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# Chemical profile, antioxidant and photoprotective activities of essential oil and crude extracts of Algerian *Thymus serpyllum*

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**Abstract**

*Thymus serpyllum* L. is an aromatic and medicinal plant widely used in Algerian folk medicine. It was collected from the Mascara region in the North-West of Algeria and studied with the aim to provide more knowledges and update about chemical composition, antioxidant and skin-protective activities of essential oil, ethanolic and infusion extracts. The chemical analysis of investigated *T. serpyllum* essential oil (EO) was performed for the first time in this research work. It was carried out by GC/MS for identifying of 25 components where the dominated compounds were carvacrol (66 %) and γ–terpinene (11.5 %). Ethanolic and infusion extracts were analyzed using HPLC/DAD detector type chromatography and revealed that benzoic acid and rosmarinic acid were found as the major compounds. The antioxidant activity was determined using the DPPH, galvinoxyl radical (GOR), CUPRAC, reducing power, and *O*-phenanthroline methods. All extracts showed a significant antioxidant capacity with different mechanisms. However, ethanol and infusion extracts showed stronger capacity than EO. Moreover, the photo-protective (skin-protective) activity of *T. serpyllum* extracts was explored for the first time in our study. Extracts exhibited high values of Sun Protective Factors (SPF) with 38.34 ± 2.29 and 38.82 ± 2.23 for ethanol and infusion extract, respectively. These results suggest a potential use of *Thymus serpyllum* as a source of bioactive compounds with antioxidant and skin-protective properties.



## Introduction

A permanent exposure to various stimulus and aggressors can generate the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) with higher concentrations. These reactive species are produced by a variety of biochemical processes (e.g. production of energy ATP, biosynthetic and detoxification reactions) and can be neutralized by molecules called "antioxidants" (Martins *et al*. 2015; Yang *et al*. 2018). An excess of production with a low level of

antioxidants can lead to the appearance of almost all pathologies such as inflammations, cancer, Alzheimer, aging and skin diseases (Kindl *et al*. 2015). Therefore, some studies are in quest for natural products that can be applied orally or topically to ameliorate skin reactions, reverse and block UV radiations (Korać and Khambholja 2011). In food processing, one of the main problems is lipid oxidation that can occur especially during storage and distribution, leading to the development of disagreeable flavors and potential occurrence of toxic substances (Shahidi and Ho 2007). As a preventive strategy the antioxidants are remarkably used as additives. Therefore, an increasing interest in natural antioxidants provides aromatic and medicinal plants as an alternative (Zehiroglu and Sarikaya 2019). Among these plants, those belonging to *Thyme* genus comprising of 215 species, mainly prevalent in Mediterranean regions, North Africa, South Europe, and Asia (Jarić *et al*. 2015). *Thymus serpyllum* L. known as wild thyme and called "Zaatar" in regional language, grows naturally in the Mascara Mountains. Since antique times, infusions and decoctions of wild thyme were used in folk medicine. Fresh and dry leaves are used in Mediterranean kitchen as flavoring and preservative food agent (Nikolić *et al*. 2014). Based on the bioactivity of wild thyme reported in literature and no such investigations were performed about *T. serpyllum* of the Mascara region, the aim of this study was to explore the chemical profile and to determinate different mechanisms of antioxidant potential of EO, ethanol and infusion extracts. Further a skin-protective activity was investigated to estimate the sun protection factor (SPF).

## Experimental

*Plant material and reagents*

The aerial parts of investigated *Thymus serpyllum* L. were gathered during the flowering stage (May – July 2019) in the North-West of Algeria (Mascara region). Botanical identification was achieved by Prof. Benhassaini Hachemi and the voucher specimen (E01/LBV/UDL/2019) has been deposited at the Herbarium. The collected material (leaves and flowers) was dried in shade at a temperature 28 – 30 °C for 10 days, then grinded into fine powder.

The chemical products and reagents used in all experiments were: 1,1-diphenyl-2-picrylhydrazyl (DPPH), neocuproine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), trichloroacetic acid (TCA), 1,10-phenanthroline, potassium ferricyanide, ethanol, methanol, acetone (all were obtained from Sigma-Aldrich GmbH, Sternheim, Germany. The iron (III) chloride ($FeCl_3$), ammonium acetate, and copper (II) chloride ($CuCl_2$) were obtained from Biochem Chemopharma (Cosne-Cours-sur-Loire, France).

Magnetic stirrer and Clevenger were used for preparation of crude extracts and essential oil respectively. Antioxidant and photoprotective activities were carried out on a 96-well microplate reader EnSpire Multimode Plate Reader (PerkinElmer, Inc., Walthman, USA).

*Extracts preparation*

Essential oil (EO) was isolated by hydro-distillation using Clevenger apparatus for 3 h. The yield (%) was given as weight of EO on weight of 100 g plant powder (w/w). In order to prepare an ethanolic extract, 20 g of plant powder was extracted by 200 mL of ethanol for 24 h under stirring in the dark. Taking in consideration that wild thyme is traditionally used as a tea infusion; 200 mL of boiling distilled water is poured over 20 g of plant powder. The mixture was left for 30 min with occasional agitation. Extracts were filtered and evaporated under reduced pressure until dryness. The obtained EO, ethanol and infusion extracts were stored in dark glass bottles in the freezer (-20 °C) until characterizations (GC/MS, HPLC) and antioxidant evaluations.

*GC/MS analysis*

Extracted EO was analyzed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Analytical gas chromatography-flame ionization detector (GC/FID) was performed using an Agilent chromatograph fitted with the Agilent 7683B autosampler (Agilent Technologies, Inc., Santa

Clara, USA) (1 : 20 split ratio), fused silica column and dual flame ionization detectors (FID). The operation conditions were as follows: volume of 1 µl sample (diluted in n-hexane 1 : 100, w/w) was injected and helium was used as a carrier gas. EO analysis was carried out by GC/MS system consisted of a gas chromatograph (Agilent Model 6890N, Santa Clara, CA) with HPL capillary column and connected to a quadruple mass spectrometer detector. The operating conditions for GC analysis were the same as described above for GC/FID. The MS conditions were as follows: ionization energy voltage 70 ev; quadruple temperature 150 °C; interface temperature 205 °C and mass spectra scan at a rate of 5 scan/S. Identification of sample compounds was performed by making comparison of their mass spectra with NIST 02 data and Adams mass spectra libraries. Premised on GC/FID peak areas without FID response factor correction, the percentage of each component was estimated.

*HPLC characterization*

Phenolic characterization was achieved following the method described by Caponio *et al*. (1999). With minor modifications qualitative and quantitative evaluation were performed using the HPLC instrument HP-Agilent 1292 infinity on C18 column with DAD diode array detector. Extracts were prepared in a concentration of 20 $mg.mL^{-1}$ and the injected volume into the column was 10 µL. The mobile phase used was a 3 % acetic acid (v/v) in (A) water and (B) methanol. Elution gradient was used as follows: 93 % A/ 7 % B for 0.1 min, 72 % A/ 28 % B for 20 min, 75 % A/ 25 % B for 8 min, 70 % A/ 30 % B for 7 min and 15 min a similar gradient was 67 % A/ 33 % B for 10 min, 58 % A/ 42 % B for 2 min, 50 % A/ 50 % B for 8 min, 30 % A/ 70 % B for 3 min, 20 % A/ 80 % B for 2 min and 100 % B in 5 min till the end of the run Abdulqadir *et al*. (2018). Detection of eluates was carried out at 278 nm and retention times of analyzed phenolic compounds were compared with the following available pure standards: gallic acid, (+)-catechin, chlorogenic acid, caffeic acid, hydroxybenzoic acid, epicatechin, syringic acid, coumaric acid, transferrulic acid, sinapic acid, benzoic acid, hesperidin, rosmarinic acid, cinnamic acid and quercetin. The quantitative analysis was determined using external calibration curve of each standard and the results were expressed as $\mu g.g^{-1}$ of extract.

*DPPH free radical scavenging assay*

The antioxidant effect of extracts in scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical was evaluated according to El Aanachi *et al.* (2020). About 40µL of sample ranging at different concentrations was added to160 µL DPPH methanol solution (0.1 mM). After 30 min of incubation, absorbances were read at 517 nm. The antioxidant standard used is Trolox. The results were expressed as 50 % inhibition concentration ($IC_{50}$) and mentioned as means ± SD data.

*Galvinoxyl free radical scavenging activity (GOR)*

GOR scavenging assay was determined according to the described method of Shi *et al*. (2001). A volume of 40 µL of sample at different concentrations was added to 160 µL of methanolic solution of galvinoxyl (0.1 mM). After 120 min incubation, the absorbance was evaluated at 428 nm. Methanolic galvinoxyl solution was used as a control and Trolox as a standard.

*Reducing power assay*

The reducing power of tested samples was performed according to Gali and Bedjou (2019), with minor modifications. 10 µL of sample was mixed with 40 µL of phosphate buffer 0.2 M (pH 6.6), 50 µL of 1 % $K_3Fe(CN)6$ and then the plate was incubated at 50 °C for 20 min. 50 µL of 10 % TCA (trichloroacetic acid), 40 µL $H_2O$ and 10 µL of 0.1 % $FeCl_3$ (ferric-chloride) were added. The absorbance of the resulting mixture was measured at 700 nm and results were expressed as absorbance $A_{0.50}$ $\mu g.mL^{-1}$ which represented the concentration producing 0.5 absorbance.

*CUPRAC assay*

The cupric reducing antioxidant ability was performed according to the CUPRAC method of Apak *et al*. (2004). In each well of the microplate a

mixture was constituted with 40 µL of sample, 60 µL ACNH4 (ammonium acetate) buffer, pH 7.50, 50 µL neocuproine and 50 µL of $CuCl_2 \cdot 2H_2O$ (copper II chloride solution). The absorbance was measured at 450 nm after 60 min of incubation. Results had been given as absorbance $A_{0.5}$ µg.mL$^{-1}$.

*Phenanthroline assay*

The evaluation of phenantroline activity was carried out in accordance with the method described by Szydlowska-Czerniaka *et al*. (2008). Briefly, into 96 well round-bottomed plate 10 µL of extract at various concentrations were placed, then 30 µL of *O*-phenanthroline (0.5 %), 50 µL of $FeCl_3$ (0.2 %), and 110 µL of methanol were added. After 20 min of incubation in the dark at 30 °C, the absorbance was measured at 510 nm. The values of the measurements have been expressed as $A_{0.5}$ µg.mL$^{-1}$.

*Photoprotective (skin-protective) activity*

The photoprotective activity was achieved following Mansur *et al*. (1986) and El Aanachi *et al*. (2021). The samples were prepared at a concentration of 2 mg.ml$^{-1}$ diluted in absolute ethanol and then analyzed at wavelengths from 290 to 320 nm (UV) with spans of 5 nm, using ethanol as a blank. SPF was calculated using the formula (Eq. 1):

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

Where: EE(λ) is the erythemal effect spectrum.
I(λ) is solar intensity spectrum.
Abs(λ) is absorbance.
CF is the correction factor (= 10).
EE(λ) and I(λ) are steady values.

*Statistical analysis*

Experimental tests were achieved in triplicate (n = 3) and results were given as means with standard deviation (mean ± SD). Values of $IC_{50}$ and $A_{0.5}$ for *in vitro* antioxidant activities were estimated by linear regression analysis and statistical significances were established at P≤0.05 using variance analysis ANOVA One-way and Tukey test (multiple range comparison).

## Results

*Chemical composition of essential oil*

The chemical profile of *T. serpyllum* EO from the Mascara region was described for the first time in this research and the chromatogram is presented in (Fig. 1). The obtained EO was light yellow in color, had a strong smell and yielded at 5.66 % (w/w).

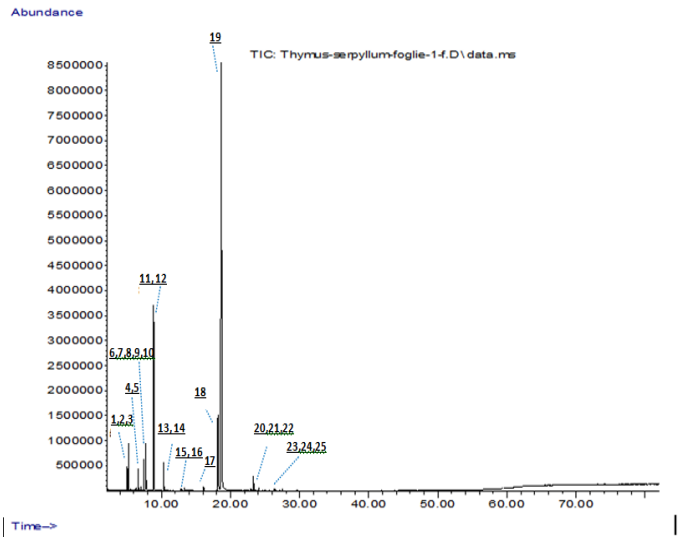


**Fig. 1.** Chromatogram of *Thymus serpyllum* essential oil.

Identified compounds with relative amounts and retention times are illustrated in Table 1 where, twenty-five compounds were found. Carvacrol was detected as the main component at 66 % of the essential oil. The γ-terpinene was detected at (11.5 %), thymol (7.5 %) and *p*-cymen (3.9 %). In lower percentages, other components were present as α-pinene, linalool, myrcene, and α-thujene.

*Determination of phenolic profile*

Ethanolic and infusion extracts of *T. serpyllum* were analyzed by HPLC-DAD detector type. The investigation of the phenolic pattern was performed using 15 phenolic standards. Results are in Table 2 and were expressed in µg.g$^{-1}$ of extract. Rosmarinic acid was the main component of infusion (17,250 µg.g$^{-1}$), followed by benzoic acid (5,200 µg.g$^{-1}$)

and hydroxybenzoic acid (1,020 µg.g$^{-1}$). Besides the abundant compounds in ethanolic extract were benzoic acid and hydroxybenzoic (2,450 µg.g$^{-1}$, 1,340 µg.g$^{-1}$). Syringic acid, coumaric acid, and sinapic acid were detected (50 µg.g$^{-1}$, 60 µg.g$^{-1}$, and 70 µg.g$^{-1}$, respectively) in the ethanolic extract and not identified in the infusion. Quercetin was not found in both extracts.

*Antioxidant activities*

For the anti-radical scavenging ability test, the DPPH and galvinoxyl free radicals were used. According to results of the DPPH scavenging assay mentioned in Table 3, the ethanolic and infusion extracts proved that both presents an excellent scavenging activity with an $IC_{50}$ values of (25.12 ± 0.23 and 29.14 ± 0.41 µg.mL$^{-1}$, respectively). The EO have shown the lowest radical scavenging activity with $IC_{50}$ (>100 µg.mL$^{-1}$). These results are less than antioxidant standard Trolox (5.12 ± 0.21 µg.mL$^{-1}$). For GOR free radical assay, the results are indicated in Table 3, where ethanolic and infusion extract revealed a similar activity with values of $IC_{50}$ (24.56 ± 1.69 and 25.13 ± 0.82 µg.mL$^{-1}$). The results of the ferric reducing power presented in Table 3 showed that the ethanolic extract exhibited the lower $A_{0.5}$ (31.70 ± 2.31 µg.mL$^{-1}$) in meaning, the highest activity in comparison with infusion extract and EO (33.04 ± 1.84 and >100 µg.mL$^{-1}$, respectively). Analysis data of CUPRAC assay mentioned in Table 3 indicated that ethanolic extract showed the best reducing copper transition metals with an $A_{0.5}$ (17.83 ± 0.54 µg.mL$^{-1}$) close moderately to Trolox (8.69 ± 0.14 µg.mL$^{-1}$), and ascorbic acid (8.31 ± 0.15 µg.mL$^{-1}$). Infusion and EO presented $A_{0.5}$ values of (23.25 ± 0.46 and 37.30 ± 2.20 µg.mL$^{-1}$) respectively.

**Table 1.** Chemical composition in (%) of essential oil from *Thymus serpyllum* by GC/MS with retention indices on HP-5MS capillary column.

| Peak | Tr [sec] | Tr [min] | RI [log. Kovats] | Compound | Formula | Class | Area [%] |
|---|---|---|---|---|---|---|---|
| 1 | 303 | 5.0422 | 929 | α-Thujene | $C_{10}H_{16}$ | MH | 1.0 |
| 2 | 313 | 5.2171 | 937 | α-Pinene | $C_{10}H_{16}$ | MH | 2.0 |
| 3 | 335 | 5.5843 | 952 | Camphene | $C_{10}H_{16}$ | MH | 0.1 |
| 4 | 378 | 6.3056 | 979 | β-Pinene | $C_{10}H_{16}$ | MH | 0.1 |
| 5 | 400 | 6.6685 | 991 | Myrcene | $C_{10}H_{16}$ | MH | 1.1 |
| 6 | 425 | 7.0838 | 1005 | γ-Phellandrene | $C_{10}H_{16}$ | MH | 0.2 |
| 7 | 436 | 7.2630 | 1012 | iso-Sylvestrene | $C_{10}H_{16}$ | MH | 0.1 |
| 8 | 448 | 7.4597 | 1018 | α-Terpinene | $C_{10}H_{16}$ | MH | 1.7 |
| 9 | 463 | 7.7089 | 1027 | p-Cymene | $C_{10}H_{14}$ | MH | 3.9 |
| 10 | 471 | 7.8444 | 1031 | Limonene | $C_{10}H_{16}$ | MH | 0.6 |
| 11 | 509 | 8.4827 | 1050 | (E)-β-Ocimene | $C_{10}H_{16}$ | MH | 0.1 |
| 12 | 532 | 8.8717 | 1062 | γ-Terpinene | $C_{10}H_{16}$ | MH | 11.5 |
| 13 | 594 | 9.9034 | 1089 | Terpinolene | $C_{10}H_{16}$ | MH | 0.1 |
| 14 | 61 | 10.3187 | 1099 | Linalool | $C_{10}H_{18}O$ | MO | 2.0 |
| 15 | 771 | 12.8542 | 1167 | Borneol | $C_{10}H_{18}O$ | MO | 0.2 |
| 16 | 801 | 13.3439 | 1178 | Terpinene-4-ol | $C_{10}H_{18}O$ | MO | 0.2 |
| 17 | 966 | 16.1023 | 1246 | Carvacrol methyl ether | $C_{10}H_{16}O$ | Phpr | 0.3 |
| 18 | 1090 | 18.1701 | 1293 | Thymol | $C_{10}H_{14}O$ | MO | 7.0 |
| 19 | 1121 | 18.6903 | 1305 | Carvacrol | $C_{10}H_{14}O$ | MO | 66.0 |
| 20 | 1376 | 22.9395 | 1407 | α-Gurjunene | $C_{15}H_{24}$ | SH | 0.1 |
| 21 | 1399 | 23.3242 | 1418 | (E)-Caryophylene | $C_{15}H_{24}$ | SH | 1.1 |
| 22 | 1446 | 24.1023 | 1438 | Aromadendrene | $C_{15}H_{24}$ | SH | 0.1 |
| 23 | 1582 | 26.3668 | 1493 | Viridiflorene | $C_{15}H_{24}$ | SH | 0.2 |
| 24 | 1650 | 27.5034 | 1522 | δ-Cadinene | $C_{15}H_{24}$ | SH | 0.1 |
| 25 | 1772 | 29.5318 | 1572 | Spathulenol | $C_{15}H_{24}O$ | SO | 0.1 |

Notes: Total identified = 99.9%; MH: monoterpenes hydrocarbons = 22.5 %; MO: oxygenated monoterpenes = 75.4 %; SH: sesquiterpene hydrocarbons = 1.6 %; SO: sesquiterpene oxygenated = 0.1 %; Phpr: phenylpropanoids = 0.3%; Tr: retention time; RI: retention indices.

**Table 2.** Qualitative and quantitative phenolic composition of *T. serpyllum*.

| Phenolic compounds | Ethanol extract [µg.g$^{-1}$] | Infusion extract [µg.g$^{-1}$] |
|---|---|---|
| Gallic acid | 80 | 80 |
| (+)-Catechin | 630 | 680 |
| Chlorogenic acid | 60 | 170 |
| Caffeic acid | 100 | 20 |
| Hydroxybenzoic acid | 1340 | 1020 |
| Epicatechin | 130 | 630 |
| Syringic acid | 50 | ND |
| Coumaric acid | 60 | ND |
| Trans-ferrulic acid | 250 | 110 |
| Sinapic acid | 70 | ND |
| Benzoic acid | 2450 | 5200 |
| Hesperidin | 300 | 610 |
| Rosmarinic acid | 480 | 17250 |
| Cinnamic acid | 10 | 190 |
| Quercetin | ND | ND |

ND: Not Determined

As seen in the Table 3 for the phenanthroline assay, the EO expressed the best antioxidant capacity with value of (12.77 ± 1.19 µg.mL$^{-1}$) followed by ethanol extract $A_{0.5}$ (13.40 ± 0.73 µg.mL$^{-1}$) and infusion with (21.42 ± 0.61 µg.mL$^{-1}$).

**Table 3.** Antioxidant activity of *T. serpyllum* extracts.

| Extracts | DPPH $IC_{50}$[µg.mL$^{-1}$] | GOR $IC_{50}$[µg.mL$^{-1}$] | Reducing power $A_{0.5}$[µg.mL$^{-1}$] | CUPRAC $A_{0.5}$[µg.mL$^{-1}$] | Phenanthroline $A_{0.5}$[µg.mL$^{-1}$] |
|---|---|---|---|---|---|
| Ethanolic extract | 25.12 ± 0.23$^{b}$ | 24.56 ± 1.69$^{a}$ | 31.70 ± 2.31$^{a}$ | 17.83 ± 0.54$^{c}$ | 13.40 ± 0.73$^{b}$ |
| Infusion extract | 29.14 ± 0.41$^{a}$ | 25.13 ± 0.82$^{a}$ | 33.04 ± 1.84$^{a}$ | 23.25 ± 0.46$^{b}$ | 24.27 ± 5.40$^{a}$ |
| Essential oil | >100 | >100 | >200 | 37.30 ± 2.20$^{a}$ | 12.77 ± 1.19$^{b}$ |
| Trolox | 5.12 ± 0.21$^{c}$ | 4.31 ± 0.05$^{b}$ | 5.25 ± 0.20$^{b}$ | 8.69 ± 0.14$^{d}$ | 5.21 ± 0.27$^{c}$ |
| Ascorbic acid | 4.39 ± 0.01$^{d}$ | 5.02 ± 0.01$^{b}$ | 3.62 ± 0.29$^{b}$ | 8.31 ± 0.15$^{d}$ | 3.08 ± 0.02$^{c}$ |

Notes: $A_{0.5}$ and $IC_{50}$ are mentioned as concentrations at 0.5 absorbances and concentration making 50 % inhibitions percentages respectively. Values of $A_{0.5}$ and $IC_{50}$ are indicated as Means ± SD of three tests and the values stated in the unchanged column with different superscripts (**a, b, c, d**) presents significant differences at ($P<0.05$).
(>100) indicates that values of $IC_{50}$ and $A_{0.5}$ are higher than 100 µg.mL$^{-1}$.

*The photo-protective (skin-protective) activity*

The dermoprotective activity of *T. serpyllum* extracts is investigated for the first time by measuring of the Sun Protection Factor (SPF). The SPF values for wild thyme extracts are presented in Table 4. Infusion and ethanolic extracts showed similar values with SPF (38.82 ± 2.23 and 38.34 ± 2.29, respectively). EO exhibited a weak activity with (4.81 ± 0.25).

**Table 4.** SPF values of *T. serpyllum* extracts.

| Extracts | SPF |
|---|---|
| Ethanolic | 38.34 ± 2.29 |
| Infusion | 38.82 ± 2.23 |
| Essential oil | 4.81 ± 0.25 |

Values of SPF are indicated as means ± SD of three tests.

## Discussion

Essential oils extracted from aromatic and medicinal plants are constituted of various volatile and lipophilic compounds, obtained from various chemical classes (Turek and Stintzing 2013).

The yield of extracted essential oil by hydrodistillation from *T. serpyllum* was in accordance to the European Pharmacopoeia (2010) (a minimum of 0.3 % (w/w)) as mentioned by (Wesołowska *et al*. 2012). Reported yields from Poland and Jordan were 2.5 % and 1.05 %, respectively, according to studies of Abu-Darwish *et al*. (2009) and Wesolowska *et al*. (2014). Comparing these results with ours, the content (5.66 %) of extracted EO was higher. The chemical composition after analysis by GC/MS revealed that carvacrol was the main component. Thus, it agreed with those reported by Kirillov *et al*. (2016). However, the amount of carvacrol detected (66 %) was higher in comparison with other studies. Significant differences were observed about a major component of *T. serpyllum* where it was found p-cymene in the Italian wild *Thyme* (D'Auria and Racioppi 2015). The difference in percentages of carvacrol, γ-terpinene, and p-cymene in *T. serpyllum* can be affected by climatic factors following reports of Wesołowska *et al*. (2012). The results of the GC/MS analysis showed a wide variation in main components and their amount from those mentioned in other works. According to Banaeva *et al*. (1998), these variations can be attributed to the geographical source, collecting season, climatic conditions and extraction methods.

Polyphenol compounds such as phenolic acids, flavonoids, tannins, and coumarins can be extracted from plants using several techniques and different solvents (Perron and Brumaghim 2009). The chromatographic analysis in the conducted study detected the rosmarinic acid, hydrobenzoic acid, and benzoic acid as the main components. Our results are similar to those of Janiak *et al*. (2017) who have reported that rosmarinic acid is the main component in aqueous extract of wild thyme. Furthermore, it was found with higher amounts than their results. Quercetin was not found in both extracts which is in conformity with Miron *et al*. (2011) studies. According to Jovanović *et al*. (2016) the concentration of phenolic compounds and their structure can influence their bioactive properties. Studies reported by Jovanović *et al*. (2019) confirmed that the phenolic pattern of thyme species can be influenced by variability of experimental extraction methods including solvent type (polarity, ratio and mixture), temperature also bound and free phenolic extracts.

Evaluation of antioxidant ability for crude extracts must be performed using more than one method due to the complexity of the antioxidant process (Aruoma 2003). For the anti-radical scavenging ability test, DPPH and galvinoxyl free radicals were used. The GOR and DPPH radical scavenging power were evaluated in term of hydrogen atom and/or electron donating capacity and determined by $IC_{50}$ where low values represent high antioxidant capacities.

The infusion presented a high amount of rosmarinic acid in the HPLC analysis than ethanol extract but slightly low antioxidant potential. This can be explained by reports of Kulišić *et al*. (2006) who mentioned that the power of compounds in water infusions may decrease a little due to their dilution and combination with other substances in the mixture of infusion. The ethanolic and infusion extracts showed the best free radical scavenging activity using the galvinoxyl free radical than DPPH. These results are consistent with works of Tirzitis *et al*. (2010) who revealed that galvinoxyl is more reactive against phenolics and tightly allied to physiological oxygen radical action than DPPH. The CUPRAC and reducing power antioxidant assays were assessed in order to associate properties of *T. serpyllum* bioactive compounds with their antioxidant potential (iron binding capacity). Obtained results about the reducing power of iron indicated the ability of *T. serpyllum* polyphenols to act as electron donors. Eghbaliferiz (2016) mentioned that polyphenols are able to form a stable complex with transition metals. Regarding the CUPRAC, the antioxidant activity is determined on measurement of absorbance for a yellow-orange complex (copper (I)-neocuproine) obtained by reduction of copper (II) into copper (I) in the presence of antioxidant compounds at 450 nm. This method was developed by Apak *et al*. (2004; 2007) who reported that this reaction can estimate lipophilic and hydrophilic antioxidants (α-tocopherol and β-carotene) at the same time. Külcü *et al*. (2019) reported a lower cupric reducing activity than our results for ethanolic extract of Turkish *T. serpyllum*. Basing on our results and according to Apak *et al*. (2004) the CUPRAC reagent was sensitive against thiol-kind oxidants

and entails faster kinetics than reducing power method. Furthermore, Kim and Choe (2018) stated that the metal chelating activity depended on structure, location and number of hydroxylgroups, pH, and the concentration of polyphenols. Evaluation of antioxidant activity with phenanthroline method is based on the iron chelating ability of extracts by reacting with formed complex ferrous-*O*-phenanthroline and reducing the Fe (III) to Fe (II) (Berker *et al*. 2007). This method was used for extracts and edible oils as reported by Szydłowska-Czerniak *et al*. (2008) but as far as we could possibly know that was not tested for essential oils. Depending on our results, essential oil revealed the higher reducing ability and was quite similar to ethanolic extract. This can be explained by its richness of phenols (carvacrol 66 % and thymol 7.5 % as mentioned above in GC/MS results). These results can be in conformity with Christodouleas *et al*. (2014) who reported that lipophilic extracts contributed more than 90 % to the total reducing of whole oil using phenanthroline-Fe method.

The sunburn, skin inflammation and skin cancer may appear as a result of regular exposure to UV radiation. These UV rays can promote photochemical reactions that induce the generation of free radicals such as $O_2^{-}$ , $HOO^{-}$, and $OH^{\cdot}$ (Batista *et al*. 2021). Some studies are in quest for natural sunscreen products to reverse and block these UV radiations. The photo-protective capacity of plant extracts is investigated by measuring the sun protection factor (SPF). The levels of protection were mentioned by Schalka *et al*. (2011) where SFP values were minimum (2-12), moderate (12-30), high (30-50), and maximum at >50. SPF values of ethanolic and infusion extracts indicates high skin-protective activity due to their polyphenols that can absorb at wavelength between 280nm and 320nm. These results can make the *T. serpyllum* extracts as potential skin-protective agents that can be used as additives in dietary and in the production of sunscreens for better photo-protection.

## Conclusion

*Thymus serpyllum* extracts were evaluated for their chemical profile, antioxidant, and skin-protective activities. Experimental results of antioxidant assays (DPPH, GOR, CUPRAC, Reducing power, and phenanthroline) revealed that both of ethanol and infusion extracts showed high antioxidant activities. Although the richness of infusion extract with phenolic acids was mainly by rosmarinic acid, the active compounds of infusion can be slightly affected by dilution and their reaction with other compounds in mixture and this has been proven by other studies. In this study, we reported for the first time the good potential of *T. serpyllum* as a photo-protective agent which can offer an initiation point for therapeutic research to use these extracts in a formulation of dermo-protective products for skin disorders. Basing on our results, it can be concluded that *T. serpyllum* from the Mascara region represented an effective source of antioxidant components that may be used as an alternative to synthetic antioxidants for treatment of pathologies associated with free radical damages and in food industries as additives to retard food deterioration and to upgrade the storage length of food products.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

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