

## *Cedrus atlantica* essential oil: Antimicrobial activity and effect on the physicochemical properties of cedar wood Surface

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### Abstract

This study aimed to investigate the chemical composition and antimicrobial effects of *Cedrus atlantica* essential oil, against two bacteria and six fungi that cause the degradation of cedar wood. The effects of *C. atlantica* essential oils, applied for different treatment times, on the physicochemical properties of cedar wood were also explored. Gas chromatography (GC)/mass spectrometry (MS) analysis of the studied essential oil showed that cedranone and iso-cedranol were the major components of *C. atlantica* essential oil. The minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations were determined, using broth microdilution assays, and the physicochemical properties of cedar wood were determined using the contact angle measurement. The results demonstrated antibacterial activity against the two bacteria tested, with MICs ranging from 1% to 2%, and antifungal activity against all fungi tested, with MICs ranging from 0.5% to 1%. The cedar wood maintained its hydrophobic character, as assessed quantitatively, after treatment with *C. atlantica* essential oil, increasing its electron donor character after 15 min and 1 h of treatment.

**Keywords:** *Cedrus atlantica*, antimicrobial activity, cedar wood, contact angle, physicochemical properties.

### Introduction

Native to the Rif and Atlas Mountains of North Africa, especially Morocco and Algeria (Renau-Morata *et al.*, 2005; Maya *et al.*, 2017), *Cedrus atlantica* Manetti a large conifer, found at altitudes ranging from 1,500 to 2,600 m. *C. atlantica* is the most important timber resource in Morocco, occupying a surface area of 132,000 ha and representing 2.3% of the national forest (Renau-Morata *et al.*, 2005). Essential oils derived from *C. atlantica* are used in various products, as drugs and perfumes (Adams, 1991). *C. atlantica* essential oils have been already studied and shown to possess larvicidal (Ez Zoubiet *et al.*, 2017), antiviral (Loizzo *et al.*, 2008), antifungal, and antibacterial activities (Hammer *et al.*, 1999; Chebli *et al.*, 2003; Satrani, 2006; Derwich *et al.*,

2010; Rhafouri *et al.*, 2014; Zrira *et al.*, 2016). However, the study of its antifungal and antibacterial activities, especially against bacteria and fungi isolated from cedar wood, and the use of *C. atlantica* essential oils for wood protection have never been reported in the literature, to our knowledge.

Biological organisms, such as bacteria and fungi adhere to different materials, including cedar wood, which was often used as a raw material during the building of historical monuments in imperial cities, such as the old medina of Fez. The growth of these latter is associated with aesthetic degradation (Dickinson, 1972; Chedgy *et al.*, 2007; Gobakken & Vestøl, 2012), biodeterioration, and the reduction of wood

durability (Blanchette, 2000; Sterflinger *et al.*, 2013), and have been associated with health risks due to the release of fungal mycotoxins (Görs *et al.*, 2007). Adhesion is an important step in the biofilm formation process and is governed by van der Waals forces, electrostatic properties, and acid-base interactions, which depend on the hydrophobicity and electron donor–electron acceptor properties of the material and the microbial surface.

Recently, to protect the wood, environmental concerns have required the use of non-biocidal solutions, instead of traditional methods, such as toxic chemicals, that can provide decay resistance but are associated with environmental effects. Thus, the

## Materials and methods

### Plant materials and essential oil extraction

The *C. atlantica* specimens were collected in the region of Azrou, located in the Middle Atlas (Morocco). The essential oil extraction was performed by the hydrodistillation of 200 g of sawdust, with a Clevenger-type apparatus. The obtained oil was dried with anhydrous sodium sulfate and stored in sealed glass vials, at 4°C.

### Essential oil analysis

The chemical composition of the tested oil was determined, using gas chromatography-mass spectrometry (GC-MS). The analysis was performed using a Trace GC Ultra gas chromatograph, equipped with an apolar capillary TR-5 column (60 m × 0.32 mm ID, 0.25-µm film thickness), coupled to a mass detector (MS Quadrupole). The analysis of *C. atlantica* essential oil was performed by employing the following GC conditions: initial temperature of 40°C for 2 min, increasing by 5°C per min until 280°C, followed by a 10-min hold. The injector was maintained at a temperature of 220°C. Helium was the carrier gas, at 1.2 ml/min; the sample (1 µL) was injected in the split ratio mode

identification of natural resources, such as essential oils, that are able to prevent microbial and fungal adhesion, by modifying the physicochemical properties of the cedar wood surface, combined with antimicrobial activities against the microorganisms that can degrade wood, is our priority.

Thus, the purpose of this study was, first, to investigate the chemical composition of *C. atlantica* essential oils; second, to examine their antimicrobial activities against bacteria and fungi associated with the deterioration of historical wood; and finally, to evaluate the effects of essential oils and treatment times on the cedar wood physicochemical properties.

(10:1). The detector temperature was 300°C. The ionization energy was 70 eV. Compounds were identified by comparing their retention index (RI) and mass spectra with those of components identified in the literature and the National Institute of Standards and Technology (NIST) Library. RIs were calculated using a homologous series of n-alkanes, C<sub>8</sub>-C<sub>18</sub> (Sigma-Aldrich, St Louis, MO).

### Antibacterial activity test

The antibacterial activity of *C. atlantica* essential oil was tested against two Gram-positive bacteria: *Bacillus safensis* and *B. subtilis*, which are known for their abilities to deteriorate cedar wood. They were isolated by Sadiki *et al.* (2017-a), from decaying cedar wood found in an old wooden house, located in the old medina of Fez. The bacterial strains were subcultured in Luria-Bertani (LB) agar and incubated overnight, at 37°C. Then, the bacterial inoculum was prepared, at a final concentration of 2 × 10<sup>6</sup> colony-forming units (CFU)/ml, using a physiologic saline solution.

The minimum inhibitory concentration (MIC) of the essential oil was determined using the broth

microdilution assay (Bouhdid *et al.*, 2009; Tianet *et al.*, 2014), with some modifications. The MICs defined as the lowest concentration of an antibacterial agent that inhibits bacterial growth (Lancini *et al.*, 1993).

To determine the MIC, 50  $\mu\text{L}$  of Mueller Hinton Broth (MHB), supplemented with bacteriological agar (0.15% w/v), was deposited from the second to the twelfth well. *C. atlantica* essential oil was dissolved in Mueller Hinton Broth (MHB), containing 0.15% of agar and diluted until the 11<sup>th</sup> well, so that the final concentration was ranged between 8-0.00781% (v/v). Finally, 50  $\mu\text{L}$  of bacterial suspension, prepared at a concentration of  $2 \times 10^6$  CFU/ml, was added to each well.

The twelfth well was considered the growth control, containing only the bacterial suspension and Mueller Hinton Broth, supplemented with agar (0.15 % w/v).

The incubation was performed at 37°C, for 20 h. Then, 5  $\mu\text{L}$  of resazurin was added to each well, followed by further incubation for 2 h, to determine the MIC of *C. atlantica* essential oil (Bouhdid *et al.*, 2009). The minimum inhibitory concentration of the essential oil corresponded with the lowest concentration that prevented the reduction of the blue resazurin dye into pink resorufin.

A 5- $\mu\text{L}$  volume from each negative well was deposited on an LB plate and incubated for 24 h, at 37°C, to determine the minimum bactericidal concentration (MBC), which is defined as the lowest essential oil concentration that results in negative subcultures.

#### Antifungal activity test

*Thielavia hyalocarpa*, *Aspergillus niger*, and four fungi of the genus *Penicillium*: *P. commune* (PDLd<sup>7</sup> and PDLd10), *P. crustosum*, and *P. expansum* were selected for their abilities to rot cedar wood. They were isolated by Zyani *et al.*

(2009) and El Abed *et al.* (2010), and identified in our laboratory. The fungal strains were subcultured in Malt-Extract (ME) agar medium, at 25°C for 10 days. Then, the fungal spores were harvested by scraping the culture surface, using a sterile physiologic saline solution containing 1% dimethyl sulfoxide (DMSO). The spore suspensions were concentrated to a final concentration of  $2 \times 10^4$  spores/ml, by centrifugation at 7,000 rpm, for 15 min at 4°C (CLSI document M38-A2. 2008).

The determination of the MIC was performed as indicated in the antibacterial, test using the broth microdilution assay, except that Malt Extract Broth (MEB), supplemented with bacteriological agar (0.15% w/v) was used instead of MHB. The 96-well plate was incubated at 25°C, for 48 h. Then, 5  $\mu\text{L}$  from negative wells was deposited on ME plates and incubated at 28°C for 72 h, to determine the minimum fungicidal concentration (MFC).

#### Wood preparation

The investigated substrate in this study was cedar wood (*C. atlantica*), which was obtained from a woodworking shop in Fez City, Morocco. The roughness of the wood samples (30 mm  $\times$  10 mm  $\times$  4 mm) was set in a range from 0.8 to 1 $\mu\text{m}$ , using a rugosimeter. Then, the samples were cleaned with distilled water, oven-dried, and autoclaved at 121°C for 20 min.

#### Wood treatment

A 20- $\mu\text{L}$  volume of the tested essential oil was deposited on the cedar wood surface, at room temperature (25 $\pm$ 2°C) (Sadiki *et al.*, 2014). The samples were analyzed with contact angle measurements, after 15 min and after 1h, to evaluate the effects of the essential oil treatment time on the cedar wood physicochemical properties. Experiments were conducted in duplicate.

#### Contact angle measurements and surface energy components

The Lifshitz-van der Waals, acid-base, and surface free energy values of

untreated and treated cedar woods were calculated from contact angle measurements, which were performed by the sessile drop method, using a goniometer (GBX Instruments) (De Meijer *et al.*, 2000). Three contact angle measurements were made on each wood sample, using three liquids (two of which must be polar), with well-known surface energy components (Table 1) (Van Oss *et al.*, 1988). Once the contact angles have been measured, the Lifshitz-van der Waals

and acid-base surface tension components can be obtained, using the following three equations (Van Oss, 1993):

$$\gamma_L(\cos \theta + 1) = 2(\gamma_S^{LW}\gamma_L^{LW})^{1/2} + 2(\gamma_S^+\gamma_L^-)^{1/2} + 2(\gamma_S^-\gamma_L^+)^{1/2} \quad (1)$$

$\theta$ : The contact angle,

$\gamma^{LW}$ : The van der Waals free energy component,

$\gamma^+$ : The electron acceptor component,

$\gamma^-$ : The electron donor component,

(S) and (L) represent the solid surface and liquid phases, respectively.

**Table 1.** Surface tension properties of pure liquids used to measure contact angles (Van Oss, 2003).

| Liquid   | $\gamma^{LW}$ (mJ/m <sup>2</sup> ) | $\gamma^+$ (mJ/m <sup>2</sup> ) | $\gamma^-$ (mJ/m <sup>2</sup> ) |
|--|------------------------------------|---------------------------------|---------------------------------|
| Water (H <sub>2</sub> O)                       | 21.8                               | 25.5                            | 25.5                            |
| Formamide (CH <sub>3</sub> NO)                 | 39                                 | 2.3                             | 39.6                            |
| Diodomethane (CH <sub>2</sub> I <sub>2</sub> ) | 50.5                               | 0                               | 0                               |

The Lewis acid-base component is expressed as follows:

$$\gamma_S^{AB} = 2(\gamma_S^-\gamma_S^+)^{1/2} \quad (2)$$

The wood sample hydrophobicity was evaluated by the approach described by Van Oss *et al.* (1988), through contact angle measurements. In this approach, the degree of hydrophobicity for a given material is

$$\Delta G_{iwi} = -2\gamma_{iw} = -2 \left[ \left( (\gamma_i^{LW})^{1/2} - (\gamma_w^{LW})^{1/2} \right)^2 + 2 \left( (\gamma_i^+\gamma_i^-)^{1/2} + (\gamma_w^+\gamma_w^-)^{1/2} - (\gamma_i^+\gamma_w^-)^{1/2} - (\gamma_w^+\gamma_i^-)^{1/2} \right) \right] \quad (3)$$

## Results and discussion

### Chemical composition of *Cedrus atlantica* essential oil

The analysis of *C. atlantica* essential oil resulted in twenty-two components, constituting 100% of the total composition (Table 2). The sesquiterpenes represented the major constituents (80.26%), among which, 47.17% were oxygenated sesquiterpenes, and 33.09% were hydrocarbon sesquiterpenes. The sesquiterpene fraction was principally constituted by  $\gamma$ -himachalane,  $\beta$ -himachalane,  $\gamma$ -calamenene,  $\delta$ -cadinene, iso-cedranol, cedranone, cedrol, and caryophyllene oxide. Cedranone and iso-cedranol were identified as the primary

expressed as the free energy of the interaction between two entities of this material when immersed in water (w):  $\Delta G_{iwi}$ . Therefore, the material is considered to be hydrophilic when the interaction between the two entities is lower than the interaction of each entity with water ( $\Delta G_{iwi} > 0$ ); otherwise, the material is considered to be hydrophobic ( $\Delta G_{iwi} < 0$ ).  $\Delta G_{iwi}$  is calculated as follows:

components in the sesquiterpenes fraction, with percentages of 19.35% and 13.78%, respectively. The monoterpenes represented 15.08% of the total identified volatiles, of which 10.76% were oxygenated monoterpenes, and 4.32% were hydrocarbon monoterpenes, represented by a single compound (sabinene). The chemical composition of *C. atlantica* essential oil has been the subject of some investigations in Morocco, especially the study by Derwich *et al.* (2010), who reported  $\alpha$ -pinene as the major component of *C. atlantica* leaf oil (14.85%), followed by himachalane (10.14%),  $\beta$ -himachalane (9.89%), and  $\sigma$ -himachalane (7.62%). The percentages of

**Table 2.** Chemical composition of *C. atlantica* essential oil.

| N° | Compounds                        | RI   | % Area |
|----|----------------------------------|------|--------|
| 1  | Sabinene                         | 969  | 4.32   |
| 2  | Rose oxide                       | 1127 | 1.15   |
| 3  | $\alpha$ -terpineol              | 1142 | 2.62   |
| 4  | Borneol                          | 1163 | 1.32   |
| 5  | p-cymen-8-ol                     | 1183 | 2.42   |
| 6  | Trans-carveol                    | 1217 | 1.45   |
| 7  | Bornylacetate                    | 1285 | 1.8    |
| 8  | Tetradecane                      | 1398 | 3.93   |
| 9  | Epi-Cedrane                      | 1441 | 0.58   |
| 10 | $\gamma$ -himachalene            | 1476 | 4.05   |
| 11 | $\beta$ -himachalene             | 1499 | 7.23   |
| 12 | $\gamma$ -Cadinene               | 1513 | 0.48   |
| 13 | $\gamma$ -Calamenene             | 1520 | 7.77   |
| 14 | $\delta$ -Cadinene               | 1524 | 7.34   |
| 15 | $\gamma$ -Dehydro-ar-Himachalene | 1526 | 1.81   |
| 16 | NI                               | -    | 0.73   |
| 17 | $\alpha$ -calacorene             | 1542 | 3.83   |
| 18 | Oxido-Himachalene                | 1574 | 0.87   |
| 19 | Caryophyllene oxide              | 1591 | 8.73   |
| 20 | Cedrol                           | 1611 | 4.44   |
| 21 | Cedranone                        | 1620 | 19.35  |
| 22 | Iso-cedranol                     | 1661 | 13.78  |
|    | Hydrocarbon monoterpenes         |      | 4.32   |
|    | Oxygenated monoterpenes          |      | 10.76  |
|    | Hydrocarbon sesquiterpenes       |      | 33.09  |
|    | Oxygenated sesquiterpenes        |      | 47.17  |
|    | Others                           |      | 4.66   |
|    | Total identified compounds       |      | 100    |

Notes: RI: Retention index; NI: Not Identified.

$\beta$ -himachalane and  $\sigma$ -himachalane identified in their study were relatively similar to our percentages. Rhafouri *et al.* (2014) have shown that  $\alpha$ -pinene, manool, and bornyl acetate represent the primary constituents of cedar wingless seeds, with percentages of 46.16%, 25.47%, and 10.18%, respectively. Zrira & Ghanmi (2016) reported the chemical composition of *C. atlantica* sawdust-derived essential oil [ $\alpha$ -(E)-atlantone (19.3%),  $\beta$ -himachalane (15.1 %), 8-cedren-13-ol, (13.1%),  $\alpha$ -himachalane (5.1%), cedroxyde (4.6%), and deodarone (4.6 %)], and recently, Ez Zoubi *et al.* (2017) have reported the presence of  $\alpha$ -himachalane (35.34%),  $\beta$ -himachalane (13.62%),  $\gamma$ -himachalane (12.6%), cedrol (10.32%), iso-cedranol (5.52%), and  $\alpha$ -pinene (5.5%), in the aerial parts of *C. atlantica*. The same components were found in

*Cedrus libani*, with different percentages [himachalol (22.50%),  $\beta$ -himachalane (21.90%), and  $\alpha$ -himachalane (10.50%)] (Loizzo *et al.*, 2008). Other studies have shown the chemical composition of oleoresin on the cones of *Cedrus libani*, which is grown in Turkey [ $\alpha$ -pinene (24.78%), abieta-7,13-diene (16.67%), abieta-8,11,13-triene (6.85%), manool (5.83%), terpinen-4-ol (3.74%),  $\alpha$ -terpineol (3.42%), p-cymene (2.89%), and limonene (2.69%)] (Necmettin *et al.*, 2005).

The quantitative differences observed among the chemical compositions of the various *C. atlantica* essential oils, and the absence of some major constituents in our *C. atlantica* essential oil, such as  $\alpha$ -(E)-atlantone (Zrira & Ghanmi 2016),  $\alpha$ -himachalane (Ez Zoubiet *et al.*, 2017), himachalol (Loizzo *et al.*, 2008), and  $\alpha$ -pinene (Necmettin *et al.*, 2005; Derwich *et al.*, 2010; Rhafouriet *al.*, 2014), could be explained by differences in geographical factors and climatic conditions, that are specific to each region (Mansouri *et al.*, 2010), differences in the parts of the plants being extracted, and differences in the harvest time (Marcum & Hanson, 2006; Muñoz-Bertomeu *et al.*, 2007).

### Antibacterial activity

Table 3 shows MIC and MBC values obtained in the antibacterial test for cedar wood essential oil. The results showed that the essential oil possessed good antibacterial activity against the studied bacterial strains studied, as the MIC values ranged between 1% and 2%.

*B. safensis* and *B. subtilis* were both found to be susceptible to *C. atlantica* essential oil, with MIC values of 2% and 1%, respectively. The essential oil exhibited abacteriostatic effect against *B. subtilis* (MBC/MIC>4), and a bactericidal effect against *B. safensis* (MBC/MIC=4) (CLSI document M07-A9. 2012).

Few studies examining the effects of *C. atlantica* essential oil have been

**Table 3.** The minimum inhibitory concentrations and the minimum bactericidal/fungicidal concentrations of cedar wood essential oil.

|          | Strains                                   | MIC<br>%(v/v) | MBC-MFC<br>%(v/v) | MBC/MIC<br>MFC/MIC |
|----------|---|---------------|-------------------|--------------------|
| Bacteria | <i>B. safensis</i>                        | 2             | 8                 | 4                  |
|          | <i>B. subtilis</i>                        | 1             | 8                 | 8                  |
| Fungi    | <i>P. commune</i><br>(PDLd <sup>o</sup> ) | 1             | >8                | -                  |
|          | <i>P. commune</i><br>(PDLd10)             | 1             | >8                | -                  |
|          | <i>P. expansum</i>                        | 0.5           | 8                 | 16                 |
|          | <i>P. crustosum</i>                       | 0.5           | >8                | -                  |
|          | <i>T. hyalocarpa</i>                      | 0.5           | 8                 | 16                 |
|          | <i>A. niger</i>                           | 1             | >8                | -                  |

published. According to these reports, *C. atlantica* essential oils have demonstrated effective antibacterial activity, with MIC values of 0.4 µl/ml against *Escherichia coli* and *Bacillus cereus* and 0.2 µl/ml against *B. Subtilis* (Zrira & Ghanmi 2016). Derwich *et al.* (2010) revealed a low to moderate antibacterial activity for *C. atlantica* leaf oil against a range of bacteria tested, with MIC values between 0.25 mg/ml and 1.62 mg/ml (MIC=0.98mg/ml for *Pseudomonas aeruginosa*, and MIC=1.31 mg/ml for *Enterococcus faecalis*). Satrani (2006) also concluded that *C. atlantica* essential oil has antimicrobial activity against *Escherichia coli*, *B. subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*. A similar study demonstrated that the essential oils derived from Atlas Cedar winged and wingless seeds were able to inhibit the growth of *Escherichia coli*, at a concentration of 1/100 v/v (Rhafouri *et al.*, 2014).

The antibacterial activity of the hydromethanolic extract of *C. atlantica* cones and its purified compounds were also investigated. Maya *et al.* (2017) revealed the interesting antimicrobial activity of hydromethanolic extract against a large panel of bacterial strains. Indeed, among the purified compounds, dehydroabiatic acid was the most active, with MIC values of 15.1 and 31.2 µg/ml against *Enterococcus faecalis* and *Staphylococcus aureus*, respectively.

The antibacterial activity of our essential oil can be attributed to its chemical composition, especially the presence of terpene alcohols (isocedranol, cedrol, trans-carveol, p-cymen-8-ol, borneol, and α-terpineol), which represent 26.03% of the oil (Satrani, 2016). Other studies have shown that the essential oils that possess the strongest antibacterial properties are rich in phenolic compounds (Baydar *et*

*al.*, 2004; Rota *et al.*, 2008). Thus, the presence of phenolic compounds, especially hydroxyl groups, play an important role in antimicrobial activity (Zinoviadou *et al.*, 2009).

### Antifungal activity

*C. atlantica* essential oil showed antifungal activity, as reflected by the obtained MIC values. The MICs determined for all fungal strains tested in this study oscillated between 0.5% and 1% (v/v) (Table 3). The essential oil tested has fungistatic activity against almost all fungal strains studied. Thus, similar susceptibility levels were identified for *P. commune* (PDLd<sup>o</sup> and PDLd10) and *A. niger*, with MIC values of 1% (v/v). *P. expansum*, *P. crustosum*, and *T. hyalocarpa* showed similar levels of susceptibility with MIC values of 0.5% (v/v).

The lipophilicity of essential oils enables their penetration into the membrane structures of the fungi, causing membrane expansion, increased membrane permeability and fluidity, the disruption of membrane-embedded proteins, and changes in the ion transport process in fungi (Burt, 2004; Oonmetta-aree *et al.*, 2006; Khanet *et al.*, 2010; Fadli *et al.*, 2012). Decreased lipids, which are major components of the cell membrane, suggest a reduction in membrane stability and the increased permeability of water-soluble materials (Helal *et al.*, 2007).

Terpenes, which are the primary constituents of essential oils, reportedly disrupt or penetrate the lipid structures of cells, by saturating the cell membrane (Prashar *et al.*, 2003). Some studies have shown that the treatment of fungi with essential oils decreased the lipid contents, affected the cell membrane structure, and inhibited fungal growth (Helal *et al.*, 2006; Helal *et al.*, 2007; Tao *et al.*, 2014).

The fungi toxicity and antifungal activities of *C. atlantica* essential oil could be attributed to fungal membrane disruption, due to the accumulation of the essential oil compounds on the cytoplasmic membrane.

### Effects of *Cedrus atlantica* essential oil on the physicochemical properties of cedar wood

According to Vogler (1998), and the approach of Van Oss *et al.* (1988, 1989), the untreated cedar wood surface was qualitatively and quantitatively hydrophobic, with values of  $\theta_w = 81.5 \pm 0.73^\circ$  and  $\Delta G_{iwi} = -64.38 \text{ mJ/m}^2$ . These results are consistent with those obtained by Meijer *et al.* (2000), who also reported the qualitative hydrophobic character of cedar wood, with  $\theta_w = 69 \pm 2^\circ$ . Table 4 shows that untreated cedar wood has an electron donor character,  $\gamma^-$ , more than an electron acceptor character,  $\gamma^+$ .

The cedar wood essential oil had remarkable effects on cedar wood surface hydrophobicity and the electron donor/electron acceptor properties after treatment. The results showed that the degree of hydrophobicity did not change much, quantitatively, even after 15 min of treatment, with  $\Delta G_{iwi} = -41.46 \text{ mJ/m}^2$ . In addition, the electron donor character, using *C. atlantica* essential oil was on average 2.5-fold higher than that of untreated wood.

Similar results were reported in our recent study, focused on the treatment of the cedar wood surface, using *Rosmarinus officinalis* essential oil (Bennouna *et al.*, 2018). After 15 min of treatment, the

physicochemical properties of the wood surface were modified, maintaining its hydrophobic character, quantitatively, with an increase in the electron donor character ( $\Delta G_{iwi} = -26.49 \text{ mJ/m}^2$ ;  $\gamma^- = 16.29 \pm 0.58 \text{ mJ/m}^2$ ). Similar results were reported by Barkai *et al.* (2015, 2016), after treatment with  $\beta$ -ionone ( $\Delta G_{iwi} = -7.52 \text{ mJ/m}^2$ ;  $\gamma^- = 27.52 \pm 0.41 \text{ mJ/m}^2$ ) and carvone ( $\Delta G_{iwi} = -5.31 \text{ mJ/m}^2$ ;  $\gamma^- = 29.11 \pm 0.43 \text{ mJ/m}^2$ ). However, unlike our results, several studies have shown that untreated cedar wood samples can become hydrophilic following treatments with *Mentha pulegium* and *Cananga odorata* essential oils (Bennouna *et al.*, 2018), essential oil components (Carvacrol and 1.8-cineol) (Barkai *et al.*, 2015, 2016), *Myrtus communis*, and *Thymus vulgaris* extracts (Sadiki *et al.*, 2014, 2015, 2017-b).

The effects of essential oil treatment time were also evaluated in this study. Contact angle measurements and surface energy components were calculated after 1 h of essential oil treatment. The results showed that the cedar wood always retained its hydrophobic character, quantitatively, with  $\Delta G_{iwi} = -11.62 \text{ mJ/m}^2$ . The electron donor/electron acceptor properties were also affected. Treatment for 1 h resulted in a 6-fold increase compared with that of the untreated wood, and a 2.5-fold increase compared with the 15-min treatment. The values of the electron acceptor character were almost negligible for both untreated and treated cedar wood.

The modification of surface properties that were noted in this study can be attributed to the chemical composition of *C. atlantica* essential oil. The maintenance of the hydrophobic character of cedar wood can be explained by the low percentage of terpene alcohols (26.03%), which have a hydrophilic character, due to the presence of hydroxyl groups, compared with the percentages of other hydrophobic compounds in essential oils (73.97%).

**Table 4.** Contact angle measurements, surface energy parameters (Lifshitz–van der Waals ( $\gamma^{LW}$ ), electron donor ( $\gamma^-$ ) and electron acceptor ( $\gamma^+$ )) of untreated and treated cedar wood.

|                | Contact angles(°) |                |                | Surface energy: components and parameters (mJ/m <sup>2</sup> ) |               |                | $\Delta G_{iwi}$ |
|----------------|-------------------|----------------|----------------|--|---------------|----------------|------------------|
|                | $\theta_W$ (°)    | $\theta_F$ (°) | $\theta_D$ (°) | $\gamma^{LW}$  | $\gamma^+$    | $\gamma^-$     |                  |
| Untreated wood | 81.50<br>±0.73    | 54.50<br>±0.57 | 21.9<br>±0.2   | 47.1   | 0.44          | 3.74           | -64.38           |
| HEC-15 min     | 62.60<br>±0.03    | 30.30<br>±0.56 | 15.9<br>±0.9   | 48.76<br>±0.22   | 0.85<br>±0.03 | 10.19<br>±0.20 | -41.46           |
| HEC-1h         | 50.60<br>±0.33    | 33.60<br>±0.15 | 10.20<br>±0.61 | 49.89<br>±0.09   | 0.07<br>±0.01 | 25.46<br>±0.41 | -11.62           |

## Conclusion

The analysis of *C. atlantica* essential oil revealed cedranone (19.35%) and iso-cedranol (13.78%) to be the major components, followed by caryophyllene oxide (8.73%),  $\gamma$ -calamenene (7.77%),  $\delta$ -cadinene (7.34%),  $\beta$ -himachalane (7.23%), cedrol (4.44%), sabinene (4.32%), and  $\gamma$ -himachalane (4.05%). All of the bacterial and fungal strains that were isolated from decaying cedar wood and were tested in this study were found to be susceptible to *C. atlantica* essential oil. The physicochemical properties of cedar wood surfaces were found to change after

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treatment with *C. atlantica* essential oil, although the wood retained its hydrophobic character, quantitatively, after both 15 min and 1h of treatment. An increase in the electron donor/electron acceptor properties was noticed, and after 1 h of treatment, they were 2.5-fold than that of the 15-min treatment. Therefore, the *C. atlantica* essential oil, as a natural product, can be used as an alternative to synthetic chemical products, to produce an anti-adhesive and antimicrobial cedar wood surface and prevent biofilm development.

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