Chemical Composition, Antioxidant Activity Of The Essential Oil Of
Thymus algeriensis Boiss, North Algeria

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ABSTRACT The leaves of Thymus algeriensis Boiss. collected from middle-North of Algeria (Sidi Aissa, M'sila). Essential oil from the stem bark of Algerian species of Thymus algeriensis Boiss. of the family of Lamiaceae was obtained by hydro-distillation using Clavenger apparatus, possessed an essential oil in 1.3 % (v/w) yield. GC and GC/MS analysis were carried out on the essential oil and was found to contain Seventy-one compounds, representing 95.99 % (area percent) of the total oil composition. Oil was rich in Oxygenated Monoterpenes (45.14 %), exhibited higher percentage of Camphor (22.60 %) followed by Camphene (12.78 %), Borneol (11.16 %), 1,8-Cineole (5.94 %), Acorenone (5.84 %) and α-Pinene (5.01 %). The principal constituents are hydrocarbons, ketones, alcohols, esters and aldehydes. The antioxidant activity of essential oil was investigated by measuring the decrease in absorption at 517 nm of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in a UV/visible spectrophotometer. The oil exhibited weak antioxidant activity by DPPH free radical scavenging bioassay (IC_{50} = 83.8 mg/ml).

1. INTRODUCTION

The Lamiaceae family (Labiatae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide. It is one of the most diverse and widespread plant families in terms of ethnomedicine [1].

The genus Thymus is one of the largest and economically most important genera within the Lamiaceae (= Labiatae) family. Thymus species are distributed throughout the arid, temperate and cold regions of the Old World north of the equator, and on the coasts of Greenland. The number of species within this genus is assumed to be more than 200. Many Thymus species are extensively used, dry or fresh, as culinary herbs. Also, essential oils obtained from these species were utilized as flavor ingredients in a wide variety of foods, beverages and confectionery products, as well as in perfumery for the scenting of soaps and lotions [2].

Aromatic plant species of genus Thymus are important medicinal plants, highly recommended due to a range of therapeutic properties of their essential oils, commonly known as thyme oil: antirheumatic, antiseptic, antispasmodic, antimicrobial, cardiac, carminative, diuretic and expectorant. The oil is also beneficial in boosting the immune system and helps to fight colds, flu, infectious diseases and chills. It is proved to be a urinary antiseptic, being very helpful for cystitis and urethritis. Scientific validation of traditional uses, and phytochemical and bioactivity evaluation of essential oils from Thymus algeriensis were performed [3].

This study to identify the chemical composition of the essential oil of the Thymus algeriensis commonly used in folk medicine Algerian folkloric medicine, as well as their antioxidant.

2. MATERIALS AND METHODS

2.1. Plant material collection and oil extraction

Flowering aerial parts of plant were collected from North middle of Algeria (Sidi Aissa M'sila) (latitude of 35°43'41" North and longitude of 3°18'27" to 6°43' East at an elevation of about 720 m
above sea level), at the flowering stage April, 2014. The harvested samples size does not exceed 20 cm. After harvest, the fresh vegetable matter was first weighted and then dried on the shadow, until constancy of the weight (20 days). Finally, leaves and flowers, were separated from stems and subjected for essential oil extraction.100 g of dried plant parts were cut in small pieces and the essential oil was obtained by hydro-distillation in 300 ml H₂O for 4 h using Clevenger’s apparatus [4]. The oil content (v/w %) was estimated on dry weight basis. The essential oil obtained was dehydrated over anhydrous sodium sulphate and was stored at 4 ºC for further study.

2.2. Gas chromatography analysis (GC-FID)

The gas chromatographic analyzes were performed using a Agilent Technologies 7890A Network system gas chromatograph equipped with a non-polar column HP5MS (30 x 0.25 mm d.i., Film thickness 0.25 μm) and a flame ionization detector (FID).

The analytical conditions were as follows: the essential oils were diluted with hexane at about 1/10. The flow of the carrier gas helium was kept constant at 1 ml/min. The temperatures of injector and detector were 250 and 280 ºC, respectively. The injected volume was 1 μl with a split of 1/20. The temperature was maintained at 60 ºC for 5 min, then increased by a gradient of 3 ºC /min to reach 250 ºC; this temperature was kept constant for 5 min.

A series of n-alkanes from C₅ to C₂₈ was injected under the same analytical conditions as the samples, for the measurement of retention indices following Van den Dool and Kratz [5].

2.3. Gas chromatography-mass spectrometry(GC-MS)

The volatile compounds were analyzed by coupled to mass spectrometry brand Hewlet Packard 5973A, equipped with an non-polar capillary column (HP-5MS, 30 m x 0.25 mm, phase thickness: 0.25 μm). GC–MS spectra were obtained using the following conditions: He (helium) as carrier gas at flow rate of 1ml/min; split model: 20; 1 μl as injected volume; 250 ºC as injection temperature. The oven temperature program was 60 ºC for 5 min increasing at 3 ºC /min toward 250 ºC and held at 250 ºC during 10 min the ionization mode used was electronic impact at 70 eV. Most constituents were identified by comparison of their GC linear retention indices (RI), determined with reference to a homologous series of C₅–C₂₈ n-alkanes. The Identification was Confirmation by comparison of the mass spectral with those stored in the MS database (National Institute of Standards and Technology NIST08 and Wiley libraries and also by comparison with mass spectra from literature data [5]). The percentage composition was calculated from the summation of peak areas of the total oil, the relative amounts of the essential oils calculated by GC-FID integration.

2.4. DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical scavenging bioassay

In this method, the antioxidant activity of the essential oil extract is evaluated in term of the capacity to scavenging free radicals of DPPH formed, according to a method described by [6]. A solution of 4 mg of the radical DPPH dissolved in 100 ml of methanol was prepared. Then 2 ml of this solution was reacted with 1 ml of oil diluted extract (dissolved in methanol). The mixture was incubated in dark room for 30 min at room temperature. The absorbance was measured at 517 nm with Helios Omega UV/VIS Thermo Scientific Merk and Co. Spectrophotometer. The percentage inhibition activity was calculated by Eq. (1):

\[ I\% = \left( \frac{A_0 - A_t}{A_0} \right) \times 100 \]  

where \( A_0 \) is the absorbance of the control sample (without essential oil) and \( A_t \) the absorbance of the extract with DPPH at 30 min [7]. Tocopherol (VitE) and BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxy toluene) was used as reference and all analyses. Sample concentration providing 50 % inhibition (IC₅₀) was obtained plotting the inhibition.
The essential oil of *Thymus algeriensis* Boiss. was extracted by hydro-distillation of the aerial parts and the percentage yield was calculated to be 1.3 % (v/w). The oil was yellow in colour, soluble in methanol, ether and ethanol, and having a characteristic strong fragrance of mint.

3. RESULTS AND DISCUSSION

The essential oil of *Thymus algeriensis* Boiss. was extracted by hydro-distillation of the aerial parts and the percentage yield was calculated to be 1.3 % (v/w). The oil was yellow in colour, soluble in methanol, ether and ethanol, and having a characteristic strong fragrance of mint.

3.1. Results of GC-MS analysis

The identified constituents with their respective percentages and Kovat’s indices are summarized in Table 1. GC and GC-MS analysis of the oil revealed recognition of Seventy-one major and minor compounds, representing 95.99 % of the total oil content. These compounds were divided into five classes that are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and other compounds (Table 1). This oil was characterized by very high percentage of monoterpenes (68.69 %) and especially the oxygenated ones (45.14 %) which constituting the predominant class as was found for the majority of *T. algeriensis* exhibited higher percentage of Camphor (22.61 %) followed by Camphene (12.78 %), Borneol (11.16 %), 1,8-Cineole (5.94 %), Acorenone (5.84 %) and α-Piine (5.01 %).

### Table 1. Chemical composition of *Thymus algeriensis* Boiss essential oil.

| NO | Compounds                     | KI¹ | KI² | (%)³ in the oil³ | Identification method⁴ |
|----|--------------------------------|-----|-----|------------------|------------------------|
| 1  | Tricyclene                     | 926 | 921 | 0.62             | GC, GC/MS              |
| 2  | α-Thujene                       | 930 | 925 | 0.21             | GC, GC/MS              |
| 3  | α-Pinene                        | 939 | 934 | 5.01             | GC, GC/MS              |
| 4  | Camphene                        | 954 | 949 | 12.78            | GC, GC/MS              |
| 5  | verbenene                       | 967 | 962 | tr               | GC, GC/MS              |
| 6  | Sabinene                        | 975 | 966 | tr               | GC, GC/MS              |
| 7  | β-Piine                         | 979 | 970 | 1.22             | GC, GC/MS              |
| 8  | β-Myrcene (Myrcene)             | 990 | 981 | 1.01             | GC, GC/MS              |
| 9  | α-Terpinene                     | 1017| 1010| tr               | GC, GC/MS              |
| 10 | p-Cymene (p-Cymol)              | 1024| 1018| tr               | GC, GC/MS              |
| 11 | Limonene                        | 1029| 1022| tr               | GC, GC/MS              |
| 12 | 1,8-Cineole                     | 1031| 1025| 5.94             | GC, GC/MS              |
| 13 | (Z)-β-ocimene                   | 1037| 1031| tr               | GC, GC/MS              |
| 14 | (E)-β-ocimene                   | 1050| 1043| 1.73             | GC, GC/MS              |
| 15 | γ-Terpinene                     | 1059| 1055| 0.11             | GC, GC/MS              |
| 16 | cis-Sabinene hydrate            | 1070| 1065| 0.66             | GC, GC/MS              |
| 17 | cis-Linaloloxide (furanoid)     | 1072| 1071| 0.13             | GC, GC/MS              |
| 18 | Camphenilone                    | 1082| 1083| 0.24             | GC, GC/MS              |
| No. | Compound                        | Retention Time | Area | Method       |
|-----|---------------------------------|----------------|------|--------------|
| 19  | α-Terpinolene (Terpinolene)     | 1088           | 0.21 | GC, GC/MS    |
| 20  | trans-Sabinene hydrate          | 1098           | 0.87 | GC, GC/MS    |
| 21  | Linalool                        | 1096           | 1.03 | GC, GC/MS    |
| 22  | Cis-p-menth-2-en-1-ol            | 1121           | tr   | GC, GC/MS    |
| 23  | α-Campholenal                   | 1126           | tr   | GC, GC/MS    |
| 24  | Nopinone                        | 1140           | tr   | GC, GC/MS    |
| 25  | trans-Pinocarveol               | 1139           | tr   | GC, GC/MS    |
| 26  | **Camphor**                     | 1146           | **22.61** | GC, GC/MS |
| 27  | Camphene hydrate                | 1149           | tr   | GC, GC/MS    |
| 28  | **Borneol**                     | 1169           | **11.16** | GC, GC/MS |
| 29  | Terpinene-4-ol                  | 1177           | 0.70 | GC, GC/MS    |
| 30  | p-Cymen-8-ol                    | 1182           | 0.10 | GC, GC/MS    |
| 31  | α-Terpineol                     | 1188           | 0.49 | GC, GC/MS    |
| 32  | Myrtenal                        | 1195           | 0.21 | GC, GC/MS    |
| 33  | Verbenone                       | 1205           | 0.05 | GC, GC/MS    |
| 34  | trans-Carveol                   | 1216           | 0.14 | GC, GC/MS    |
| 35  | Isobornyl formate               | 1239           | 0.14 | GC, GC/MS    |
| 36  | Neral (Z-Citral)                | 1238           | 0.08 | GC, GC/MS    |
| 37  | Piperitone                      | 1252           | tr   | GC, GC/MS    |
| 38  | Geraniol                        | 1252           | 1.36 | GC, GC/MS    |
| 39  | Geranial(E-Citral)              | 1267           | 0.26 | GC, GC/MS    |
| 40  | Bornyl acetate                  | 1285           | 3.86 | GC, GC/MS    |
| 41  | α–Copaene                       | 1376           | tr   | GC, GC/MS    |
| 42  | β-Bourbonene                    | 1388           | tr   | GC, GC/MS    |
| 43  | Geranyl acetate                 | 1381           | 2.65 | GC, GC/MS    |
| 44  | β-Elemene                       | 1390           | 0.25 | GC, GC/MS    |
| 45  | α–Gurjunene                     | 1409           | 0.28 | GC, GC/MS    |
| 46  | (E)-β-Caryophyllene             | 1419           | 0.13 | GC, GC/MS    |
| 47  | α–Humulene                      | 1454           | tr   | GC, GC/MS    |
| 48  | Aromadendrene                   | 1441           | 0.56 | GC, GC/MS    |
|   | Compound                        | KI | KI 2 | %   | Detection Method |
|---|---------------------------------|----|------|-----|------------------|
|49 | α-Amorphene                     | 1484 | 1475 | 0.30 | GC, GC/MS        |
|50 | Valencene                       | 1496 | 1485 | 0.15 | GC, GC/MS        |
|51 | Bicyclogermacrene               | 1500 | 1493 | 1.04 | GC, GC/MS        |
|52 | α-Muurolene                     | 1500 | 1496 | 0.11 | GC, GC/MS        |
|53 | α-Bulnesene                     | 1509 | 1500 | 0.33 | GC, GC/MS        |
|54 | γ-Cadinene                      | 1513 | 1511 | 0.18 | GC, GC/MS        |
|55 | Cis-dihydroagarofuran           | 1520 | 1517 | 0.35 | GC, GC/MS        |
|56 | δ-Cadinene                      | 1523 | 1521 | 0.72 | GC, GC/MS        |
|57 | α-cadinene                      | 1538 | 1534 | tr   | GC, GC/MS        |
|58 | α-Agarofuran                    | 1550 | 1546 | 0.27 | GC, GC/MS        |
|59 | Palustrol                       | 1568 | 1564 | 0.13 | GC, GC/MS        |
|60 | Germancrene D-4-ol              | 1575 | 1574 | 1.2  | GC, GC/MS        |
|61 | spathulenol                     | 1578 | 1576 | 0.84 | GC, GC/MS        |
|62 | Caryophyllene oxide             | 1583 | 1581 | 0.28 | GC, GC/MS        |
|63 | Ledol                           | 1602 | 1598 | 0.31 | GC, GC/MS        |
|64 | α-epi-Cadinol (tau-Cadinol)     | 1640 | 1639 | 0.86 | GC, GC/MS        |
|65 | α-epi-Muurolol (tau-Muurolol)   | 1642 | 1644 | 0.53 | GC, GC/MS        |
|66 | α-Cadinol                       | 1654 | 1655 | 2.17 | GC, GC/MS        |
|67 | 7-epi-α-Eudesmol                | 1663 | 1659 | 2.63 | GC, GC/MS        |
|68 | Acorenone                       | 1692 | 1692 | 5.84 | GC, GC/MS        |
|69 | Acorenone B                     | 1697 | 1697 | 0.43 | GC, GC/MS        |
|70 | Nootkatol                       | 1715 | 1714 | 0.25 | GC, GC/MS        |
|71 | Eudesn-11-en-4-α,6-α-diol       | 1808 | 1808 | 0.31 | GC, GC/MS        |

**Total identified (%)**  
95.99

| Class composition of the oil | Monoterpene Hydrocarbons | Oxygenated Monoterpene | Sesquiterpene Hydrocarbons | Oxygenated Sesquiterpene | Other Compounds |
|-----------------------------|---------------------------|------------------------|---------------------------|--------------------------|----------------|
|                             |                           | 23.55                  | 45.14                     | 4.03                     | 16.4           | 6.88           |

KI 1: Kovats (retention) indices on the non-polar column of literature [5]; KI 2: Kovats (retention) indices measured relative to n-alkanes (C₅ – C₂₈); (%); percentage calculated by GC-FID on non-polar (HP-5MS) capillary column; GC-MS: identification based on a high match of mass spectra; Components listed in order of their Kovats indices on same column. tr = traces (compounds % <0.01%).
3.2. Results of the Antioxidant Activities

The antioxidant activity of the essential oil of *Thymus algeriensis*, has been evaluated for its DPPH radical scavenging activity. The oil reduced the stable, purple-colored DPPH radical to a yellow-colored DPPH-H with an IC$_{50}$ value of 83.8 mg/ml. Reduction of the DPPH absorbance was concentration dependent. Tocopherol (Vit E) and BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxy toluene), which was used as a standard, showed an IC$_{50}$ value of 15.5 µg/ml, 22.5 µg/ml and 180 µg/ml respectively.
Table 2. Antioxidant activity of aerial parts essential oils extracted by hydro-distillation (HD-EO) and other antioxidant references.

| Samples     | IC\textsubscript{50} (µg/ml) |
|-------------|-----------------------------|
| HD-EO (Vit E) | 8379.03 ± 15               |
| BHA         | 15 ± 0.12                   |
| BHT         | 22.5 ± 0.35                 |
|             | 180 ± 1.42                  |

It is well-known that the antioxidant activity of plant essential oils containing terpenes is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests, used to prove the ability of the components of the *Thymus algeriensis* oil to act as donors of hydrogen atoms. The obtained results are shown in Figure 3. The *Thymus algeriensis* oil showed a weak effect in inhibiting free radicals produced by DPPH, reaching up to 66.7% at 50 mg/ml and IC\textsubscript{50} value was found as 83.8 mg/ml. This capability was decreased with the decrease of oil concentration 50 (66.71), 10 (54.46), 1 (39.36) and 0.8 mg/ml (20.78). These findings suggested that oil was not able to reduce the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to the transparent diphenyl picryl hydrazine, because it dose not contain high amounts of phenolic compounds. The strong antioxidant activities of species of Thymus with high amount of carvacrol and thymol has been previously reported [8–10].

![Graph](image-url)

Fig. 3. Variation of the inhibition percentage of *Thymus algeriensis* essential oil (DPPH test).
Fig. 4. Variation of the inhibition percentage of BHT, Tocopherol (V.E) and BHA in relation to its concentration (DPPH test).

4. CONCLUSION

In order to prolong the storage stability of foods and to reduce the damage to the human body, synthetic antioxidants are used for industrial processing. But according to toxicologists and nutritionists, the side effects of some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have already been documented. For example, these substances can show carcinogenic effects in living organisms [11, 12]. From this point of view, governmental authorities and consumers are concerned about the safety of their food and about the potential effects of synthetic additives on health [13]. When compared to the antioxidative potential of the standard compounds used in this study (BHT, Tocopherol and BHA), essential oil of T. algeriensis exhibited weak antioxidant activity. On the other hand, further studies are urgently needed for better clarifying the cytotoxicity and other biological properties of the plant species presented here. In conclusion, to drive health benefits from these highly effective natural antioxidants, their application in the dairy industry may be very valuable and desirable.

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