International Journal of Secondary Metabolite
2023, Vol. 10, No. 2, 297–312
https://doi.org/10.21448/ijsm.1047122
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

The effect of extraction methods on the yields, chemical composition and antifungal activity of sawdust *Cedrus atlantica* Manetti essential oils

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Abstract: The aim of this work is to determine the effect of the extraction method on the yield, the chemical composition, and the antifungal activity of cedarwood essential oils (EOs) from sawdust of Moroccan *Cedrus atlantica (C. atlantica)*. EOs were extracted by different methods: hydrodistillation, soxhlet, maceration, and ultrasound. The chemical composition was determined using gas chromatography mass spectrometry (GC-MS) analysis. The yields of EOs were 5.60%, 11.68%, 4.82% and 9.33% for hydrodistillation, soxhlet, maceration and ultrasound, respectively. GC-MS revealed a diversity of chemical compounds depending on extraction methods. Indeed, the main compound of EOs obtained by soxhlet, maceration, and ultrasound was copalic acid methyl ester by a rate of 28.41%, 20.24%, and 24.17%, respectively. However, β-himachalene (21.32%) followed by α-himachalene (9.40%), β-Copaen-4α-ol (7.71%) and longifolene (6.74%) are the main compounds of EO extracted by hydrodistillation. The antifungal activity of cedarwood EO was tested in vitro on two pathogenic fungi*: Fusarium culmorum* (*F. Culmorum)* and *Botrytis cineria (B. Cinerea)*. The minimum inhibitory concentration (MIC) was determined by successive dilutions of the stock solutions. The extracted EOs by soxhlet, maceration and ultrasound showed the important inhibitory effect against *B. cinerea* (MIC=1.25 mL/L). However, *F. culmorum* showed resistance towards all tested EOs. The finding of this study showed clearly that the volatile composition of EOs can be variable according to extraction. methods. Moreover, antifungal effects are depending on chemical composition of EOs but also to tested staris.

ARTICLE HISTORY
Received: Dec. 31, 2021
Revised: July 26, 2022
Accepted: Aug. 21, 2022

KEYWORDS
Cedrus atlantica,
Essential oils,
Fusarium culmorum,
Botrytis cinerea,
Antifungal activity.

1. INTRODUCTION

Medicinal plants are an important source of significant secondary metabolites with therapeutic potential (Al-Rimawi *et al*., 2020; Newman *et al*., 2020). The growing need for new treatments for a variety of health issues, as well as the emergence of multidrug-resistant bacteria, cancer chemotherapy resistance, adverse effects of commercial drugs, and economic pressures, have

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all contributed to an increase in interest in natural product chemistry research (Belkacem *et al*., 2021; Bouyahia *et al*.,2022). Eos are the volatile components of plants that are responsible for their aroma. They have biological properties, which include antimicrobial (Abers *et al*., 2021), anticancer (Fitsiou & Pappa, 2019), anti-inflammatory (Ribeiro *et al*., 2018, antiparasitic (Mancianti & Ebani, 2020), and antioxidant (Pateiro *et al*., 2018) activities.

We were interested in the species *C. atlantica* in the context of this work because it is an important forest tree species distributed in northern Africa and one of the most economically and ecologically important species in Morocco's Mediterranean mountains belonging to the Pinaceae family (Moukrim *et al*., 2020). Numerous pharmacological investigations have been reported that *C. atlantica* exhibited several activities such as anticancer (Huang *et al*., 2020; Hung *et al*., 2020), molluscicidal (Lahlou, 2003), antioxidant (Belkacem *et al*., 2021; Bouyahia *et al*., 2022), antimicrobial (Derwich *et al*., 2010a; Zrira *et al*., 2016), larvicidal (Zoubi *et al*., 2017), antiviral (Loizzo *et al*., 2008), insecticidal (Ainane *et al*., 2019; Emer *et al*., 2018), and antifungal (Bouchra *et al*., 2003; Fidah, 2016) effects.

Phytopathogenic fungi are the cause of several plant diseases that cause yield losses of up to 20% per year on average (Tasei, 1996). To compensate these losses, synthetic fungicides are the primary means of control. However, these chemicals exhibit serious biological side effects on ecosystems (Tasei, 1996; Yu *et al*., 2011). Furthermore, the use of natural bioactive compounds such as aromatic and medicinal plants is considered actually as a promising strategy (AMPs) (Benkherara *et al*., 2011).

In Morocco, growing awareness of the harmful effects of pesticides on the environment has prompted studies on the development of alternative methods such as organic farming and the use of botanical extracts and EOs in plant protection (El Guilli *et al*., 2009; Habybellah, 2006). The main methods used to obtain the EOs from the plant materials are hydrodistillation, maceration, steam distillation, expression and empyreumatic (or destructive) distillation. Hydrodistillation has been the most widely used method (Stahl-Biskup & Sáez, 2002). Ultrasound assisted extraction (Da Porto *et al*., 2009), Microwave-assisted extraction (Wang *et al*., 2010), and supercritical fluid extraction (Li *et al*., 2009) are examples of new approaches that have been used to improve extraction yield, reduce extraction time, and lower operational costs. Many studies on medicinal plants have found that harvest season, geographical origin and extraction method all have an impact on the chemical composition and functional activities of EOs (Govrin & Levine, 2000; Yesil *et al*., 2007).

However, no comparative work exposing the effect of different extraction methods on the chemical composition of EOs as well as the antifungal activity has been published to our knowledge. As a result, the purpose of this study was to investigate the effect of the extraction methods (hydrodistillation, soxhlet, maceration et ultrasound) on the yield, chemical composition and antifungal activity of EOs extracted from *C. atlantica* wood.

2. MATERIAL and METHODS

2.1. Plant material

The sawdust samples were collected at Azrou sawmill (Middle Atlas). Grinding was performed until a fine and homogeneous powder was obtained.

2.2. Essential oil extraction

2.2.1. *Extraction with an organic solvent*

The oils studied were extracted with an organic solvent at the rate of 3 tests to express the values of the yields relative to the dry matter. During each test, 100 g of the raw material was mixed with 700 mL of hexane. The EOs were extracted using the soxhlet, maceration and ultrasound.

- **Soxhlet**

A mass of cedar wood powder was placed in a porous cartridge which was introduced into a soxhlet extractor with a balloon in the base where the hexane was introduced for 6 h

- **Maceration**

A quantity of the powder of cedar wood was macerated in cold for 12 h, with the hexane which retains the chemical compounds we intend to extract. Once the time had elapsed, the mixture had been filtered through a filter paper and the maceration obtained was stored in a bottle.

- **Ultrasound**

Ultrasound-assisted extraction was performed with a powder sonicated in hexane for 10 minutes. After this treatment, the extract was filtered and conserved in a bottle.

The mixture collected by the different extraction methods mentioned above was subjected to vacuum pressure using a rotary evaporator, to separate the solvent and the EOs.

2.2.2. *Extraction by hydrodistillation*

A quantity of water and 100 g of sawdust were immersed in a balloon. installed at the Clevenger device for 6 hours. The mixture (oil and water) was separated by density difference. The recovered oil was placed in a small opaque bottle and stored at fridge.

2.3. GC–MS analysis

The constituents were identified using a gas chromatography coupled to a mass spectroscopy. Perkin Elmer Version ClarusTM GC-680 with Q-8 MS is the device. It is equipped with an auto-sampler which gives access to the automatic injection of samples into the injector and an HP-5MS type capillary column traversed by Helium gas. The mass spectrometer was powered by a source of electronic ionization SMART source which allowed to ionize and vaporize the different molecules as well as a quadrupole filter to separate the different ions according to their m/z ratio. The GC-MS system was controlled by a computer device accompanied by Turbomass TM software which allowed the programming of analysis methods as well as the qualitative and quantitative identification of the species detected.

The analysis time was 82 min with a gas flow rate of 1 mL / min. The ionization energy was 70 eV. The volume injected was 0.5 µL (10%of the EO was dissolvedwith 90% of hexane) at 280 °C. The oven temperature was programmed to start at 60°C for 1 minute, followed by a temperature gradient of 2°C/min up to 200°C, where it remained for 1 minute. Subsequently, the temperature was raised to 300°C with a ramp of 20°C/min and held for 5 minutes.

2.4. Fungal Material

B. cineria is a pathogenic ubiquitous and polyphagous ascomycete. This fungus is responsible for rots on a large number of economically important host plants in agriculture. It attacks more than 200 crop species worldwide (Govrin & Levine, 2000; Lecompte *et al*., 2013). *F. culmorum* is a fungus found in all cereal growing regions around the world that causes a destructive disease that not only reduces yield and grain quality but also contaminates grain with various mycotoxins (Kang *et al.*,2000). The two fungal strains studied, *F. culmorum* and *B. cineria*, came from the phytopathology laboratory of the INRA in Rabat. They were regularly transplanted and maintained on PDA (Potato Dextrose Agar) medium.

2.5. Antifungal Effects of Essential Oils

PDA medium with a concentration of 1.25 ml/L of each EO was prepared for the two fungi. After homogenization using 0.5 % of tween 80 as an emulsifier, both solutions and control medium were poured into 9 cm diameter Petri dishes and allowed to solidify. After 24 hours, a

mycelial fragment of 5 mm in diameter was placed in the center of each Petri dish. The plates were incubated at 20°C for 7 days. The diameter of the fungal colonies was measured daily. MIC is relative to the EO whose initial concentration completely controls the growth of the fungus. Culture media at concentrations of 0.624, 0.312 and 0.156 ml/L corresponding to dilutions of 1/2, 1/4 and 1/8 to stock solutions (1.25 ml/L) respectively, plus a control medium were prepared and poured into 9 cm diameter Petri dishes. After solidification, the dishes were inoculated and incubated at 20°C for 7 days. Each treatment was repeated 3 times, and the growth of the fungus was monitored daily in each dish.

To investigate the effect of the EOs on the two fungi, mycelial growth was plotted against the variation of radial mycelial growth with days of incubation. The reduction rate was calculated according to the equation:

$$\boldsymbol{I = (dt - dc/dt) \times 100}$$

Where: I = Rate of reduction of mycelial growth.

dt = Maximum diametrical growth (cm) of mycelium on control medium

dc = Maximum diametrical growth (cm) of mycelium on the culture medium at a certain concentration of EO.

According to Laib *et al.* (2012), the concentration of an EO is highly active with an inhibition between 75 and 100% and then the fungal strain is highly susceptible. The concentration of an EO is active when the inhibition is between 50 and 75% and the fungal strain is said to be sensitive; moderately active when it has an inhibition between 25 and 50% and the strain is said to be limited; and not very or not at all active when it has an inhibition between 0 and 25%; the strain is said to be little sensitive or resistant.

3. RESULTS and DISCUSSION

3.1. Yields of extraction

The extraction yield values ranged from 4.82 % for the maceration to 11.68 % for the soxhlet (Table 1). The yield for the EOs obtained by soxhlet and ultrasound methods was higher than that of the classical methods (hydrodistillation and maceration). This confirms the advantages of the new extraction methods in terms of yield. These yields provided by the sawdust are higher than those obtained by the needles (1.8%) (Derwich *et al*.,2010b), winged (2.6%) and wingless (3.6%) seeds (Rhafouri *et al*., 2014). The studies led by Aberchane *et al*. (2001); Fidah *et al*. (2016) on the cedarwood, originating from Azrou provided a yield did not exceed 3.41%. EOs yields are subject to fluctuations and variations that can be attributed not only to the extraction method or the part of the tree used, but also to the provenance or age and harvesting period (Duval, 2012).

Table 1. The extraction yield of the different extraction methods.

Extracts	Extraction time	Yield (%)
Hydrodistillation	6 h	5.60
Soxhlet	6 h	11.68
Maceration	12 h	4.82
Ultrasound	10 min	9.33

3.2. Chemical Composition of The EOs

The chemical composition of the extracted EOs of various methods is given in Table 2. These analyses allowed us to identify approximately 33 components, which represent about 76.19%, 81.14%, 72.84% and 74.03% of the total EOs composition for hydrodistillation, soxhlet, maceration and ultrasound respectively. The obtained results showed that the chemical

composition of the oils obtained by hexanic solvent is different to those extracted by hydrodistillation (Figure 1). The main compound found by soxhlet (Figure 2), maceration (Figure 3) and ultrasound (Figure 4) is: Copalic acid methyl ester (28.41%, 20.24%, and 24.17%, respectively). On the contrary, we find the major constituent one for the oil obtained by hydrodistillation being: β-himachalene (21.32%) followed by α-himachalene (9.40%). These differences can be due to the influence of the polarity of the solvent and the extraction method.

The chemical analysis obtained by hydrodistillation has constituents relatively similar to those of other cedarwood EO analyzed by Aberchane *et al*. (2001). They found that the major compounds are β-himachalene (39.72%), α-himachalene (15.78%) γ-himachalene (9.56%) and E-a-atlantone (9.15%). In addition, in a recent study realized by Jaouadi *et al*. (2016) about EO of *C. atlantica* (in the provinces of the Middle Atlas of Morocco in the forest of Itzer) approximately comparable to the composition of our EO, the main compounds identified were: β-himachalene (24.25%), α-himachalene (13.76%), methyl-1,4-cyclohexadiene (9.06%) trans-cadina-1(6),4-diene (7.65%), and 6-camphenol (7.44%).

Figure 1. GC-MS chromatogram of *C. atlantica* EO extracted by hydrodistillation.

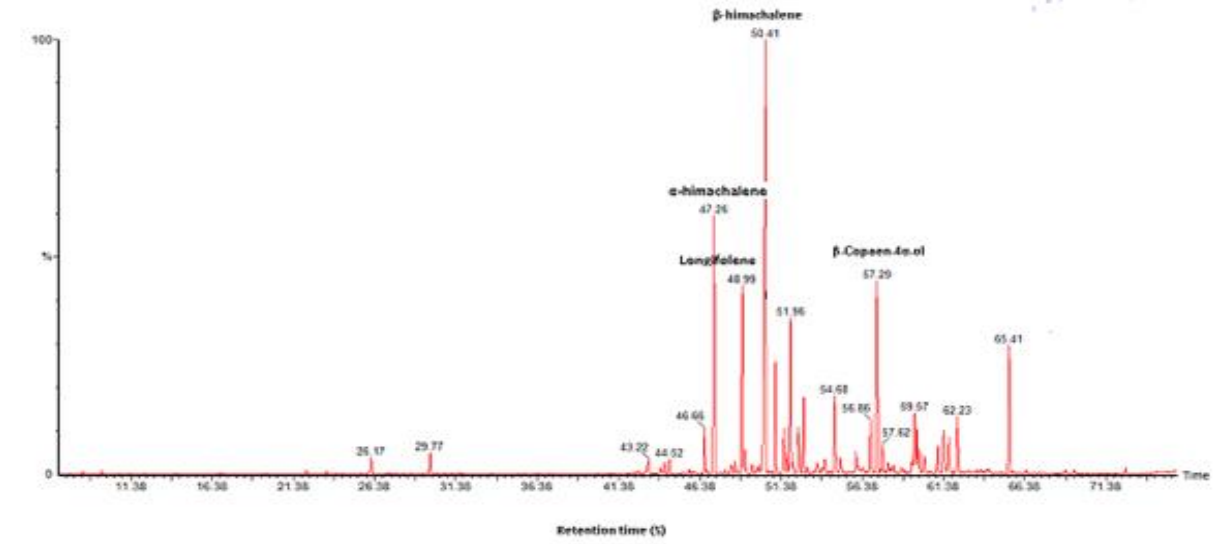


Figure 2. GC-MS chromatogram of *C. atlantica* EOextracted by soxhlet.

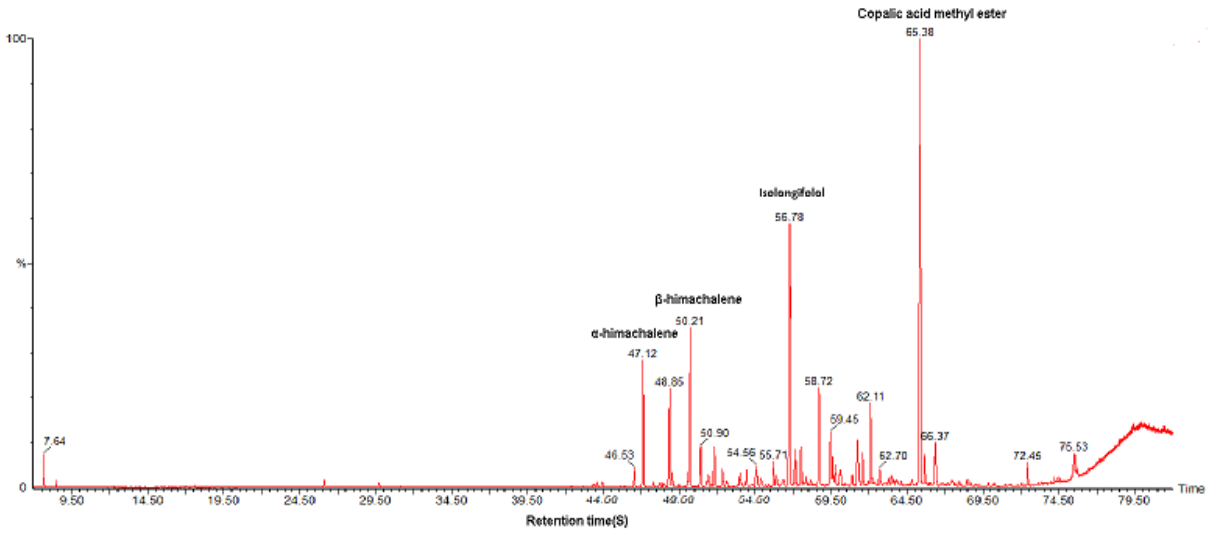


Figure 3. GC-MS chromatogram of *C. atlantica* EO extracted by maceration.

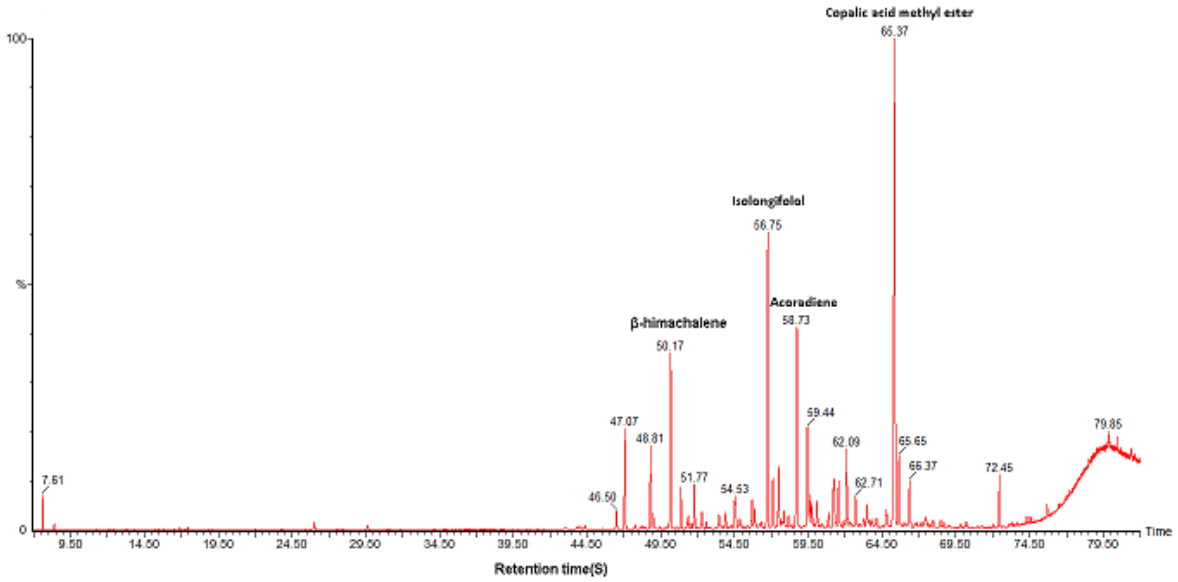


Figure 4. GC-MS chromatogram of *C. atlantica* EO extracted by ultrasound.

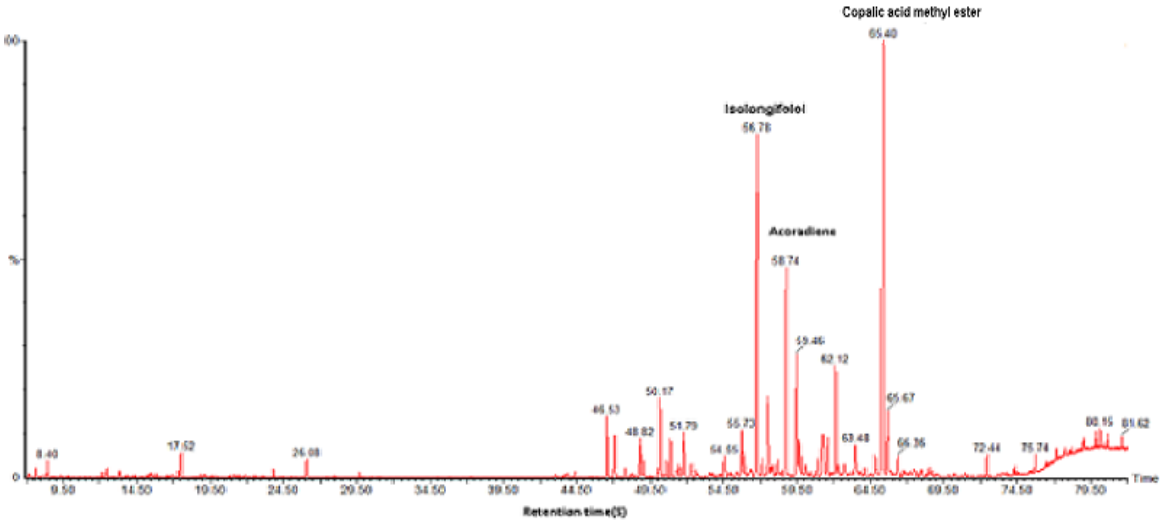


Table 2. Chemical composition of cedarwood EOs.

Compound	Hydrodistillation		Soxhlet		Maceration		Ultrasound	
	TR	%	TR	%	TR	%	TR	%
P-Cresol	22.18	0.12	nd	nd	nd	nd	Nd	nd
Endo-borneol	28.59	0.02	nd	nd	nd	nd	Nd	nd
Calarene	44.21	0.37	nd	nd	nd	nd	44.36	0.18
Farnesol	46.65	1.43	46.53	0.83	46.49	0.5	46.52	1.85
shouldα -himachalene	47.26	9.4	47.11	5.48	47.06	2.71	47.07	1.34
Longipinene	nd	nd	48.85	4.12	48.81	2.31	48.81	1.19
Longifolene	48.99	6.74	49.02	0.66	48.98	0.43	48.99	0.71
β-himachalene	50.41	21.32	50.20	7.3	50.17	5.14	50.17	2.52
1-Mesitylbuta-1.3-diene	51.03	3.77	50.89	1.79	50.86	1.12	50.87	1.22
δ-Cadinene	nd	nd	51.40	0.73	51.36	0.5	51.38	0.54
E-Calamenene	51.54	1.73	nd	nd	nd	nd	51.6	0.37
α-Calacorene	51.75	0.62	51.80	1.71	51.76	1.21	51.78	1.54
Androstenediol	55.02	0.66	nd	nd	nd	nd	55.03	0.07
Longiborneol	56.00	0.91	nd	nd	nd	nd	Nd	nd
Isolongifolol	56.86	2	56.77	12.99	56.74	9.25	56.78	13.65
β-Copaen-4α-ol	57.28	7.71	nd	nd	nd	nd	Nd	nd
α-Cubebene	57.62	0.9	nd	nd	nd	nd	57.67	0.16
γ-Gurjunene	58.28	0.24	nd	nd	nd	nd	Nd	nd
Acoradiene	58.78	0.28	58.72	4.79	58.73	6.74	58.74	8.58
β-humulene	59.57	1.83	59.45	2.35	59.44	2.82	59.46	4.05
Carveol	59.73	1.01	59.75	0.66	59.74	0.49	59.75	0.77
Ledane	59.87	0.65	nd	nd	nd	nd	Nd	nd
Cadalene	60.20	0.66	nd	nd	60.09	0.76	Nd	nd
Cedreanol	61.25	3.32	61.25	3.3	61.24	2.42	61.25	2.31
Curlone	61.37	3.69	nd	nd	nd	nd	Nd	nd
Tumerone	62.05	0.03	62.10	3.57	62.09	2.23	62.11	3.55
Tujopsene	62.22	1.95	nd	nd	nd	nd	62.31	0.34
Longipinane	62.77	0.04	62.70	0.83	62.7	0.96	62.71	0.49
β-cis-caryophyllene	63.4	0.11	63.49	0.44	63.46	0.64	63.47	1.3
Copaen-15-ol	64.86	0.07	nd	nd	64.79	0.72	64.8	0.9
Copalic acid methyl ester	65.40	4.54	65.38	28.41	65.37	20.24	65.4	24.17
Trans-Calamenene	65.59	0.07	65.64	1.18	65.64	1.75	65.66	2.02
Octasiloxane	nd	nd	nd	nd	79.85	9.9	79.85	0.21
Total identified		76.19%		81.14%		72.84%		74.03%

Table 2. *Continues.*

Sesquiterpene	54.37%	31.38%	27.09%	26.55%
Calarene	0.37	nd	nd	0.18
α-himachalene	9.4	5.48	2.71	1.34
Longipinene	nd	4.12	2.31	1.19
Longifolene	6.74	0.66	0.43	0.71
β-himachalene	21.32	7.3	5.14	2.52
1-Mesitylbuta-1.3-diene	3.77	1.79	1.12	1.22
δ-Cadinene	nd	0.73	0.5	0.54
E-Calamenene	1.73	nd	nd	0.37
α-Calacorene	0.62	1.71	1.21	1.54
α-Cubebene	0.9	nd	nd	0.16
γ -Gurjunene	0.24	nd	nd	nd
Acoradiene	0.28	4.79	6.74	8.58
β-humulene	1.83	2.35	2.82	4.05
Ledane	0.65	nd	nd	nd
Cadalene	0.66	nd	0.76	nd
Curlone	3.69	nd	nd	nd
Tujopsene	1.95	nd	nd	0.34
Longipinane	0.04	0.83	0.96	0.49
β-cis-caryophyllene	0.11	0.44	0.64	1.3
Trans-Calamenene	0.07	1.18	1.75	2.02
Alcohols	17.25%	17.78%	13.38%	19.55%
P-Cresol	0.12	nd	nd	nd
Endo-borneol	0.02	nd	nd	nd
Farnesol	1.43	0.83	0.5	1.85
Androstenediol	0.66	nd	nd	0.07
Longiborneol	0.91	nd	nd	
Isolongifolol	2	12.99	9.25	13.65
Carveol	1.01	0.66	0.49	0.77
β-Copaen-4α-ol	7.71	nd	nd	nd
Cedreanol	3.32	3.3	2.42	2.31
Copaen-15-ol	0.07	_	0.72	0.9
Tumerone	0.03%	3.57%	2.23%	3.55%
Copalic acid methyl ester	4.54%	28.41%	20.24%	24.17%
Octasiloxane	nd	nd	9.9%	0.21%

nd: not detected.

3.3. Antifungal Activity

3.3.1. *Fusarium culmorum*

Mycelial growth was slowed for the concentrations of 1.25mL/L, 0.624 mL/L, 0.312 mL/L, and 0.156 mL/L of *C. atlantica* EOs extracted by hydrodistillation, soxhlet, maceration and ulrasound (Table 3). At the stock concentration of 1.25 mL/L, *C. atlantica* EOs did not control the development of the fungus, but they significantly slowed its growth compared to the control (Figure 5).

Figure 5. Effects of stock concentrations (1.25 mL of the EO/L of culture medium) of *C. atlantica* EOs on the radial growth of *F. culmorum*.

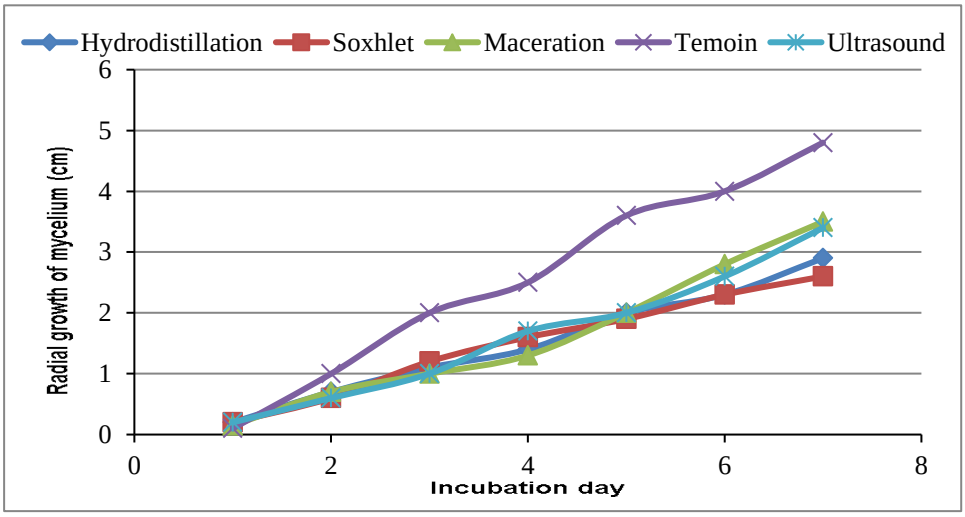


Figure 6. The effect of concentration (1. 25 mL/L) of EOs (H: oil extracted by hydrodistillation, S: oil extracted by soxhlet, M: oil extracted by maceration and U: oil extracted by ultrasound) on growth of *F. culmorum* after 7 days of incubation.

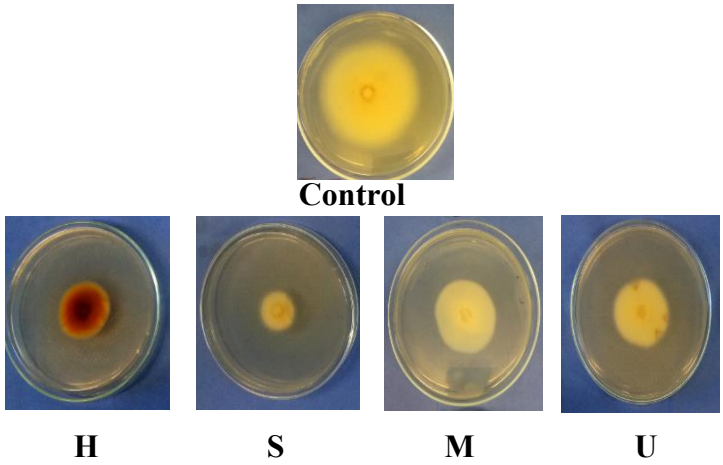


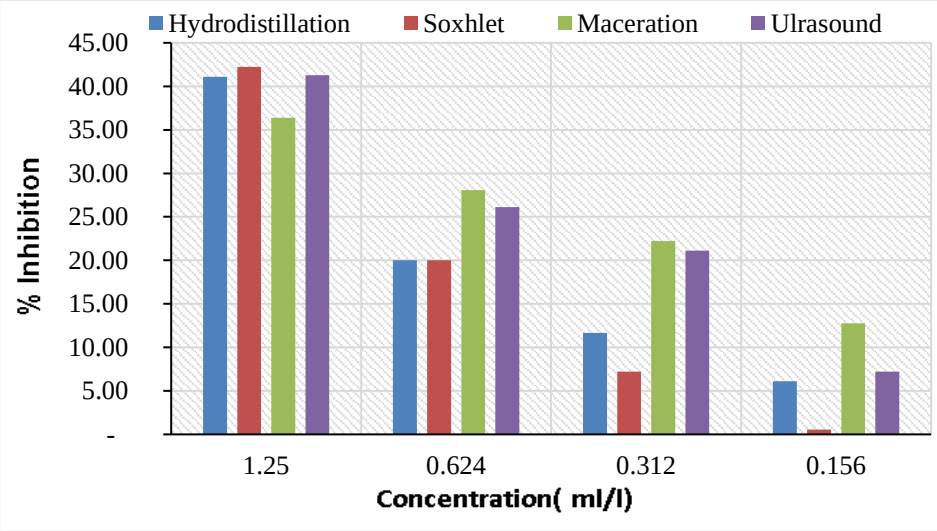
Figure 6 shows the effect of concentration (1. 25 mL/L) of EOs on growth of F. culmorum after 7 days of incubation. Our results confirmed other authors' works on the antifungal power of *C. atlantica* EOs. Indeed, Uwineza *et al*. (2016) found that the EO of *C. atlantica* bark did not control the development of the fungus, but it only inhibited the growth of *F. culmorum*. According to the formula 1, the mode of action for each concentration is presented in Figure 7. It confirms the fact that *F. culmorum* is limited to the highest concentration (1.25 mL/L) of *C. atlantica* EO.

Table 3. Average growth (in cm) of F. *culmorum* on culture medium PDA supplemented with different concentrations of cedar EOs.

F.Culmorum	Concentration (mL/L)	D1	D2	D3	D4	D5	D6	D7
Hydrodistillation	1.25	0.2	0.7	1.1	1.4	2	2.3	2.9
	0.624	0	0.5	1.5	2.1	2.9	3.3	4.1
	0.312	0	0.7	1.7	2.3	3.1	3.6	4.5
	0.156	0.1	0.9	1.8	2.5	3.1	3.8	4.7
Soxhlet	1.25	0.2	0.6	1.2	1.6	1.9	2.3	2.6
	0.624	0	0.4	1.5	2.1	2.9	3.5	4
	0.312	0	0.8	1.8	2.4	3.3	3.9	4.5
	0.156	0.1	1	1.9	2.5	3.6	4	4.8
Maceration	1.25	0.15	0.7	1	1.3	2	2.8	3.5
	0.624	0	0.3	1.1	1.6	2.15	3.5	4.3
	0.312	0	0.4	1.2	1.7	2.2	3.8	4.7
	0.156	0	0.7	1.5	2.5	2.3	3.9	4.8
Ultrasound	1.25	0	0 .3	0.7	1.4	2	2.9	3.3
	0.624	0	0.5	0.9	1.7	2.2	3.4	4.6
	0.312	0	0.6	1	1.8	2.3	3.8	4.7
	0.156	0	0.8	1.8	2.5	2.8	4	4.8
Control	0	0.1	1	2	2.5	3.6	4	4.8

D: Incubation day after inoculation

Figure 7. Reduction rate of radial growth of *F. culmorum* by different concentrations of *C. atlantica* EOs.



3.3.2. *Botrytis cinerea*

The stock solution (1.25 mL/L) of *C. atlantica* EOs extracted by hexanic solvent (soxhlet, maceration and ultrasound) completely inhibited mycelial growth, whereas the EO extracted by hydrodistillation slowed down the development of this fungus considerably but did not inhibit it completely (Figure 8). The successive dilutions 1/2, 1/4 and 1/8 were used for each PDA-EO mixture, corresponding to oil concentrations of 0.624 mL/L, 0.312 mL/L and 0.156 mL/L respectively. All these concentrations decreased the growth compared to the control (Table 4).

Figure 8. Effects of stock concentrations (1.25 ml/L) of EOs of *C. Atlantica* on the radial growth of *B. cinerea*.

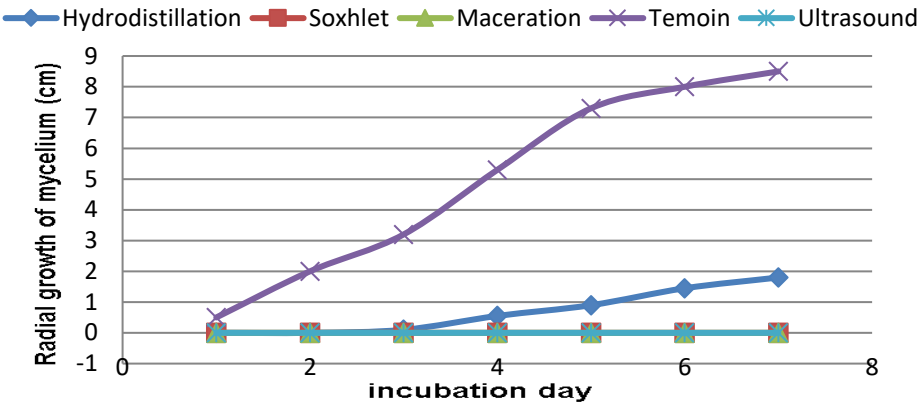


Figure 9. The effect of concentration (1.25 mL/L) of EOs (H: oil extracted by hydrodistillation, S: oil extracted by soxhlet, M: oil extracted by maceration and U: oil extracted by ultrasound) on growth of *B. cinerea* after 7 days of incubation.

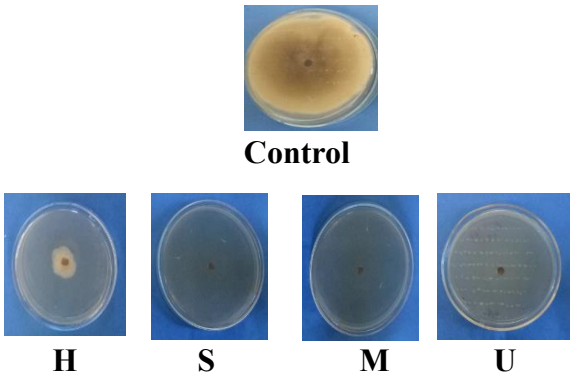


Table 4. Average growth (in cm) of *B.cinerea* on culture medium PDA with different concentrations of EOs of *C. atlantica*.

B. cinerea	Concentration (mL/L)	D1	D2	D3	D4	D5	D6	D7
Hydrodistillation	1.25	0	0	0.1	0.55	0.9	1.45	1.8
	0.62	0	0	1	1.5	1.7	2.5	3.1
	0.31	0	0.1	1.1	2	2.8	3	3.5
	0.15	0.1	0.8	3	5.3	7.2	8	8.5
Soxhlet	1.25	0	0	0	0	0	0	0
	0.62	0	0	0	0	0.2	0.8	1.1
	0.31	0	0.1	1.1	1.6	2.1	2.5	3.3
	0.15	0.3	1.3	2.7	5.3	6.5	8	8.5
Maceration	1.25	0	0	0	0	0	0	0
	0.62	0	0	0.9	1.9	2.9	3.3	3.8
	0.31	0	0.1	1	2.1	2.7	3.5	4.5
	0.15	0.1	0.5	1.1	3	4.4	5.3	6.2
Ultrasound	1.25	0	0	0	0	0	0	0
	0.62	0	0.1	1.3	2.3	3.4	3.8	4.1
	0.31	0.1	1.2	2.7	4.2	4.5	5	5.2
	0.15	0.2	1.5	3.2	4.4	4.8	5.2	5.9
Control	0	0.5	2	3.2	5.3	7.3	8	8.5

D: Incubation day after inoculation

Figure 10. Rate of reduction of radial growth of *B. cinerea* by different concentrations of *C. atlantica* EOs for the two extraction methods.

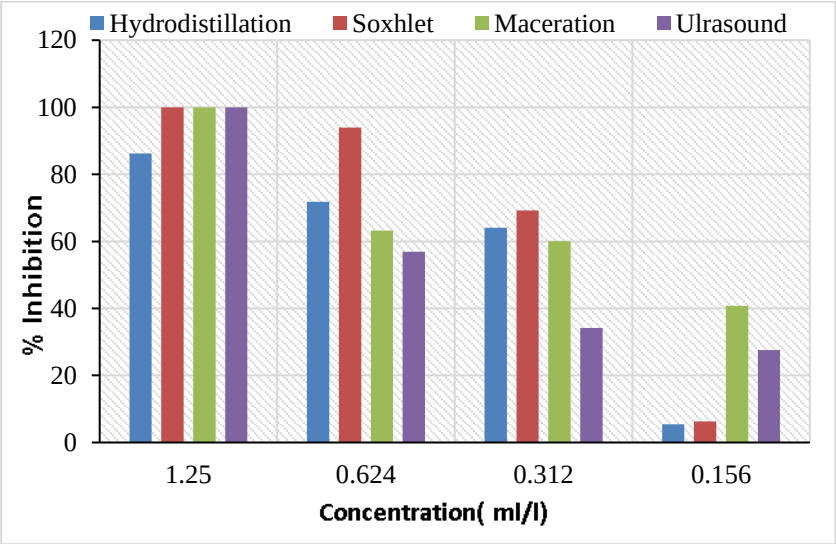


Figure 9 shows the effect of concentration (1.25 mL/L) of EOs on growth of B. cinerea after 7 days of incubation. The 1.25 mL/L concentration of *C. atlantica* EOs extracted by soxhlet. maceration and ultrasound (Figure 10) corresponds to the MIC for B. cinerea. The studies led by Chebli *et al*. (2004) of *C. atlantica* leaf oil showed inhibition of mycelial growth against *B. cinerea* at 250 ppm. Figure 6 shows that. *B. cinerea* is very sensitive to 1.25 mL/L of the *C. atlantica* EOs.

C. atlantica EOs extracted by soxhlet. maceration and ultrasound with hexanic solvent had stronger antifungal activity than EOs extracted with hydrodistillation. This is related to their majority compounds. This was confirmed with the studies by Nakamura *et al*. (2017). which showed that copalic acid has a greater antifungal property. The research condcuted by Rhafouri *et al*. (2014) showed the antifungal power of *C. atlantica* EO extracted by hydrodistillation with a concentration of 1/100 (v/v). and this allowed us to assume that for the EO extracted by hydrodistillation. Higher concentrations could give better results than those obtained in this study.

All these biological properties of EOs could well be attributed not only to their majority compounds (Bourkhiss *et al*., 2007; Kellouche & Soltani, 2004). But also to the synergistic effect of minority compounds (Bouzouita *et al*., 2008; Kordali *et al*., 2008).

4. CONCLUSION

The yields of EO extracted by hyrodistillation. soxhlet. maceration and ultrasound were 5.60%. 11.68%. 4.82%, and 9.33%, respectively. The main constituent of EOs obtained by hexanic solvent (soxhlet, maceration, and ultrasound) is copalic acid methyl ester (28.41%. 20.24% and 24.17% respectively). While, the major compound of the EO obtained by hydrodistillation is: β-himachalene (21.32 %), according to the results of the antifungal activity evaluations. the EOs of *C. atlantica* extracted from the hexanic solvent were the best against *B. cinerea* with the minimum fungicidal concentration of 1.25 mL/L. While for the same concentration of *C. atlantica* EO. it was found that *F. culmorum* slowed the development of the fungus without stopping it completely. Our study demonstrated the potential of *C. atlantica* EO as a conservative antifungal agent in vitro against two pathogens *B. cinerea and F. culmorum*.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Chaimaa Bouyahia and **Maria Benbouzid**: Investigation, Software, Resources, and Writing - original draft. **Souad El Hajjaji** and **Miloudia Slaoui**: Methodology, Supervision, and Validation. **Fatiha Bentata**, **Mustapha Labhilili**, and **Abdelhakim Bouyahya**: visualization, editing the original draft.

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