Influence of drying plant material on yield, chemical composition, antioxidant and anti-inflammatory activities of essential oil extracted from the Tunisian *Cupressus sempervirens* leaves.

Hayfa Argui[#1], Fatiha Bradai[*2], Oumaima Ben Youchret-Zallera[#1], Coskun Yalçin[#3], Mossadok Ben-Attia[#1], Marie-Laure Fauconnier[#4], Hechmi Said[#1]

[#]*Department of Chemistry, University of Carthage, 7021 Jarzouna, Tunisia.*
[1]arguihayfa.tn@gmail.com, [1]oumayma_2005@yahoo.fr, [1]Mossadok.benattia@fsb.u-carthage.tn, [1]hechmi.said@gmail.com

[#]*Department of Plant Production and Animal Husbandry, Canakkale Onsekiz Mart University, 17800 Çanakkale, Turkey*
[3]yalcin33coskun@gmail.com

[#]*Agro-Bio Chem Department, University of Liege, Gembloux Agro-Bio Tech, 2, Passage of deportees, B-5030 Gembloux, Belgium.*
[4]Marie-Laure.Fauconnier@uliege.be

[*]*Science of nature and life Department, University Center of Tipaza Morsli Abdellah, 42 00 Tipaza Algérie*
[2]fetihabradai@gmail.com

***Abstract*— The present study aims to assess the effectiveness of the drying time on the yield, chemical composition, antioxidant and anti-inflammatory properties of the essential oils (EOs) obtained from the Tunisian *Cupressus sempervirens* leaves. Adequate oils extraction conditions were determined. The drying time affected significantly the EOs yield of this plant. The major compounds found were; α-pinene, β-selinene, δ-3-carene, α-cubebene, β-caryophyllene and α-cedrol. Also, oils extracted from dried (60 and 90 days) leaves have high antioxidant properties due to the high content of (α-pinene and β-caryophyllene). Those constituents were known for their antioxidant activities. For the anti-inflammatory study, the inhibition was higher for the 60 days of drying at 100 µg mL^{-1}.**

***Keywords*— *Cupressus sempervirens;* leaf; essential oils; Microwave-assisted hydrodistillation; antioxidant activity; anti-inflammatory**

I. INTRODUCTION

In Tunisia, the *Cupressus sempervirens* L. was involved in traditional medicine and its fruit was known for the treatment of diabetes and as an antiseptic. EOs occupy a large place in scientific research due to their antioxidant, antibacterial and antifungal activities [1]. Many studies have been conducted on the biological activities of Cupressus sempervirens EO [2] but no studies or little were done on the effect of drying *Cupressus sempervirens* leaves on the EO yields. This work aimed to study the effect of drying *Cupressus* leaves on the qualitatifs and quantitatifs caracteristics of EO by applying a new method of distillation assisted by microwaves.

II. MATERIAL AND METHODES

A. *Plant material collection*

Fresh leaves of *Cupressus sempervirens* were collected manually from Forest Rimmel located in the North of Tunisia. After, they were washed and divided in batches.

B. *Determination of moisture content*

The moisture content was determined according to the method of Twidwell et al. [3] Fresh plant material was dried in an oven at 105°C for 24 hours. It was calculated according to the following equation: **H (%) = ((mf – md)/md)*100 (1)**

C. Determination of dry matter

The dry matter content was determined according to the equation: **MS (%) = (m2/m1)*100 (2)**

D. *Extraction of EOs by Microwave-assisted hydrodistillation (MAHD)*

After drying in the shade for (30, 60 and 90 days), crushed leaves (200g) were introduced in a reactor of 2L capacity and all microwave parameters were adjusted. - *Influence of Heating Power on EO Yield*. In this assay, *MAHD* was performed at 400 rpm for 5 powers (250, 300, 400, 500 Watt).

- *Influence of the number of turns on the EO Yield*. This test was carried out for 3 revolutions (250, 300 and 400).

E. *Evaluation of antioxidant activity of EOs*

Antioxidant activity of EOs was determined by the DPPH (2, 2-diphenyl-I-picrylhydrazyl) test [4] and the FRAP method (Ferric reducing) [5]. The IC50 index was defined following Sharififar et al. [6].

F. *Evaluation of anti-inflammatory activity*

The in vitro inhibitory effect of EOs was assessed using the protein denaturation modified protocol of [7]. The inhibition of albumin denaturation was calculated following [8]: **I (%) = ((Ac – At)/Ac)*100**

III. RESULTS AND DISCUSSION

A. Moisture content and dry matter

The moisture content and the dry matter rate of *Cupressus sempervirens* leaf were shown in the Table 1. Analysis of the

Cupressus sempervirens samples reveals a high humidity level (62%), which means that the majority of the weight of the plant is fresh.

TABLE 1. Moisture content of *Cupressus sempervirens*.

Plant	Moisture content (%)	Dry matter (%)
Cupressus sempervirens	62	38

A. Optimal conditions for Cupressus EOs extraction

Results have shown that the optimal conditions obtained were 20 min as time of extraction, Heating power at P = 600 W, turns number of 400 rpm and a water/plant material ratio at approximately (200mL/200g).

B. Essential oils yields

Increasing the *Cupressus* EOs expressed in dry weight during the 30 days of drying can be explained by a physiological activity in response to water stress (Fig. 1).

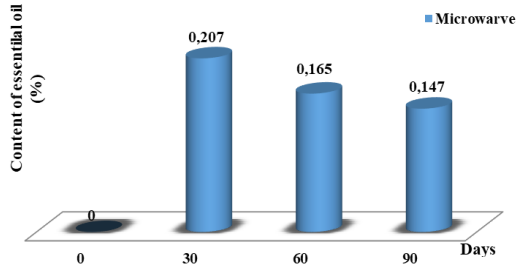

Fig. 1. Evolution of EO content of the leaves of *Cupressus sempervirens* during drying

C. Chemical composition of Cupressus EOs

The chemical constituents confirmed that this oil is rich in essential phytochemicals. A remarkable modification of the chemical composition of Cupressus EOs, at the level of two major compounds (α-Pinene and α-Cedrol) was observed. Where α-Cedrol rate increases from (4.357±0.052%) to (5.540±0.076%) while α-pinene drops from (24.877±0.138%) to (15.087±0.171%) after 90 days of drying in shade (Fig. 2).

This variation of α-pinene and α-Cedrol contents in the EOs of Cupressus sempervirens have been proven to be mainly due to geographic, climatic, harvest season and soil conditions [9].

D. Antioxidant activity

The inhibition (%) of samples dried for 60 and 90 days was higher than 30 days (Fig. 3). Treatment (60 d) has a higher antioxidant activity (IC50 30.743 µg.mL^{-1}), which was almost

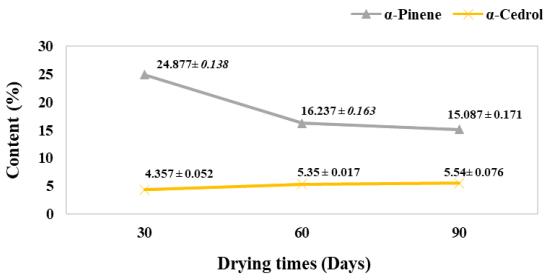

Fig. 2 Variation of α-pinene and α-Cedrol contents in the EOs of *Cupressus sempervirens* according to the drying time of the plant material.

similar to the 90 (IC50 36.554 µg.mL^{-1}). These results could be due to the high content in α-pinene [4] and β-caryophyllene[10].

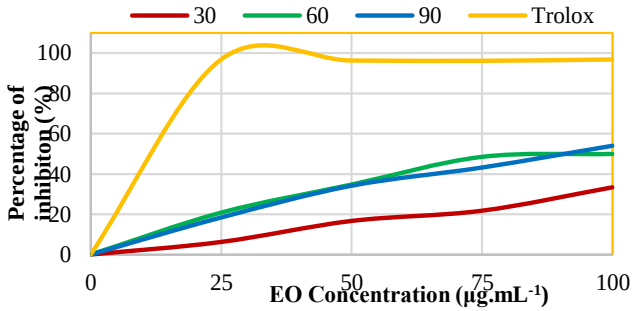

Fig. 3 Inhibition (%) of DPPH radicals by EOs from leaves of *Cupressus sempervirens* at different drying times and Trolox as a function of concentration.

The reducing power of Cupressus EO was high for the 60 days treatment at high concentrations. β-caryophyllene is one of those compounds that may interact synergistically or antagonistically to create an effective system against free radicals [11].

Anti-inflammatory activity

The EO leaves denatured the protein at 88±3.079% for the treatment (60 days) and 49.765±1.248% for (90 days) at a concentration of 100 µg.mL^{-1}.

IV. CONCLUSION

In this study it was shown that for better industrial use of EO of this specie, it will be appropriate to extract it from leaves of *Cupressus sempervirens* after one month of drying, since the content of this oil would be at its maximum. Beyond this period, this oil loose its qualitative characteristic.

ACKNOWLEDGMENTS

This research work was carried out in the Laboratory of Volatolomics, Unit of General and Organic Chemistry, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium. A part of this work was funded by the Tunisian Ministry of Higher Education and Scientific Research and the University of Carthage (Bizerte).

REFERENCES

[1] D.R. Batish et al. *Forest Ecology and Management* 2008, 256(12):2166-2174.
[2] G. Sacchetti et al. *Food chemistry* 2005, 91(4):621-632.
[3] E.K. Twidwell et al. 2002, Extension extra, Paper 280.
[4] H. Argui, O.B. Youchret-Zalleza et al. *Journal of Essential Oil Bearing Plants* 2021, 24(3):439-452.
[5] S.A. Lamia, B. Moussa et al. *The Natural Products Journal* 2020, 10(3):193-199.
[6] F. Sharififar, M. Moshafi et al. *Food control* 2007, 18(7):800-805.
[7] H. Rahman, M.C. Eswaraiah et al. *Am Eurasian J Agric Environ Sci* 2015, 15(1):115-121.
[8] T.B. Thanh, Duc LV et al. *Journal of basic and clinical physiology and pharmacology* 2017, 28(1):79-84.
[9] S.A. Fayed. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 2015, 43(2):320-326.
[10] R. Torres-Martínez, Y.M. García-Rodríguez et al. *Pharmacognosy magazine* 2017, 13(Suppl 4):S875.
[11] I. Laib, M. Barkat. Agriculture 2011, 2(2) : 89-101