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Antioxidant and Anti-inflammatory Potential of *Thymus vulgaris* L. Essential Oil from Souk-Ahras (Northeast Algeria)

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Abstract
The aims of the present study were to determine the chemical composition of the essential oils (EO) extracted from the aerial parts of *Thymus vulgaris* collected in the Souk-Ahras . Phytochemical analysis showed that *T. vulgaris* EO exhibited a high antioxidant power, confirmed by DPPH and FRAP concentrations of 22.517 ± 4.09 µg/ml and 21.342 ± 4.735 µg/100 ml, respectively. Our results underline the importance of the biological activities of *T. vulgaris* essential oil from the Souk Ahras region**,** which may represent a promising reservoir of bioactive compounds, offering a potential therapeutic alternative for the treatment of various types of diseases caused by oxidative stress.

Key words: *Thymus vulgaris* essential oils, Souk ahras, phytochemical analysis, biological activity.

Introduction

Lately, there's been a lot of exploration into traditional medicine and how it's applied. What we've found is that it's essentially a rich mix of knowledge, skills, and practices deeply rooted in the unique beliefs and experiences of different cultures. People use these traditional approaches not just to stay healthy, but also to prevent, identify, treat, and improve various physical and mental health issues (Hemmami et al., 2023).

There is growing interest in plant-based products in the health, food, perfume, and cosmetics sectors. Among all these plant-based products, essential/volatile oils have attracted a great deal of interest because of their significant biological usefulness (Mancini et al., 2015). The chemistry of essential oils is influenced by local geography and weather conditions, as well as the season and time of day during plant harvesting, and the methods of processing, packaging, and storage (Moghaddam and Mehdizadeh, 2017). Algeria is known for its wealth of medicinal plants, due to its surface area and bioclimatic diversity.

The Thymus genus is one of the most common medicinal plants found all along the coast and even in inland regions and arid zones. This genus includes several botanical species, including *Thymus vulgaris*, commonly called thyme or locally known as "Zaatar". This medicinal and aromatic plant is native to the Mediterranean basin and is widely present in north-eastern Algeria, particularly in the Souk Ahras region.

This species exhibits several biological activities that have been demonstrated by several researchers, including antifungal and antibacterial activity (Lemos et al., 2017; Ozogul et al., 2020; Boukhatem et al., 2020; Diniz et al., 2023); antispasmodic (Salmalian et al., 2014); antioxidant (Hamzawy et al., 2012; Mancini et al., 2015; Aljabeili et al., 2018); anti-inflammatory (Abdelli et al., 2017; Abdelhamed et al., 2022). All these activities are linked to the high content of phenolic compounds, particularly thymol and carvacrol (Rodrigues et al., 2019).

The aim of this study is to determine the antioxidant and anti-inflammatory activity of the EO of *T. vulgaris* harvested in Souk ahras, Algeria, to identify new natural sources in food products and drugs.

MATERIEL AND METHODS

Plant collection

Fresh aerial parts of *T. vulgaris* (endemic in Algeria) were collected during flowering in the region of Ouled moumen, Souk ahras (Northeast Algeria) with an amount of 1.5 kg in Jun 2023. Fresh aerial parts of *Thymus vulgaris* were harvested during flowering at a rate of 1.5 kg in the region of Ouled moumen, Souk ahras (North-East Algeria), in June 2023.The plant was identified by Dr. Ketfi Louiza, botanist at the University of Souk ahras, Algeria, and a voucher specimen (V. THv-5) was deposited in the herbarium of the Department of Botany, at the Faculty of Science, University of Mohamed Cherif Messaadia, Souk ahras, Algeria. The plant material was ear-dried in the shade for 7 days before extraction.

Extraction of Essential oil

The fresh aerial parts of the plant were thoroughly washed with water to eliminate any residues, and any damaged portions were removed. The sample was placed on paper for drying in the open air.

An amount of 200 g of the dry plant was transferred to hydrodistillation and one liter of distilled water was added. The extraction was carried out using a Clevenger-type apparatus for 4 hours. At the end of the distillation process, the oil in liquid form was collected using a separating funnel. The isolated oil was dried with sodium sulfate to remove all traces of water, then collected and stored in an amber bottle at 4°C. until it was tested for biological activities.

The yield of essential oil is the ratio between the weight of the oil extracted and the weight of the plant's dry matter. The essential oil yield expressed as a percentage was calculated according to the following formula:

$$R = PB/PA \times 100$$

R: Oil yield in %.

PA: Weight of the dry matter of the plant in g.

PB: Weight of the oil in g

Antioxidant Activity

Free radical-scavenging activity

The anti-radical activity of essential oil was studied as described by Brand-Williams et al (1995). DPPH (diphenylpicrylhydrazyl) was used as a relatively stable free radical. The antioxidants reduce the violet-coloured DPPH to a yellow compound, the intensity of the color of which was inversely proportional to the capacity of the antioxidants present in the environment to produce protons. A 100 µl volume of crude sample solutions was added to 2 ml of DPPH (2.4 mg prepared in 100 ml of methanol). In parallel, a negative control was prepared by combining 100 µl of methanol with 2 ml of DPPH methanol solution. The absorbance of the solution was measured against a reference solution at 517 nm after incubation for 30 minutes at room temperature in darkness. Ascorbic acid was prepared as a standard antioxidant solution, and its absorbance was measured under identical conditions to the samples. The assay was conducted three times for each concentration. Free radical scavenging activity was estimated according to the equation below:

$$\mathbf{I\% = Ac - At/AC \times 100}$$

Where, At is the optical density of test samples, while Ac is that of control.

Ferric reducing antioxidant power assay (FRAP)

The obtained iron reductive activity of the phenolic extracts was determined according to Mohamadi et al. (2023), the amount of 500 µl of approximately 10 µg/ml concentration sample was mixed with 1250 µl of a phosphate buffer solution (pH 6.6) and 1250 µl of potassium ferrocyanide ($K_3Fe(CN)_6$) (1%). The mixture was incubated at 50°C for 20 min. To stop the reaction, the mixture was cooled to room temperature, then added to the 2500 µl mixture of trichloroacetic acid (10%). The mixture was centrifuged at 3000 turns for 10 min. An amount of 1250 µl of the recovered supernatant was mixed with the same volume of distilled water and 250 µl of a 0.1% $FeCl_3$ solution. The absorbance was measured using spectrophotometry at 700 nm against a control that had been manufactured in the same way. The same experiment was carried out for ascorbic at different concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml in distilled water). The tests were repeated 3 times for all the tested compounds.

Evaluation of anti-inflammatory activity

The anti-inflammatory activity of the sample was evaluated according to the protein thermal denaturation inhibition assay described by Karthik et al. (2022). Concentrations in different ranges, from 0.0625 to 1 g/mL, were prepared for use in these assays. Solutions composed of 1 mL of each dilution and 1 mL of 0.2% ovalbumin (diluted in phosphate-buffered saline [PBS]) were incubated for 5 min at 72°C. After vortexing and cooling, absorbance was measured at a wavelength of 660 nm against a blank sample free of extract. The reference anti-inflammatory agent, diclofenac, was prepared using the same procedures as the extract. The inhibition level (T%) of protein denaturation was calculated using the following formula: $\% = 100 - ODs / ODc \times 100$,

where ODs: Optical Density of brine extract ODc: Ovalbumine solution without extract

Statistical analysis

For all experiments, the analyses were performed in triplicate, and the values are reported as the mean ± standard deviation (SD).

Results and Discussion

The yield of *Thymus vulgaris* essential oil (EO) obtained in our study was 1.18%, which aligns with previous findings from similar regions in Algeria, such as the Chria region, Blida, reported by Hassani et al. (2017). However, this yield is relatively lower compared to yields from north-western Algeria, specifically Tlemcen and Mostaganem, where Abdelli et al. (2017) documented EO yields of 2.2% and 4.2%, respectively. Such discrepancies in EO yield can be attributed to a variety of factors that influence both the quantity and quality of essential oil extraction.

Several studies confirm that essential oil yield can significantly vary based on multiple factors. For instance, variations in geographical location, climate, and soil conditions are known to play a pivotal role in the biosynthesis of secondary metabolites, including essential oils (Ghasemi et al., 2013). Previous research suggests that altitude plays a crucial role in determining oil content in ***Origanum vulgare*** *ssp.* ***hirtum*** and ***Thymbra spicata*** *var.* ***spicata*** L. (Kizil, 2010), with higher essential oil concentrations observed at lower altitudes. Adverse environmental conditions can limit photosynthesis and disrupt nutrient absorption, as well as affect carbon, sugar, amino acid, and inorganic ion flow within the plant. Plants grown in more favourable climates or under stress conditions (e.g., drought or temperature extremes) often produce higher quantities of essential oils as a defence mechanism (Sangwan et al., 2001). This could explain the higher EO yield observed in Tlemcen and Mostaganem, which may have environmental conditions more conducive to the production of volatile oils than the Souk-Ahras region.

Additionally, the time of harvesting plays a crucial role. According to Abu-Darwish et al. (2012), the concentration of essential oils in *T. vulgaris* is significantly affected by the phenological stage of the plant, with peak yields typically observed at the flowering stage. The plant's age, as well as the time of day and season of harvest, also influence the essential oil yield and composition (Pirbalouti et al., 2013). These factors could explain differences in oil yield across various studies, as harvesting practices vary widely among regions.

Moreover, the method of essential oil extraction greatly impacts yield. Steam distillation is the most commonly used method, but parameters such as distillation time, pressure, and temperature can all affect the efficiency of oil recovery. In a comparative study, variations in EO yield due to extraction technique were documented by Dhotre and Sathe (2023), emphasizing that optimization of the extraction process is critical for maximizing yield.

Finally, post-harvest factors like the drying process of plant material before extraction also contribute to differences in EO yield (Rohloff.et al.,2005). Lalami et al. (2013) noted that drying conditions (air-drying vs. oven-drying) and the duration of storage before oil extraction can lead to changes in oil content and quality. Improper drying techniques can cause degradation of essential oil components, resulting in reduced yields.Abdelli et al. (2017) also pointed out that differences in cultivation practices, such as irrigation, fertilization, and pruning, may further affect the oil yield and composition.

In light of these considerations, the yield of 1.18% obtained in our study falls within the expected range for *T. vulgaris* grown under varying environmental and cultivation conditions.

Antioxidant activity

At the first stage, we examined *in vitro* antioxidant activities of the essential oils of *Thymus vulgaris* using the free radical scavenging capacity using DPPH method and FRAP (ferric reducing antioxidant power).

The Reduction of ferric iron ($Fe3+$) to ferrous iron ($Fe2$) is one of the most often-applied methods for the evaluation of the antioxidant potential of medicinal plant compounds. The FRAP method was used as a significant indicator of potential antioxidant activity of oil. For this reason, the reducing power of ferric ions is examined (Table 1).

Table1: Antioxidant activity of *Thymus vulgaris* essential oil from Souk ahras, Algeria.

Species	**DPPH** [a]	**FRAP** [b]
T.vulgaris	22.517±4.09	21.342±4.735
Ascorbic acids *	1.309 ± 0.012	

[a] *Antiradical DPPH activities are expressed as IC_{50} in µg/mL for sample and compounds;* [b] *Reducing power mesured as the ability to reduce ferric ions in µg/100 ml, Data are the mean of three replicates (n=3) and represented as mean±SD * Used as standard antioxidants*

The antioxidant activity of essential oils (EOs) is a subject of significant interest in phytochemistry and food science due to their potential health benefits and applications in food preservation. In this context, the essential oil derived from *Thymus vulgaris* (thyme) has demonstrated noteworthy antioxidant properties, as evidenced by the findings of our study, which reported an IC50 of 22.517 ± 4.09 µg/ml. This level of activity surpasses previously reported findings, indicating a rich profile of bioactive compounds, especially phenolics, that contribute to its potent free radical scavenging capabilities.

The antioxidant potential of thyme EO has been documented in several studies, each employing different extraction methods and commercial sources, leading to varying results. Teixeira et al. (2013) evaluated a commercial thyme EO and found a significantly higher IC50 value of 250 ± 0.01 µg/ml. This suggests that the commercial EO tested may have lower concentrations of the active antioxidant components than those present in our study's sample. Similarly, Gedikoğlu et al. (2019) assessed antioxidant activities using different extraction methods, reporting IC50 values of 93.77 ± 13.0 µg/ml for microwave-assisted extraction (MAE) and 159.59 ± 12.79 µg/ml for hydrodistillation (HD).

These findings illustrate that the extraction method significantly influences the antioxidant capacity of EOs. MAE, for example, has been shown to yield EOs with lower phenolic content compared to traditional hydrodistillation, which can result in reduced antioxidant activity (Gedikoğlu et al., 2019). Moreover, the inherent variability in the chemical composition of *Thymus vulgaris* due to factors such as geographic origin, climate, and harvesting conditions can also contribute to discrepancies in reported antioxidant activity.

The results of the Ferric Reducing Antioxidant Power (FRAP) assay, presented in Table 1, indicate that the essential oils of *Thymus vulgaris* exhibit significantly lower FRAP values compared to those reported in other studies. For example, Crespo et al. (2019) demonstrated that mixtures of essential oils, including those from A*pium graveolens*, *Thymus vulgaris*, and *Coriandrum sativum*, displayed enhanced antioxidant activity through synergistic effects, leading to higher FRAP values. This discrepancy may suggest that the antioxidant potential of *Thymus vulgaris* essential oil, when evaluated in isolation, may not fully reflect its effectiveness when combined with other compounds. Additionally, variations in extraction methods, chemical composition, and the specific conditions of the assays used in different studies can influence FRAP results. Additionally, variations in extraction methods, chemical composition, and the specific conditions of the assays used in different studies can influence FRAP results.

The bioactive compounds within thyme EO, particularly phenolic compounds, are largely responsible for its antioxidant properties. Phenolic compounds possess hydroxyl groups that can donate hydrogen atoms to free radicals, effectively neutralizing them. The richness of these compounds in the EO tested in our study might explain its superior antioxidant activity compared to those reported by Teixeira et al. (2013) and Gedikoğlu et al. (2019). Understanding the relationship between the profile of phenolic compounds and antioxidant activity can further elucidate the mechanisms through which thyme EO exerts its protective effects. György et al. (2020) identified key compounds in *Thymus vulgaris*, emphasizing the significance of these bioactive molecules in countering oxidative stress. Among these, phenolics such as thymol and carvacrol have been extensively researched for their potent antioxidant properties. For instance, studies by Boulanouar et al. (2013) found that *T. vulgaris* essential oil, rich in thymol and carvacrol, exhibited strong free radical scavenging activity, further corroborating our study's findings.

Additionally, Bounatirou et al. (2007) indicated that the phenological stage of *T. vulgaris* has a notable impact on the antioxidant activity of its essential oil. Their research showed that oils extracted during the flowering and post-blooming stages displayed higher antioxidant activity than those obtained during the vegetative stage, which likely accounts for the robust activity observed in our study, as the plant material was harvested at an optimal time.

The high antioxidant activity of *T. vulgaris* essential oil is attributed not only to its phenolic content but also to other components such as monoterpene hydrocarbons, including p-cymene, α-pinene, sabinene, terpinene-4-ol, β-pinene, and α-terpineol. These compounds are known to exhibit significant antioxidant properties (Obame et al., 2007). Furthermore, it is essential to compare the antioxidant activity of *T. vulgaris* essential oil with other species within the *Thymus* genus, as studies have shown that various *Thymus* species display differing antioxidant capacities based on their chemical composition.

Anti-inflammatory activity

Proteins denaturation is a known source of inflammation. Therefore, as part of the investigation to assess the anti-inflammatory mechanism of EO, its inhibitory ability on BSA denaturation was calculated. Table 3 shows the inhibitory action of EO on BSA denaturation.

Table 3. Anti-inflammatory activity of *Thymus vulgaris* essential oil from Souk ahras, Algeria.

Species	**IC50 (µg/ml)**
T.vulgaris	84.06±01 µg/ml
Diclofenac sodium	11.55±0.403

Data are the mean of three replicates (n=3) and represented as mean±SD

This assay revealed that *T. vulgaris* essential oil showed a dose-dependent maximum inhibition of BSA denaturation with value of 84.06±01 µg/mL, while the IC50 value of diclofenac standard anti-inflammatory drug (diclofenac sodium) (11.55 µg/ml) showed an inhibition rate of 64.44%.

T. vulgaris essential oil has a complex composition that includes a variety of monoterpenes and phenolic compounds with anti-inflammatory properties. Indeed, these two elements can scavenge free radicals and maintain cell membranes to generate anti-inflammatory effects (Baser and Buchbauer, 2010) and prevent protein denaturation.

However, the IC50 of *T. vulgaris* essential oil is higher than that of diclofenac sodium, suggesting that, although the essential oil has anti-inflammatory properties, it is less effective than the conventional drug.

This result is in line with other research showing that the essential oil of this plant generally has moderate anti-inflammatory effects compared with synthetic drugs and conventional pharmaceuticals (Rodrigues et al., 2019; Ridaoui et al., 2022). The Boukhatem et al., (2020), assay revealed that the carvacrol-rich fraction of *Thymus vulgaris* essential oil exhibited a dose-dependent maximum inhibition of BSA denaturation of 96.35% at 0.5 µl/ml (IC50 value of 6.843 ± 0.830 µl/ml), while a standard anti-inflammatory drug (diclofenac sodium) showed a maximum inhibition of 96.89% at a concentration of 1 mg/ml. In addition, others studies have highlighted the anti-inflammatory and analgesic properties of caryophyllene (El-Sayed et al., 2017).

Conclusion

The findings from this study highlight the significant potential of *Thymus vulgaris* essential oil from souk ahras region due to its robust antioxidant and bioactive properties. These results underscore the importance of *Thymus* species as a valuable source of natural compounds with therapeutic potential. Given their ability to mitigate oxidative stress, these essential oils could serve as promising alternatives for the treatment and prevention of diseases linked to oxidative damage. Further research into the mechanisms of action and clinical applications is warranted to fully explore their efficacy in medical and health-related contexts.

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