | Phenylpropanoids (22, 32) | | | | | tr | tr | tr | tr |
| | Thiophenes (45, 47, 48) | | | | | 0.7 | 2.8 | 0.1 | 0.9 |
| | Others (3, 42, 46, 49) | | | | | 2.5 | 2.6 | 0.5 | 0.8 |

**$t_{ret}$.**: Retention time; **$RI^{lit}$** - Retention indices from literature ([a]Adams (2007); [b]Balogun et al. (2017); [c]Szarka et al. (2007)); **$RI^{exp}$**: Experimentally determined retention indices using a homologous series of n-alkanes (C8-C20 and C21-C40) on the HP-5MS column. **MS**: constituent identified by mass-spectra comparison; **RI**: constituent identified by retention index matching; **Co-I**: constituent identity confirmed by GC co-injection of an authentic sample; **tr** = trace amount (<0.05%; <0.1 mg/ml); **OFEO** - essential oil isolated from orange flower petals, **RFEO** - essential oil isolated from red flower petals

According to the results of GC/MS analysis, 30 and 42 compounds were identified in OFEO and RFEO, comprising 99.2%, and 99.1% of total EO composition, respectively.

The most abundant groups of compounds in OFEO were sesquiterpene hydrocarbons (49.9%) and oxygenated monoterpenes (38.5%). On the other side, sesquiterpene hydrocarbons with 83.9% in the total essential oil composition were the dominant group in RFEO (**Tab. 1**). Among sesquiterpene hydrocarbons (*E*)-caryophyllene was the dominant compound in both EOs, with 31.6% and 69.4%, in total OFEO and RFEO composition, respectively while β-bisabolene, with 12.5% was second most dominant compound in OFEO. β-bisabolene was not detected in RFEO. Among oxygenated monoterpenes, the dominant one was geranyl acetate with 37.6% in total OFEO composition (structures given in **Fig. 3**). It was not detected in RFEO. The results obtained are in agreement with the study by Szarka et al. (2007). The authors hydrodistilled EO from *T. patula* flowers that had been air-dried, and the most abundant compound found there was (*E*)-caryophyllene, which accounted for 50.2% (compared to 69.4% in herein study). Geographical origin plays an important role in the chemical diversity not just among species but even in the same species. For example, Krishna et al. (2002) analysed EOs of the capitula, leaves and shoots of *Tagetes patula* L. raised in the CIMAP Experimental

Farm at Lucknow (India). The main constituents of EO isolated from capitula were (*Z*)-β-ocimene (19.9%), (*Z*)-tagetenone (12.4%), (*E*)-tagetenone (10.4%), piperitenone (5.8%) and (*E*)-caryophyllene (15.1%) (Krishna et al., 2002). On the other hand, the EO isolated from cultivated *T. patula* flowers grown in Mandal (Uttarakhand, India) contained β-ocimene (22.11%), α-terpinolene (14.59%), and (*E*)-caryophyllene (12.69%) as the most abundant compounds (Negi et al., 2013). The most abundant components of the EO isolated from fresh flowers of *T. patula* grown in New Delhi (India) were (*E*)-caryophyllene (3.92-42.76%), germacrene-D (1.48-6.72%), (Z)-tagetone (1.29-4.38), caryophyllene oxide (0.68-24.3%), and piperitone oxide (0.11-1.23) (Tamut et al., 2019). In the EO isolated from *T. patula* flowers sampled during August in areas of Erbil Province (Iraq), (*E*)-caryophyllene (20.59%), and (*E*)-ocimenone (12.08%) were the most abundant components while geranyl acetate was not identified (Safar et al., 2020).

Zarate-Escobedo et al. (2018) reported the presence of geranyl acetate for genus *Tagetes* for the first time. They determined the chemical composition of the essential oil hydrodistilled from floral stems of 14 *T. lucida* populations from North and South of the State of Mexico, where six types of soils and six climatic conditions were detected. The plant material was collected from September to October 2014 and in September 2015. In Southern populations with a warm climate, the major compounds were monoterpenes: geranyl acetate (ranging from 12% to 40%) and β-ocimene (14% to 24%) depending on the location (Zarate-Escobedo et al., 2018).

It is postulated that terpenoid compounds play a role in an ecological interaction of plants with biotic and abiotic factors of their environment by defending plants from herbivores and pathogens (toxins or repellents) or being the signals and rewards to pollinators. The constant evolution of new terpenoids structures is enabled by the evolution of new genes encoding new enzymes capable of making such new metabolites (Pichersky & Raguso, 2016). What is more, according to the available literature data single compounds such as bisabolene, (*E*)-caryophyllene, camphor, (*E*)-β-farnesene, pinene, and linalol have been recognized as good repellents towards aphids and various pests (Hori, 1998; Isman, 2000; Halbert et al., 2009; Pascual-Villalobos et al., 2017; Dardouri et al., 2019). Taking into account that (*E*)-caryophyllene and β-bisabolene were present in considerable amounts in the EOs isolated in this study (**Tab. 1**), they could be considered as a potential source of natural pesticides.

It is well known that acyclic monoterpenes (ocimenones) including (*Z*)-β- and (*E*)-β-ocimene, (*Z*)- and (*E*)-tagetone and (*Z*)- and (*E*)-tagetenone are formed by chemical modification (such as hydrolysis, dehydration, oxidation and reduction which are catalyzed by specific enzymes) of either GPP or neryl pyrophosphate (NPP). On the other hand, the biosynthesis of sesquiterpenes proceeds through the precursor farnesyl pyrophosphate (FPP) which is formed by condensation of GPP with one molecule of IPP (Singh et al., 2015). Given that terpenoids' biosynthesis is genetically determined, finding the reason for the difference in chemical composition between OFEO and RFEO, regarding the most abundant components, goes beyond the scope of this paper.

Geranyl acetate is an acyclic monoterpenes ester with great economic value. It is widely used in cosmetic industry due to its „rose-like" odor. In *Cymbopogon* spp. geranyl acetate is biosynthesed by acetylation of geraniol catalyzed by geraniol acetyl transferase (GAT) (Ganjewala & Luthra, 2010). Phosphatase (GPPase) mediates the formation of geraniol while geranyl acetate esterase (GAE) catalizes deacetylation of geranyl acetate into geraniol. Thus, the content of geraniol depends on the relative activities of these three (GPPase, GAT, and GAE) enzymes. Geraniol was not identified in herein studied EO. The possible reason could be that the plant material was collected in the phase when GAE (catalyzing transformation of geranyl acetate to geraniol) had little or no activity. Having in mind that essential oils isolated from „geranyl-acetate rich" plants like palmarosa (*Cymbopogon martini* (Roxb.) Wats. var. *motia* Burk.) contained 4.3-14.8% of geranyl acetate (Rajeswara Rao et al., 2009); wild carrot (*Daucus carota* L. ssp. *carota*) mature umbels 16.5% (Staniszewska et al., 2005); lemongrass (*Cymbopogon flexuosus* (Steud) Wats.) 25.9% (Kulkarni et al., 1997); pastinocello fruits (*Daucus carota* ssp. *major*) 34.2% (Flamini et al., 2014); coriander (*Coriandrum sativum* L.) fruits 46.27% (Msaada et al., 2007); and the OFEO isolated in this paper (containing 37.6%) could be considered as a potential source of geranyl acetate.

The thiophenes, with BBT, PBT, and α-terthienyl as representatives, were also identified, with 0.7% and 2.8% in OFEO and RFEO, respectively (**Tab. 1**). Although the thiophenes are the main secondary metabolites of *Tagetes* roots in this paper they were identified in flower petals, which is in agreement with the study of Szarka et al. (2007). Namely, Szarka et al. (2007) studied the composition of EOs isolated from hairy roots, normal roots and flowers and the major thiophene of flower EO was PBT with 6.0% in the total EO composition (Szarka et al., 2007). The identity of thiophenes identified in this paper was confirmed by comparing mass spectrum from the hit

list in mass spectra libraries but also with the mass spectra given in the study of Szarka et al. (2006) as well as by comparing experimentally obtained retention indices with the retention indices given in the study of Szarka et al. (2007). On the other side, in the study of Arciniegas et al. (2020), the authors determined the antioxidant and photosensitizer activities of extracts and isolates of genus *Dyssodia* (Asteraceae, Tageteae). The antioxidant activity was determined by the interactions with copper ion ($Cu^{2+}$) observed in EPR, as well as by the DPPH and the thiobarbituric reactive substances (TBARS) methods. Their photosensitizer activities were observed as the the abilities to produce $_1O^2$ by electron paramagnetic resonance (EPR). They isolated seven thiophene derivatives, among others 2,2′:5′,2″-terthiophene, and 5-(3-buten-1-ynyl)-2,2′-bithiophene, which were identified in this study as well. Both thiophenes had no antioxidant activity determined by the DPPH assay, so they surely not contribute to the antioxidant activity of the EOs studied in this paper. On the other side, α-terthiophene and related compounds are known as photosensitizers by their activity to generate singlet oxygen ($^1O_2$) under UV irradiation regime (Arciniegas et al., 2020). What is more, these compounds, and especially α-terthienyl show enhanced nematocidal activity in the presence of sunlight (UV-A), but also antibiotic, ovicidal, algicidal, larvicidal, and antifeedant activities (D'Auria et al., 1987).

### *Antioxidant activity*

The antioxidant activity of isolated EOs was determined by the DPPH assay and compared both mutually, and with BHT as a positive control. Their DPPH radical scavenging activity determined after 20 min, 40 min, 60 min, 90 min, and 120 min incubation with the DPPH radical is shown in **Fig. 5**.

The $EC_{50}$ values obtained from the graphs given in **Fig. 5** are presented in the **Tab. 2**.

According to the results obtained, antioxidant activity of both EOs and BHT was dependant on both concentration and incubation time with the DPPH radical, as already stated (Stanojević et al., 2016). Generally, the duration of incubation time of DPPH radical with samples depends on the type of sample, taking longer time for interaction with the weak antioxidants (Bal et al., 2021). The effect of incubation time studied in this paper indicated that DPPH radical scavenging activity of both EOs and BHT reached the maximum after 120 minutes of incubation (**Tab. 2**). However BHT, as a phenolic representative of synthetic antioxidants used in this paper, reduced the 50% of the initial DPPH radical concentration (the $EC_{50}$ value) after 20 minutes of incubation and showed ~12 and ~14 times stronger

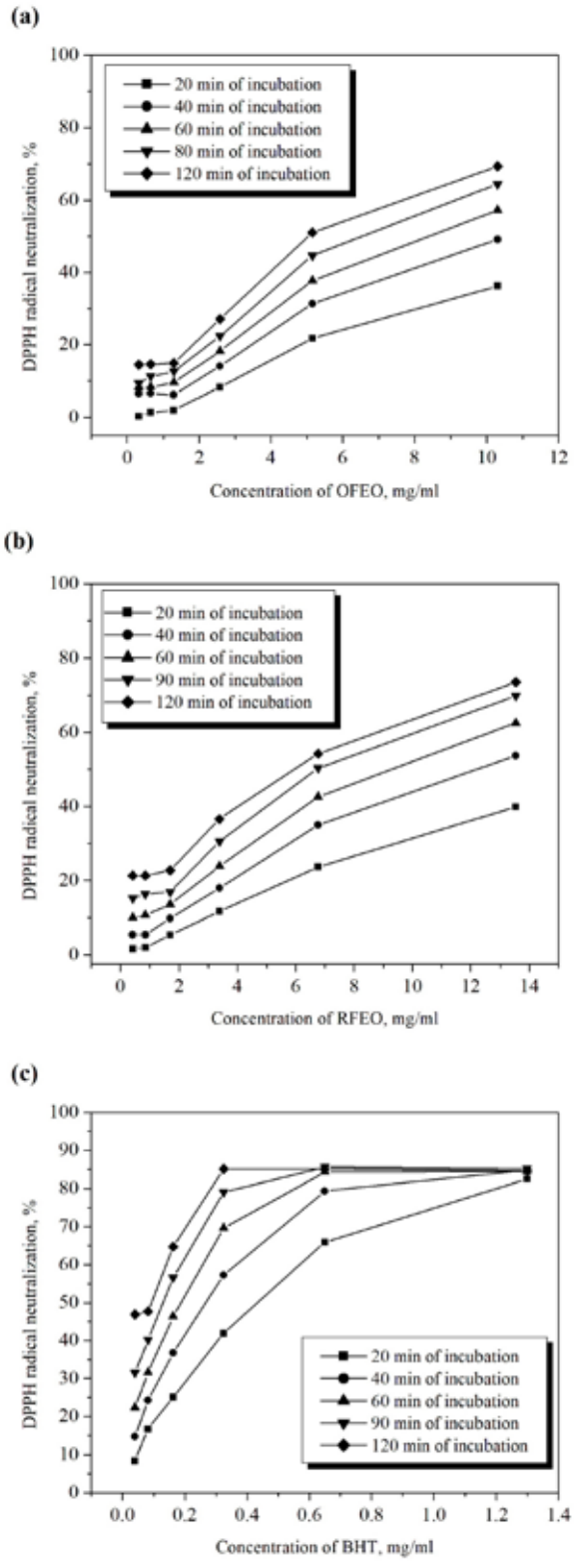



**Fig. 5.** Antioxidant activity of (**a**) OFEO, (**b**) RFEO, and (**c**) BHT

antioxidant activity in comparison to OFEO and RFEO after 120 minutes of incubation, respectively. Therefore, the isolated EOs had similar antioxidant

**Table 2.** The $EC_{50}$ values of isolated EOs and BHT

| | $EC_{50}$, mg/ml | | |
|---|---|---|---|
| **Incubation time** | **OFEO** | **RFEO** | **BHT** |
| 20 min | - | - | 0.43±0.002 |
| 40 min | - | 12.20±0.054 | 0.27±0.001 |
| 60 min | 8.41±0.014 | 9.29±0.050 | 0.19±0.001 |
| 90 min | 6.55±0.018 | 6.71±0.025 | 0.13±0.000 |
| 120 min | 5.04±0.000 | 5.96±0.000 | 0.09±0.001 |

activity and both are weaker antioxidants than BHT. Comparing the chemical composition of isolated EOs, the main groups were sesquiterpenes hydrocarbons (83.9% vs. 55.6%) and oxygenated monoterpenes (2.1% vs. 38.5%) in RFEO and OFEO, respectively. According to the study by Ruberto & Barrata (2000), oxygenated monoterpenes have better antioxidant activity in comparison to monoterpenes hydrocarbons, sesquiterpenes hydrocarbons, and oxygenated sesquiterpenes. The best antioxidant activity in the mentioned study showed phenolic compounds (such as thymol and carvacrol) due to their redox properties, and the ability to neutralize free radicals (Ruberto & Barrata, 2000). Considering that OFEO contained considerable amount of oxygenated monoterpenes (38.5%) in comparison to RFEO (2.1%), its slightly better antioxidant activity could be ascribed to their presence, especially to the presence of geranyl acetate due to its capacity to reduce free radical stability via electron or hydrogen donating mechanisms (Seema Farhath et al., 2013). On the other side, phenolic compounds (particularly eugenol) were identified in traces in both isolated EOs, so their weak antioxidant activity is not surprising.

## Conclusions

The essential oils hydrodistilled from dry red and orange *Tagetes patula* L. flower petals cultivated in south-eastern Serbia are rich sources of sesquiterpene hydrocarbons ((*E*)-caryophyllene in RFEO comprising 69.4% of total EO composition) and oxygenated monoterpenes (geranyl acetate in OFEO comprising 37.6% of total EO composition). They have also contained sulphurated tiophenes (BBT, PBT and TTP) with 2.8% and 0.7% in total RFEO and OFEO composition, respectively. Having in mind that α-terthienyl, also called terthiophene (TTP) and 5-(3-penten-1-ynyl)-2,2-bithienyl (PBT) are phototoxic compounds, they should not be used as components in cosmetic products for applications on areas of skin exposed to sunshine. Being weaker antioxidants in comparison to the synthetic antioxidant BHT, they could not be used as its natural alternative. On the other side, the overuse of synthetic pesticides causes pest resistance and makes them one of the major pollutants in soil and water, as well as toxic substances for humans and animals. Taking into account that (*E*)-caryophyllene and β-bisabolene present in considerable amounts in the EOs isolated in this study are recognized as good repellents (besides thiophenes). The authors would like to draw attention to marigolds, not just as garden ornamental flowers but also as a potential source and appropriate basis for future development of naturally based herbicides.

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